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ORAL PRESENTATIONS

O1

Future direction of pathogenesis and treatment for rheumatic disorders

Steffen Gay
Department of Rheumatology, University Hospital, Gloriastrasse 25, CH-8091 Zurich, Switzerland
E-mail: Steffen.Gay@usz.ch

Arthritis Research & Therapy 2012, **14(Suppl 1):**O1

After the breakthrough in the treatment of rheumatoid arthritis and numerous related disorders with biological therapies targeting TNFa at the Kennedy Institute in London

Millions of patients have tremendously benefitted. However, we cannot cure these diseases yet and have to search for additional therapeutic targets.

Since it was shown that synovial fibroblasts (SF) are not only effector cells responding to inflammatory stimuli, but appear endogenously activated and potentially involved into spreading the disease [1], we searched for the epigenetic modifications leading to the activated phenotype of these cells.

Epigenetics in its scientific definition "is the study of all heritable and potentially reversible changes in genome function that do not alter the nucleotide sequence within the DNA", but might be considered in simpler terms as the *regulation of gene expression*.

Epigenetic modifications include:
Acetylation,
Methylation,
Phosphorylation,
Sumoylation,
miRs or microRNAs.

Our laboratory is studying these processes and we have found that RASF reside in a hyperacetylated synovial tissue and appear hypomethylated [2]. Hypomethylation leads to the activated phenotype of RASF which is characterized by the production of matrix-degrading enzymes and of potent chemokines induced by Toll-like receptor signalling. Current strategies are designed to methylate these cells to deactivate and "normalise" them again.

miRs are about 20 nucleotide long smallRNAs acting to destroy specific mRNA.

In the race to identify specific miRs as novel targets we have identified for example, that interleukin-6 modulates the expression of the Bone Morphogenic Protein Receptor Type II through a novel STAT3microRNA cluster 17/92 pathway, which helps to explain the loss of the BMPR2 in the vascular cells in pulmonary hypertension [3]. Moreover, miR-203 is regulating the production of IL-6 [4].

Most interestingly, epigenetic therapy is also on the horizon [5].

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O2

From rheumatic diseases to cancer - role of autoantibodies as diagnostic biomarkers

Eng M Tan
Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA

Arthritis Research & Therapy 2012, **14(Suppl 1):**O2

Rheumatology has pioneered in the study of autoantibodies by showing that they are not only involved in pathogenesis but are also highly useful as diagnostic biomarkers. The diagnostic biomarker aspect of autoimmunity has gained increasing importance in cancer and many of the insights gained in Rheumatology have contributed to understanding the significance of autoantibodies in cancer.

Features of autoantibodies in rheumatic disorders: In rheumatic diseases no individual autoantibody-antigen system has sufficient combination of sensitivity and specificity to serve as a useful diagnostic biomarker. Instead, several antigen-antibody systems constructed as profiles of biomarkers are highly effective in distinguishing one disorder from another. In lupus, anti-double strand DNA and anti-Sm distinguishes it from scleroderma, where the profile is anti-DNA topoisomerase 1 and anti-centromere proteins. The autoantigensare cell components involved in universal and basic gene expression pathways, such as Sm in precursor mRNA splicing and DNA topoisomerase 1 in DNA replication and transcription [1].

Features of autoantibodies in cancer: Autoantibodies in cancer target intracellular molecules referred to as TAAs (tumor-associated antigens). As in rheumatic disorders, no individual autoantibody-antigen system has sensitivity and specificity to serve as a stand-alone diagnostic marker [2]. Most tumors show multiple antibody specificities and with panels of TAA-anti-TAAs (analogous toprofiles) the cumulative sensitivity and specificity reaches diagnostic significance. Different tumorigenesis pathways are

activated in similar cell-type tumors from the same organ and are the driving mechanisms behind the autoantibody response. The immune responses are directed to products of oncogenes and tumor-suppressor genes such as p53 and other proteins that regulate and modulate the functions of p53 [3-5].

Protein phosphatase 2A (PP2A) is an important tumor suppressor protein. It is a serine/threonine phosphatase and is a trimeric complex. The B subunit is recruited from several intracellular proteins and the type of B subunit determines the substrate of its tumor suppressor activity. One of the B subunits, p90, was identified in our laboratory with autoantibody from a patient with hepatocellular carcinoma [6]. It was found to co-immunoprecipitate with other subunits of PP2A [7] and was shown to function as an inhibitor of the tumor-suppressor activity of PP2A.

The immune system is capable of sensing dysregulation of tumorigenesis pathways. The goal of continuing research is in developingTAA-anti-TAAs for detecting cancer in individual patients and profiles which are common to specific types of tumors.

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O3

Etiology and molecular pathogenesis of RA; how can we best use European initiatives to advance our knowledge?

Lars Klareskog
Rheumatology Unit, Department of Medicine, Karolinska Institutet/Karolinska University Hospital, 171 76 Stockholm, Sweden
Arthritis Research & Therapy 2012, **14(Suppl 1):**O3

Understanding etiology and molecular pathogenesis of rheumatoid arthritis is key to the development of precise prevention and curative therapy for this disease. Recent progress on how genes and environment interact in causing immune reactions that may induce arthritis in humans as well as in mice, have provided a conceptual basis for the development of new prevention and treatment strategies which need to be different for different subsets of RA. In order to bring this emerging knowledge to the level where basic and clinical academic science can collaboratewitj industry for rapid development of the potential new therapies, there is a need for closer collaboration between basic and clinical scientists from many centers, and for increased collaboration between industry and academia in translational medicine.

In Europe, both the EU-funded framework programs and the EU and industry funder Innovative Medicine Initiative (IMI) funder programs in rheumatology are geared to accomplishing these goals. This presentation will be concerned both with the scientific basis of these programs and with a descriptions of the challenges and potential promises that these new collaborative programs offer to rheumatology.

O4

Clinically isolated syndrome in collagen diseases; approaches and treatments

Kazumasa Yokoyama*, Nobutaka Hattori
Department of Neurology, Juntendo University School of Medicine, Hongo, Tokyo, 113-8421, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O4

Background: Acute isolated neurological syndromes, such as optic neuropathy or transverse myelopathy, may cause diagnostic problems since they can be the first presentations in a number of demyelinating disorders including multiple sclerosis (MS) and collagen diseases. However, clinical presentation and lesions evidenced by magnetic resonance imaging may be similar. Collagen disease coexists in demyelinating disorders and frequently various collagen disease related autoantibodies are positive in daily practice.

Hence, the algorithm to overcome these diagnostic and therapeutic issues should be clarified.

B cell immunity in demyelinating disorders: In primary demyelinating disease, MS, a renewed interest in the role of humoral immunity in the pathophysiology has been investigated because oligoclonalIgG band in the CSF and increased intrathecalIgG synthesis are used as an auxiliary diagnosis measure. Moreover, in the secondary progressive MS, meningeal B-cell follicles are associated with early onset of the disease and severe cortical pathology. B cell but not plasma cell depletion therapy with single treatment by Rituximab in MS showed reduced inflammatory brain lesions and clinical relapses.

Oligodendropathy and astrocytopathy in demyelinating disorders: Neuromyelitisoptica (NMO) was previously considered to be a variant of MS but is now recognized as an astrocytopathy and secondary demyelinating event mimicking MS characteristics occurring due to autoantibody mediated mechanisms. Advancement of molecular biology makes it possible to differentiate MS by measuring abnormal autoantibody to aquaporin 4 (water channel). Interestingly, collagen diseases coexist more frequently with NMO than with MS. B cell depletion therapy with Rituximab has showed the same benefits, although, plasma exchange therapy is more effective with NMO than with MS.

TNF therapy and demyelinating event: A report indicates that adverse events such as the demyelinating lesion in the brain, optic neuritis, and neuropathy occurred after treatment with anti-TNF alpha therapy in collagen disease, and TNF antagonizing therapy showed worsening in a clinical trial with MS. Pathogenesis of these events such as primary or secondary demyelination are still in enigma.

In this presentation, I will decode the temporal and spatial demyelinating processes in collagen diseases and show practical approaches and treatments.

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O5

Research platform for fibromyalgia in Japan

Kusuki Nishioka
Japan College of Fibromyalgia Investigation, Japan Rheumatism Foundation, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O5

Epidemiology: Fibromyalgia (FM) is found worldwide, with an estimated prevalence of 1% to 4% of the general population. In Japan epidemiological surveillance showed number of the patient up to1.66% in population-based studies in 2003, following 2.04% by internet surveillance in 2011. 3,500 Japanese patients with our FM data base, frequent age of onset is 35 to 55 years, estimated prevalence of FM, reaching 40% in woman of age 30 to 50 years old. FM occurs in children and adolescents, although only a few epidemiologic studies.

Pathogenesis: FM was initially one the kind for inflammatory muscle pain. Following this concept FM was classified so called soft-tissue rheumatism. Now FM is recognized CNS sensitization following functional pain caused by neuroendocrine system and stress. The abundant neuroendocrine and pain regulation descending pathway and sensitization of pain receptor target molecules such as $\alpha_2\delta$ ligand, LPA and collapsing response mediator, where currently protein (CRMP) family have been revealed.

New diagnosis, provisional ACR 2010 criteria: 1990, ACR proposed FM criteria based on 18 tender point sites on digital palpation with exclusive differential diagnosis. In 2010, provisional criteria is score based on total score of severity for pain and somatic symptom (2010 ACR criteria). Based

on 2010 ACR criteria, we proposed the assessment of FM severity termed "FAS31". Here, we would like propose the assessment of FM severity score termed FAS31.
FDA approved of pregabalin in FM by double-blind, multicenter and randomized study. Both studies enrolled patients with a diagnosis of FM using the ACR criteria. Each of these studies showed a significant reduction in pain compared with placebo. In addition, improvement demonstrated based on FIQ. In Japan, this clinical trial has been developed. Sooner or later, excellent result will be revealed.
In other medication, gabapentin practical efficacy for reduced pain with FM patient. Several anti-dispersants (SSTIs, SNRIs. TCAs) NSAIDs, muscle relaxant, anti epileptics and pilocarpine hydrochloride also reduced the pain and an associated symptom. Based on with multivariant statistical analysis based on 3,500 patients, we will present several associated somatic symptoms influencing on drug response for pain and prognosis with FM.
In conclusion, FM is one the most important scientific field to understand the pain neurology and rheumatology in near.

O6

Recent advances in understanding of various chronic pain mechanisms through lysophosphatidic acid (LPA) receptor signaling

Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School Biomedical Sciences, Nagasaki 852-8521, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O6

Lysophosphatidic acid (LPA) receptor (LPA1) signaling plays the key role in initiation of nerve injury-induced neuropathic pain [1-4]. LPA, which is produced in the spinal cord following the sciatic nerve injury causes a calpain-mediated demyelination of dorsal root fibers and sprouting through LPA1 receptor, leading to an induction of synaptic reorganization underlying allodynia. The LPA1 signaling also initiates the up-regulation of $Ca_v\alpha_2\delta_1$ in DRG, leading to an enhancement of spinal pain transmission underlying hyperalgesia. Similar LPA1-mediated chronic abnormal pain and underlying mechanisms are observed in mouse models with Meth-A sarcoma surrounding sciatic nerve (cancer model) or with chemotherapy (paclitaxel). Central neuropathic pain following spinal nerve injury is now recently found to include the LPA1-mediated mechanisms. In contrast, (arthritic) inflammatory pain following Complete Freund Adjuvant treatment fails to show the involvement of LPA1 signaling. Thus it seems that many models of neuropathic pain, but not inflammatory pain model include LPA1-mediated mechanisms.
Recent studies revealed that another subtype LPA3 receptor plays a crucial role in neuropathic pain mechanisms in terms of LPA biosynthesis. Nerve injury and intrathecal administration of LPA increased the levels of lysophosphatidylcholine (LPC) and LPA in the spinal dorsal horn and dorsal root with peaks at 1 - 2 h. We obtained the evidence for in vitro LPA biosynthesis in spinal dorsal horn and dorsal root as well as in vivo one. In these studies we successfully identified the species of LPC and LPA molecules by use of Mass Spectrometery. Major species are the molecules with lipid chain 16:0, 18:0 or 18:1, and their contents were all time-dependently increased by nerve injury. Interestingly, there was an LPA-induced amplification of LPA biosynthesis through an activation of LPA3 receptor and microglia. The microglial involvement was found to play key roles as an initiation of neuropathic pain mechanisms including LPA3-mediated amplification of LPA biosynthesis.

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O7

The role of mRNA degradation in immunity and inflammation

Shizuo Akira
Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, 3-1 Yamadaoka, Suita City, Osaka 565-0871, Japan
E-mail: sakira@biken.osaka-u.ac.jp
Arthritis Research & Therapy 2012, **14(Suppl 1):**O7

The innate immune system is an evolutionally conserved host defense mechanism against pathogens. Innate immune responses are initiated by pattern recognition receptors (PRRs), which recognize specific structures of microorganisms. Among them, Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria to fungi, protozoa and viruses, and play a major role in innate immunity. Individual TLRs recognize different microbial components, and give rise to different patterns in gene expression.
We are now focusing on the role of genes induced in response to TLR stimulation, particularly the genes that are rapidly induced in a MyD88-dependent manner within 30 min after LPS stimulation. Among them, we have recently identified a novel gene named Zc3h12a which has a CCCH-type zinc finger domain. The knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. Subsequent studies showed that Zc3h12a is a nuclease involved in destabilization of IL-6 and IL-12mRNA. We renamed it Regulatory RNase-1 (Regnase-1) based on the function.
We recently found that the IKK complex controls *Il6* mRNA stability by phosphorylating Regnase-1 in response to IL-1R/TLR stimulation. Phosphorylated Regnase-1 underwent ubiquitination and degradation. Regnase-1 re-expressed in IL-1R/TLR-activated cells exhibited delayed kinetics, and Regnase-1 mRNA was found to be negatively regulated by Regnase-1 itself via a stem-loop region present in the Regnase-1 3' untranslated region. These data demonstrate that the IKK complex phosphorylates not only IkBalpha, activating transcription, but also Regnase-1, releasing the "brake" on *Il6* mRNA expression.

O8

Death receptor-induced apoptosis signalling - essential guardian against autoimmune disease

Andreas Strasser[1*], Lorraine A O'Reilly[1], Philipp Jost[1], Thomas Kaufmann[1], Stephanie Grabow[1], Elizabeth Kruse[1], Lin Tai[1], Mark Smyth[2], Philippe Bouillet[1]
[1]The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia; [2]The Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne, Victoria 3000, Australia
E-mail: strasser@wehi.edu.au
Arthritis Research & Therapy 2012, **14(Suppl 1):**O8

The FasL/Fas system is critical for deletion of autoreactive and antigen-activated T and B cells. Accordingly, mutations in these proteins result in lymphadenopathy and autoimmunity in *gld* and *lpr* mutant mice, which lack functional FasL or Fas, respectively. Upon antigenic stimulation of T cells, FasL is sythesised, directed to and stored in secretory lysosomes followed by extrusion at the immunological synapse where it is rapidly downregulated by a metalloprotease, shedding the extracellular portion (sFasL) to prevent non-specific killing. It is unclear whether the pathology observed in *gld* mutant mice is due to the loss of the membrane-bound or the secreted form of FasL or both.
We have produced a panel of mutant FasL knock-in mice to address this question. In the first mutant strain the cytoplasmic and trans-membrane domains of FasL were replaced with the signal peptide from G-CSF. Activated T cells from these mutant mice can produce cytoplasmic but no membrane bound FasL and, interestingly, they are defective in FasL-mediated cytotoxic function and undergo significantly less activation-induced cell death upon re-stimulation with anti-CD3 antibodies than wt T cells. The extent of these defects is similar to that seen in FasL mutant *gld* T cells. With age these FasL mutant knock-in mice develop lymphadenopathy and splenomegaly and $CD3^+B220^+CD4^-CD8^-$ T cells accumulate, similarly to what has been observed in *gld* and *lpr* mutant mice. In contrast to *gld* mice, the FasL mutant knock-in mice on the C57BL/6 background develop haemopoietic tumours and reticular cell sarcomas, suggesting that while

membrane-bound FasL is the guardian against autoimmunity, secreted FasL may play a critical role in tissue damage and tumour suppression.

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O9

Cancer immunotherapy; integration of T cell biology with nanogel- and vector-technology in translational research

Hiroshi Shiku
Dept. of Cancer Vaccine and Immuno-Gene Therapy, Mie University Graduate School of Medicine, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O9

Molecular definition of cancer specific antigens recognized by T cells opened an approach to develop cancer specific immunotherapy. Through a series of key findings in cancer immunology, for development of effective therapy major effort has been directed to how to induce T cells with fine specificity, sufficient quantity and high quality in hosts.

We intended to integrate immunobiological strategy of T cells with two technologies, nanogel technology and retroviral vector technology for translational research of cancer immunotherapy. Cholesterol-bearing hydrophobizedpullulan (CHP), physically cross-linked nanogels by self-assembly, form nanoparticle complex with protein in water.We found that antigen protein with multiple T cell epitopes, when complexed with CHP, was efficiently transported to lymph nodes and well captured by antigen presenting cells such as dendritic cells and macrophages leading to cross presentation. Hence, CHP-antigen protein complex may become excellent cancer vaccine to induce both $CD8^+$ killer T cells and $CD4^+$ helper T cells of high quality.

Intrinsic weakness of insufficiency in number of cancer specific T cells in hosts, prompted us to develop adoptive T cell therapy withlymphocytes engineered to possess cancer specificity. For this purpose, we developed novel retroviral vectors to highly express exogenously transduced cancer specific T cell receptor (TCR), yet suppressing expression of endogenous polyclonal TCR. This approach allowed us to prepare T cells with finer specificity of expressed TCR. In addition, use of RetroNectin®, a recombinant fragment of fibronectin opened a way to ex vivo prepare T cells of sufficient quantity and good quality for clinical use.

Translational clinical trials of these cancer vaccine and adoptive T cell therapy are now on-going.

An open innovation to promote fusion of different fields of science and technology played an essential role in our development of cancer immunotherapy.

O10

Autoimmune arthritis caused by altered thymic T-cell selection due to a mutation of the ZAP-70 gene

Yoshinaga Ito[1*], Shimon Sakaguchi[1,2]
[1]Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; [2]Laboratory of Experimental Immunology, WPI Immunology Frontier Research Center, Osaka University, Suita, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O10

SKG mouse is a murine model of autoimmune arthritis. A spontaneous point mutation of the gene encoding an SH2 domain of the ζ-associated protein of 70 kDa gene (ZAP-70), a key signal transduction molecule in T cells, causes chronic autoimmune arthritis in SKG mice that resembles human RA in many aspects. Altered signal transduction from T-cell antigen receptor through the aberrant ZAP-70 changes the thresholds of T cells to thymic selection, leading to the positive selection of otherwise negatively selected autoimmune T cells.

Based on the finding that the skg-mutation of ZAP-70 causes autoimmune arthritis, we then examined how attenuated TCR signaling affects the spectrum of autoimmune diseases. In a set of mice with the mutation, the amount of ZAP-70 protein as well as its tyrosine phosphorylation upon TCR stimulation decreased from +/+, *skg*/+, *skg*/*skg*, to *skg*/− mice in a stepwise manner. The reduction resulted in graded alterations of thymic positive and negative selection of self-reactive T cells and $Foxp3^+$ natural regulatory T cells (Tregs) and their respective functions. Consequently, *skg*/− mice spontaneously developed autoimmune arthritis even in a microbially clean environment, whereas *skg*/*skg* mice required stimulation through innate immunity for disease manifestation. After Treg depletion, organ-specific autoimmune diseases, especially autoimmune gastritis, predominantly developed in +/+, at a lesser incidence in *skg*/+, but not in *skg*/*skg* BALB/c mice, which suffered from other autoimmune diseases, especially autoimmune arthritis. In correlation with this change, gastritis-mediating TCR transgenic T cells were positively selected in +/+, less in *skg*/+, but not in *skg*/*skg* BALB/c mice. Similarly, on the genetic background of diabetes-prone NOD mice, diabetes spontaneously developed in +/+, at a lesser incidence in *skg*/+, but not in *skg*/*skg* mice, which instead succumbed to arthritis. Thus, the graded attenuation of TCR signaling alters the repertoire and the function of autoimmune T cells and natural Tregs in a progressive manner. It also changes the dependency of disease development on environmental stimuli. These findings collectively provide a model of how genetic anomaly of T cell signaling contributes to the development of autoimmune disease.

O11

Anti-Fas IgM monoclonal antibody (anti-Fas mAb) effect on haemophilic arthropathy (HA) synoviocytes

Serena Guiducci[1*], Eloisa Romano[1], Claudia Ceccarelli[1], Daniela Melchiorre[1], Mirko Manetti[2], Anna F Milia[2], Lidia Ibba Manneshi[2], Kusuki Nishioka[3], Marco Matucci Cerinic[1]
[1]Dept Medicine, Division of Rheumatology, University of Florence, Florence, Italy; [2]Dept Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy; [3]Dept Rheumatology, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O11

Background: Haemophilic arthropathy (HA), which shares some clinical and biological injury characteristics with rheumatoid arthritis (RA), is characterized by chronic proliferative synovitis and cartilage destruction. Anti-Fas mAb specifically targets the Fas molecule, which is expressed and activated on the cell surface of inflammatory synovial cells and plays a key role for induction of apoptosis. Caspases are the final executioners of apoptosis and their activation requires proteolytic processing of inactive zymogen into activated fragments.

Aim: To evaluate the effects of anti-Fas mAb on HA synoviocytes and its capacity of inducing apoptosis analysing caspase 3 activity.
Methods: HA synoviocytes were incubated with IgM 1000 ng/ml (control), TNFalpha 10 ng/ml, FGF 10 ng/ml, CH11 100 ng/ml (positive control of apoptosis) with or without anti-Fas mAb at different concentrations (from 0,1 to 1000 ng/ml) for 24 h. RA and healthy synoviocytes were used as controls. To measure cell proliferation/citotoxicity, the WST-1 assay has been performed. Caspase 3 activity has been evaluated with ELISA kit and western blot.
Results: Anti-Fas mAb induced a citotoxic effect in HA ($p < 0,001$ for any dose), healthy ($p < 0,001$ at 100 and 1000 ng/ml) and RA synoviocytes ($p < 0,05$ for any dose) reaching a maximum effect at 1000 ng/ml. After stimulation with anti-Fas mAb combined with TNFalpha, there was a citotoxic effect on healthy ($p < 0,001$ at 10, 100, 1000 ng/ml anti-Fas mAb), RA ($p < 0,001$ for any dose) and HA synoviocytes ($p < 0,005$ at 1, 10, 100 and 1000 ng/ml anti-Fas mAb). After stimulation with anti-Fas mAb combined with FGF, there was a citotoxic effect on healthy, RA and HA synoviocytes ($p < 0,001$ for any dose). Caspase 3 levels were increased in HA synoviocytes after anti-Fas mAb treatment in a dose-dependent manner, even after co-stimulation with TNFalpha ($p < 0,001$ for any stimulus). CH11 induced an increase of caspase 3 levels in HA synoviocytes more than RA synoviocytes. Western blot showed that HA synoviocytes had higher levels of activated caspase 3 compared to RA synoviocytes after stimulation with Anti-Fas mAb, CH11 and co-stimulation with TNFalpha.
Conclusion: Anti-Fas mAb has a dose-dependent citotoxic effect on HA synoviocytes, even when associated with TNFalpha and FGF. Anti-Fas mAb is effective in increasing caspase 3 levels in HA synoviocytes in a dose-dependent manner. HA synoviocytes show higher levels of activated caspase 3 compared to RA synoviocytes. Our results suggest that anti-Fas IgM mAb may favour the induction of apoptosis in HA synoviocytes.

O12

Overview of osteoimmunology: What's happened? And what's going on?

Hiroshi Takayanagi
Tokyo Medical and Dental University, ERATO, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O12

The interaction between the immune and skeletal systems has long been acknowledged, but molecular mechanisms linking the two systems have not been demonstrated until recently. Investigation into autoimmune arthritis as well as the various bone phenotypes found in mice deficient in immunomodulatory molecules has highlighted the importance of the dynamic interplay between the two systems and brought about a rapid evolution of the field of osteoimmunology [1]. In bone loss in autoimmune arthritis, IL-17-producing helper T (T_H17) cells play a major role by inducing RANKL [2]. Maintenance and mobilization of hematopoietic cells are regulated by bone cells. In addition to cellular interactions via cytokines, the immune and skeletal systems share various molecules, including transcription factors, signaling molecules and membrane receptors. RANKL stimulates osteoclastogenesis through NFATc1 in cooperation with immunoglobulin-like receptors. Here I will discuss emerging topics in osteoimmunology including the mechanisms underlying bone cell communication: osteocyte RANKL [3] and inhibition of bone formation by osteoclast Sema4D [4].
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O13

Regulation of bone mass at unloaded condition by osteocyte network

Toshihisa Komori
Department of Cell Biology, Unit of Basic Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O13

Disuse osteoporosis, which occurs commonly in prolonged bed rest and immobilization, is becoming a major problem in modern societies; however, the molecular mechanisms underlying unloading-driven bone loss have not been fully elucidated. Bone adjusts its shape and strength against mechanical stress. Osteocytes are the most abundant cells in bone and comprise the communication system through the processes and canaliculi throughout bone. The osteocyte network is considered to be an ideal mechanosensor and mechanotransduction system. We found that overexpression of *BCL2* in osteoblasts reduces the number of osteocyte processes, probably due to the function of Bcl2 that modulates cytoskeletal reorganization, and induces the apoptosis of osteocytes, in which the transgene expression was reduced, presumably caused by an insufficient supply of oxygen, nutrients, and survival factors due to the reduced osteocyte processes. Our *BCL2* transgenic mouse with accumulated dead osteocytes is a useful model to analyze the function of osteocytes, because a repair process, which replaces dead osteocytes with new osteocytes by bone resorption and formation, was not evident in the mice irrespective of the massive accumulation of dead osteocytes
We searched for the molecules responsible for disuse osteoporosis using *BCL2* transgenic mice. Pyruvate dehydrogenase kinase isozymes (Pdk1, Pdk2, Pdk3, and Pdk4) are negative regulators of pyruvate dehydrogenase complex (PDC), which converts pyruvate to acetyl-CoA in the mitochondria, linking glycolysis to the energetic and anabolic functions of the tricarboxylic acid (TCA) cycle. Pdk4 was upregulated in femurs and tibiae of wild-type mice but not of *BCL2* transgenic mice after tail suspension. Bone in *Pdk4*$^{-/-}$ mice developed normally and was maintained. At unloading, however, bone mass was reduced due to enhanced osteoclastogenesis and *Rankl* expression in wild-type mice but not in *Pdk4*$^{-/-}$ mice. Osteoclast differentiation of *Pdk4*$^{-/-}$ bone marrow-derived monocyte/macrophage lineage cells (BMMs) in the presence of M-CSF and RANKL was suppressed, and osteoclastogenesis was impaired in the coculture of wild-type BMMs and *Pdk4*$^{-/-}$ osteoblasts, in which *Rankl* expression and promoter activity were reduced. Further, introduction of *Pdk4* into *Pdk4*$^{-/-}$ BMMs and osteoblasts enhanced osteoclastogenesis and *Rankl* expression and activated *Rankl* promoter. These findings indicate that upregulation of *Pdk4* expression in osteoblasts and bone marrow cells after unloading is, at least in part, responsible for the enhancement of osteoclastogenesis and bone resorption after unloading [1].
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O14

Assessment of histological alterations in cartilage and extracellular matrix driven by collagen-induced arthritis in Macaca fascicularis

Norio Amizuka[1*], Hiromi Hongo[1], Muneteru Sasaki[1], Tomoka Hasegawa[1], Paulo Henrique Luiz de Freitas[2], Hiroshi Mori[3], Minqi Li[1]
[1]Dept of Develop Biol of Hard Tissue, Hokkaido University, Sapporo, Japan; [2]Dept of Oral/Maxillofacial Surg, Dr Mário Gatti Municipal Hospital, Campinas, Brazil; [3]Pharmacological Evaluation Section, Ono Pharmaceutical Co Ltd, Osaka, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O14

Background: Arthritis is characterized by progressive cartilage erosion, inflammation of adjoining soft tissues and collapse of subchondral bone due to enhanced osteoclastic resorption. Human joints are complex structures formed by synovial tissues, articular cartilage and subchondral bone tissue. Believing on the similarities of normal joints in humans and monkeys, we have employed a model of collagen-induced arthritis in Macaca fascicularis (or crab-eating monkey) in an attempt to evaluate the histological alterations caused by such condition in the extracellular matrix of the articular cartilage.
Materials and methods: Intermediate phalangeal proximal joints of six Macaca fascicularis suffering from collagen-induced arthritis were extracted and fixed with 4% paraformaldehyde solution. Samples were also taken from disease-free animals as controls. Tissues were embedded in paraffin or epoxy resin for histochemical and ultrastructural observations. Paraffin sections were used for alkaline phosphatase (ALP), tartrate-resistant acid

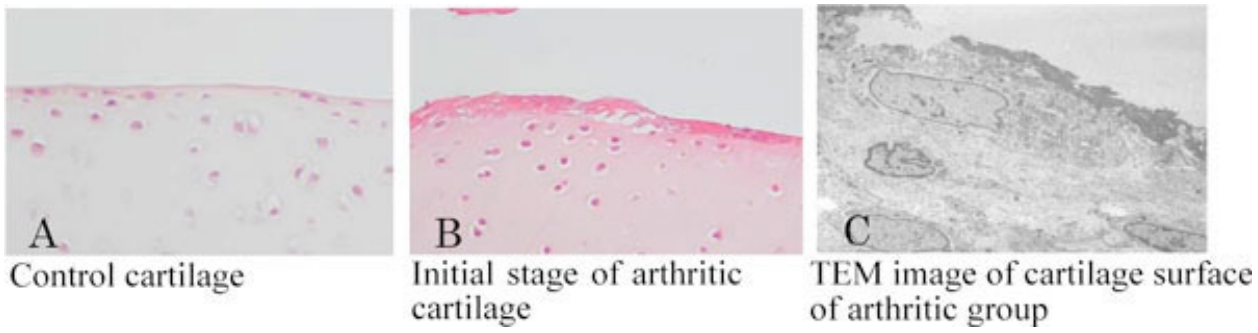

Figure 1(abstract O14)

phosphatase (TRAP), cathepsin K, MMP-1, type II collagen, CTX-II (fragmented type II collagen) and fibronectin staining assessments.
Results: Control monkeys showed faint immunoreactivity against cathepsin K and MMP-1 in cells covering the articular cartilage and synovial tissues, indicating physiological levels of collagenous degradation. In arthritic animals, more intense cathepsin K and MMP-1 staining was observed in similar locations. ALP-positive osteoblasts and TRAP-reactive osteoclasts were abundant at the subchondral bone in arthritic samples, while control ones depicted fewer osteoclasts and weakly-stained ALP-positive osteoblasts, suggesting stimulated bone turnover in the arthritic group. Interestingly, a thick cell layer covered the articular cartilage with arthritis, and cellular debris overlaid this thick cell layer; nonetheless, articular chondrocytes seemed intact (Figure 1). In arthritic joints, the synovial tissues displayed cellular debris in abundance. CTX-II was seen in the superficial layer of the articular cartilage in arthritic samples, but it was virtually absent in the control group. Fibronectin also accumulated on the surface of the arthritic cartilage.
Conclusion: Based on the evidence provided, it is possible that matrix degradation starts not from the adjacent subchondral bone, but from the most superficial region of the arthritic cartilage.

O15

Evidence for synovial fibroblasts spreading rheumatoid arthritis

Ulf Müller-Ladner[1*], Stefanie Lefèvre[1], Birgit Zimmermann[1], Ingo H Tarner[1], Robert Dinser[1], Thomas Pap[2], Steffen Gay[3], Elena Neumann[1]
[1]Dept Internal Medicine and Rheumatology, JLU Giessen, Kerckhoff-Clinic, Bad Nauheim, 61231 Germany; [2]Div Mol Med of Musculoskeletal Tissue, University Hospital Münster, Germany; [3]Ctr Exp Rheumatology, Zürich Center for Integrative Human Physiology, USZ, Zürich, 8006 Switzerland
Arthritis Research & Therapy 2012, **14(Suppl 1):**O15

Background: Active rheumatoid arthritis (RA) is characterized by continuous progression of the inflammatory process, eventually affecting the majority of joints. Thus far, molecular and cellular pathways of disease progression are largely unknown. One of the key players in this destructive scenario are synovial fibroblasts (SF) which actively attach to, invade into and degrade articular cartilage. As RASF are able to migrate *in vitro*, the current series of experiments were designed to evaluate the potential of RASF to spread the disease *in vivo* in the SCID mouse model of RA.
Methods: Healthy human cartilage was co-implanted subcutaneously into SCID mice together with RASF. At the contralateral flank, simulating an unaffected joint, cartilage was implanted without cells. To analyze the route of migration of RASF, the cells were injected subcutaneously, intraperitoneally or intravenously before or after implantation of cartilage. In addition, whole RA synovium and normal human cartilage were implanted separately in order to analyze the effects of matrix and other cells on the migratory behavior of RASF. To evaluate potential influences of wound healing, either the primary RASF-containing implant or the contralateral implant without RASF, respectively, was inserted first, followed by implantation of the corresponding other implant after 14 days. After 60 days, implants, organs and blood were removed and analyzed. For the detection of human cells, immunohisto- and -cytochemistry were performed with species-specific antibodies.
Results: RASF not only invaded and degraded the co-implanted cartilage, they also migrated to and invaded into the contralateral cell free implanted cartilage. Injection of RASF led to a strong destruction of the implanted cartilage, particularly after subcutaneous and intravenous application. Interestingly, implantation of whole synovial tissue also resulted in migration of RASF to the contralateral cartilage in one third of the animals. With regard to the route of migration, few RASF could be detected in spleen, heart and lung, mainly located in vessels, most likely resulting from an active movement to the target cartilage *via* the vasculature. With respect to functional aspects, growth factors and adhesion molecules appear to influence significantly the migratory behavior of the synovial fibroblasts.
Conclusions: The results support the hypothesis that the clinically characteristic phenomenon of inflammatory spreading from joint to joint is mediated, at least in part, by a transmigration of activated RASF, regulated by growth factors and adhesion molecules.
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O16

Skeletal involvement in the pathogenesis and outcomes of rheumatoid arthritis and osteoarthritis

Jean-Pierre Pelletier
University of Montreal and University of Montreal Hospital Centre (CHUM), Montreal, Quebec, Canada
Arthritis Research & Therapy 2012, **14(Suppl 1):**O16

Bone remodeling is a frequently observed phenomenon in musculoskeletal diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). The level of imbalance between bone resorption/deposition is responsible for the morphological changes osteopenia/bone erosion/osteosclerosis observed in these arthritic conditions.
In RA, increased osteoclastic activity is responsible for the development of focal osteopenia/erosion and systemic osteoporosis. The increased osteoclast activity in RA has been demonstrated to be linked to a dysregulation of pathways including cell-cell interactions, cytokines, and the receptor activator of nuclear factor κB (RANK)/RANK ligand (RANKL) system. Recent studies have shown that joint erosion in RA is linked to a decrease in long-term physical function.
Under OA conditions, the subchondral bone is the site of numerous dynamic morphological changes. These changes are associated with a number of local abnormal biochemical pathways related to the altered metabolism of osteoblasts and osteoclasts. At the early stages of the disease process, increased bone loss and resorption is observed with subchondral bone associated with local production of catabolic factors including cathepsin K and MMP-13. Moreover, OA osteoblasts present an abnormal phenotype resulting in increased production of growth hormones and catabolic factors. In addition, factors such as osteoprotegerin (OPG) and RANKL have been

found to be expressed and modulated over time in human OA subchondral bone. Their synthesis varies from being reduced in early OA to being increased in the late stages of the disease. This finding may explain that in the early stages of OA, bone remodeling favors resorption and in the more advanced stages of the disease, bone formation is predominant.
Magnetic resonance imaging (MRI) studies in knee OA patients have shown that the subchondral bone is frequently the site of signal alterations-bone marrow lesions (BML) - indicative of a great variety of morphological changes. BML and cartilage loss have been linked in several studies. Moreover, studies have identified, in OA patients, a number of risk factors for total knee replacement including BMLs.
The paradigms regarding the role of bone lesions in arthritic diseases raise a number of important questions. A comprehensive understanding of the factors that contribute to these changes will provide us with better knowledge of the pathophysiology of the diseases and the role of these structural alterations in patient symptoms and prognosis, as well as guiding the development of new therapeutic strategies.

O17

Fcγ receptor targeting in RA

Toshiyuki Takai[1*], Akira Nakamura[1,2], Akiko Tobinai[1], Shota Endo[1], Masanori Inui[1]
[1]Department of Experimental Immunology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-8575, Japan; [2]Department of Immunology, Kanazawa Medical University, Ishikawa 920-0293, Japan
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The activation threshold of cells in the immune system is often tuned by cell surface molecules. Among these, Fc receptors expressed on various hematopoietic cells constitute critical elements for activating or down-modulating immune responses.
IgGFc receptors (FcγRs) were originally identified as B cell surface molecules. For more than 40 years, FcγRs have continued to attract the interest of many basic researchers and clinicians due to their intriguing IgG binding ability, which provides a critical link between the humoral and cellular branches of the immune system.
Several activating-type FcγRs, which associate with homodimeric Fc receptor common γ subunits, are crucial for the onset and exacerbation of inflammatory diseases. In contrast, a unique inhibitory FcγR, FcγRIIB, plays a critical role in keeping immune cells silent. Murine models for allergic responses and autoimmune diseases including RA illustrate the indispensable roles of activating-type FcγRs and the inhibitory FcγRIIB in the initiation and suppression of inflammation, respectively [1-5].
The ultimate goals of FcγR research are to accomplish our understanding of this molecular family and to delineate novel therapeutic strategies toward the conquest of allergic and autoimmune diseases, infectious diseases, immunodeficiency, transplantation-associated immune disorders, and malignant tumors. Although many lines of evidence indicate that a part of the intravenous Ig (IVIg)-mediated anti-inflammatory effects can be attributable to the blocking of activating-type FcγRs, recent studies have pointed out an indispensable role of FcγRIIB in therapeutic benefits of IVIg in several murine models of inflammatory diseases including RA [6]. In this session, we will give a brief summary of recent knowledge on antibody biomedicine including IVIgto you, in light of exploiting FcγRs as potential therapeutic targets for various inflammatory diseases, along with the comparison withnon-FcγR-mediated mechanisms of IVIg.

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O18

Therapeutic targets for rheumatoid arthritis: lessons from animal models

Yoichiro Iwakura[1,2*], Shinobu Saijo[1], Susumu Nakae[1], Noriyuki Fujikado[1], Harumichi Ishigame[1], Masanori Murayama[1]
[1]Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan; [2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama 332-0012, Japan
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We have generated two RA models, human T-cell leukemia virus type I (HTLV-I) transgenic mice and IL-1 receptor antagonist (Ra)-deficient (KO) mice, to elucidate the pathogenic mechanisms of the disease. Both models spontaneously developed arthritis closely resembling that of RA in humans. We found that TNF-, but not IL-6-, deficiency suppressed development of arthritis in IL-1Ra KO mice, while IL-6 but not TNF was involved in the HTLV-I transgenic mouse model [1]. IL-17 was important in both models. These observations suggest that pathogenic roles of IL-6 and TNF are different and both TNF, IL-6, and IL-17 are good targets for therapeutics.
We found that the expression of C-type lectin receptor (CLR) genes was augmented in the affected joints of these models using DNA microarrays. Dendritic cell immunoreceptor (DCIR) is one of such CLRs with a carbohydrate recognition domain in their extracellular carboxy terminus and an ITIM in its intracellular amino terminus. Because human shared syntenic locus containing the *Dcir* gene is linked to several autoimmune diseases including RA and SLE, we have generated *Dcir* KO mice to examine the roles of this gene in the immune system. We found that aged *Dcir* KO mice spontaneously developed sialadenitis and enthesitis associated with elevated serum autoantibodies [2]. DCs were excessively expanded in *Dcir* KO mice after aging. *Dcir* KO mouse-derived bone marrow cells (BMCs) differentiated into DCs more efficiently than did wild-type BMCs upon treatment with GM-CSF, owing to enhanced STAT-5 phosphorylation. These findings indicate that DCIR is crucial for maintaining the homeostasis of the immune system, suggesting that Dcir is one of novel targets for the treatment of RA.
We have also found that the expression of *Muratin1*, which encodes uncharacterized and secreted protein, is specifically up-regulated in affected joins of both models. Interestingly, the development of collagen-induced arthritis was markedly exacerbated in *Muratin1* KO mice. I would like to discuss the roles of Muratin-1 in the development of arthritis.

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O19

Abnormal osteogenesis in osteoarthritis: gone with the Wnt?

Daniel Lajeunesse*, Thomas F Chan, Aline Delalandre, Jean-Pierre Pelletier, Johanne Martel-Pelletier, Élie Abed
Unité de recherche en Arthrose, CRCHUM, Université de Montréal, Québec, Canada, H2L 4M1
Arthritis Research & Therapy 2012, **14(Suppl 1):**O19

Background: Clinical and in vitro studies suggest that subchondral bone sclerosis due to abnormal osteoblast (Ob) functions, is involved in the progression and/or onset of osteoarthritis (OA). Human OA subchondral Ob show a differentiated phenotype, however they fail to mineralize normally. The canonical Wnt/b-catenin signaling pathway (cWnt) plays a key role in osteogenesis by promoting the differentiation and mineralization of Ob.

Dickkopfs (DKKs) are potent antagonists whereas R-spondins (Rspo) are newly described agonists that play key roles in cWnt signalling. However, the regulation of DKKs and Rspos in OA Ob remains unknown.

Materials and methods: We prepared primary human subchondral Ob using the sclerotic medial portion of the tibial plateaus of OA patients undergoing knee arthroplasty, or from tibial plateaus of normal individuals at autopsy. DKK1, DKK2, SOST and Rspo-1 and -2 expression and production were evaluated by qRT-PCR and WB analysis. The regulation of their expression was determined in response to transforming growth factor-ß1 (TGF-ß1) and as a function of the growth of OA Ob. Selective inhibition was performed using siRNA techniques. cWnt signaling was evaluated by measuring target gene expression using the TOPflash Tcf/lef luciferase reporter assay and intracellular ß-catenin levels by WB. Mineralization was evaluated by Alizarin red staining. TGF-ß1 levels were determined by ELISA.

Results: DKK2 expression and production were elevated in OA Ob compared to normal whereas DKK1 was similar. Rspo2 expression was reduced in OA Ob whereas Rspo1 was similar. TGF-ß1mRNA expression and protein levels were high in OA Ob. TGF-b1 stimulated DKK2 expression and production in Ob whereas it inhibited Rspo2 expression. cWnt signaling was reduced in OA compared to normal Ob. This inhibition was due in part to elevated DKK2 levels and to reduced Rspo-2 levels since correcting DKK2 by siRNA or the addition of Rspo-2 increased cWnt signaling using the TOPflash reporter assay. These treatments also increased ß-catenin levels in OA Ob. Mineralization of OA Ob was reduced compared to normal Ob and was also corrected in part by inhibiting DKK2 or by Rspo2 addition. Both elevated DKK2 and reduced Rspo2 levels contributed to abnormal expression of bone markers by OA Ob.

Conclusions: These studies demonstrate that elevated antagonist or reduced agonist levels of cWnt signalling interfere in normal Ob function and lead to abnormal mineralization. Since these are secreted soluble proteins, this could lead to potential new avenues of treatment of OA to correct their abnormal bone phenotype and mineralization.

O20

Understanding the role of Fas-Fas ligand system in bone

Ana Marusic[1*], Natasa Kovacic[2], Ivan Kresimir Lukic[3], Vedran Katavic[2], Danka Grcevic[4]

[1]Department of Anatomy and Department of Research in Biomedicine and Health, University of Split School of Medicine, Split, Croatia; [2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia; [3]Partek Inc, Zagreb, Croatia; [4]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia

Arthritis Research & Therapy 2012, **14(Suppl 1):**O20

Fas ligand (CD 178) and its receptor Fas (CD 95) are members of the TNF superfamily of ligands and receptors involved in the activation of apoptosis. Our research group demonstrated that Fas and Fas ligand were expressed during osteoblast and osteoclast differentiation, and their expression may be modified by various cytokines. The lack of functional Fas signaling in murine models leads to altered endochondral ossification, increase of the bone mass in adult mice, and resistance to ovariectomy-induced bone loss. We also showed that mice with a Fas gene knockout lose less bone during antigen-induced arthritis. These changes seem to be, at least in part, mediated by increased expression of osteoprotegerin (OPG), another member of the TNF superfamily, which acts as a decoy receptor for receptor activator for nuclear factor κB (RANK) ligand (RANKL). The bone phenotype of mice lacking Fas signaling may be related to the immunological disturbance rather than intrinsic bone disorder. To address this question at molecular level, we performed a set of parabiotic experiments in mice with non-functional Fas ligand mutation (gld mice). Mice were kept in parabiosis for 1 to 4 weeks, and for 2 weeks after separation from 4-week parabiosis. We also analyzed OPG levels in the peripheral blood of patients with autoimmune lymphoproliferative syndrome (ALPS). Joined circulation between gld and wild-type mice led to increased expression of bone protective OPG in the wild-type animal, both at the gene and protein level at 4 weeks of parabiosis. This effect was sustained even after the separation of parabiotic mice. At the same time, double-negative T lymphocytes transferred from gld into wild-type member of a parabiotic pair rapidly vanished from the periphery of both gld and control mice in parabiosis. Patients with ALPS had increased OPG mRNA level in peripheral blood mononuclear cells, as assessed by real-time PCR, in comparison to age- and sex-matched controls. These findings show that bone and immune changes are uncoupled during Fas ligand deficiency. Under the assumption that OPG also acts as a molecular brake in the immune system, downregulation of OPG in gld mice during parabiosis with wild-type mice could be considered as a molecular marker of remission. Increased expression of OPG in children with ALPS leads to the hypothesis that a similar mechanism might be at play in humans.

O21

Regulation of inflammatory immune responses leading to the development of bone destructive autoimmune disease rheumatoid arthritis by IL-27

Takayuki Yoshimoto[1*], Mingli Xu[1,2], Izuru Mizuguchi[1], Yukino Chiba[1,2], Sadahiro Kamiya[3], Masanori Matsui[4], Shiva Shahrara[5], Junichiro Mizuguchi[2]

[1]Department of Immunoregulation, Institute of Medical Science, Tokyo Medical University, Tokyo 160-8402, Japan; [2]Department of Immunology, Tokyo Medical University, Tokyo 160-8402, Japan; [3]Departments of Clinical Sciences, Josai International University, Chiba 283-8555, Japan; [4]Department of Microbiology, Saitama Medical University, Saitama 350-0495, Japan; [5]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

Arthritis Research & Therapy 2012, **14(Suppl 1):**O21

IL-27, a member of the IL-6/IL-12 family of cytokines, induces early helper T (Th)1 differentiation and generation of cytotoxic T cells and IL-10-producing type 1 regulatory T cells, while it suppresses the production of inflammatory cytokines and inhibits Th2 and Th17 differentiation [1,2]. The receptor activator of NF-kB ligand (RANKL), which is expressed by not only osteoblasts but also activated T cells, plays an important role in bone-destructive disease rheumatoid arthritis (RA). Recently, IL-17-producing Th17 cells were identified as the exclusive osteoclastogenic T-cell subset. This is because Th17 cells express RANKL, and that IL-17 not only induces RANKL expression on osteoblasts, but also increases the production of various inflammatory molecules. It was previously reported that IL-27 is detected in RA synovial membranes and that treatment with IL-27 attenuated inflammatory responses in collagen-induced arthritis (CIA), one of mouse RA models.

We have been investigating the role of IL-27 in the regulation of inflammatory responses leading to the development of bone destructive autoimmune disease. We first demonstrated that osteoclastogenesis from bone marrow cells induced by soluble RANKL is inhibited by IL-27 with reduced multinucleated cell numbers [3]. Then, other group further clarified that IL-27 directly acts on osteoclast precursor cells and suppresses RANKL-mediated osteoclastogenesis through STAT1-dependent inhibition of c-Fos, leading to amelioration of the inflammatory bone destruction. We recently investigated the mechanistic role of IL-27 in the pathogenesis of CIA and found that local injection of adenoviral IL-27 transcript into the ankles of CIA mice attenuates joint inflammation, synovial lining thickness, bone erosion and leukocyte migration [4]. IL-27 reduced the production of IL-1b and IL-6, and suppressed Th17 cell differentiation as well as IL-17 downstream target genes, which leads to decreased IL-17-mediated monocyte recruitment and angiogenesis possibly through the reduction of neutrophil and monocyte chemokines. We also elucidated that IL-27 inhibits cell surface expression of RANKL on naive $CD4^+$ T cells activated by T cell receptor ligation and secretion of its soluble RANKL as well [5]. The inhibitory effect was mediated in part by STAT3 but not by STAT1 or IL-10. In differentiated Th17 cells, IL-27 much less but significantly inhibited the RANKL expression after re-stimulation.

Taken together, these results suggest that IL-27 regulates inflammatory immune responses leading to the development of bone destructive autoimmune disease through multiple mechanisms as described above (Figure 1), and that IL-27 may be a promising target for therapeutic intervention to control disease in RA patients.

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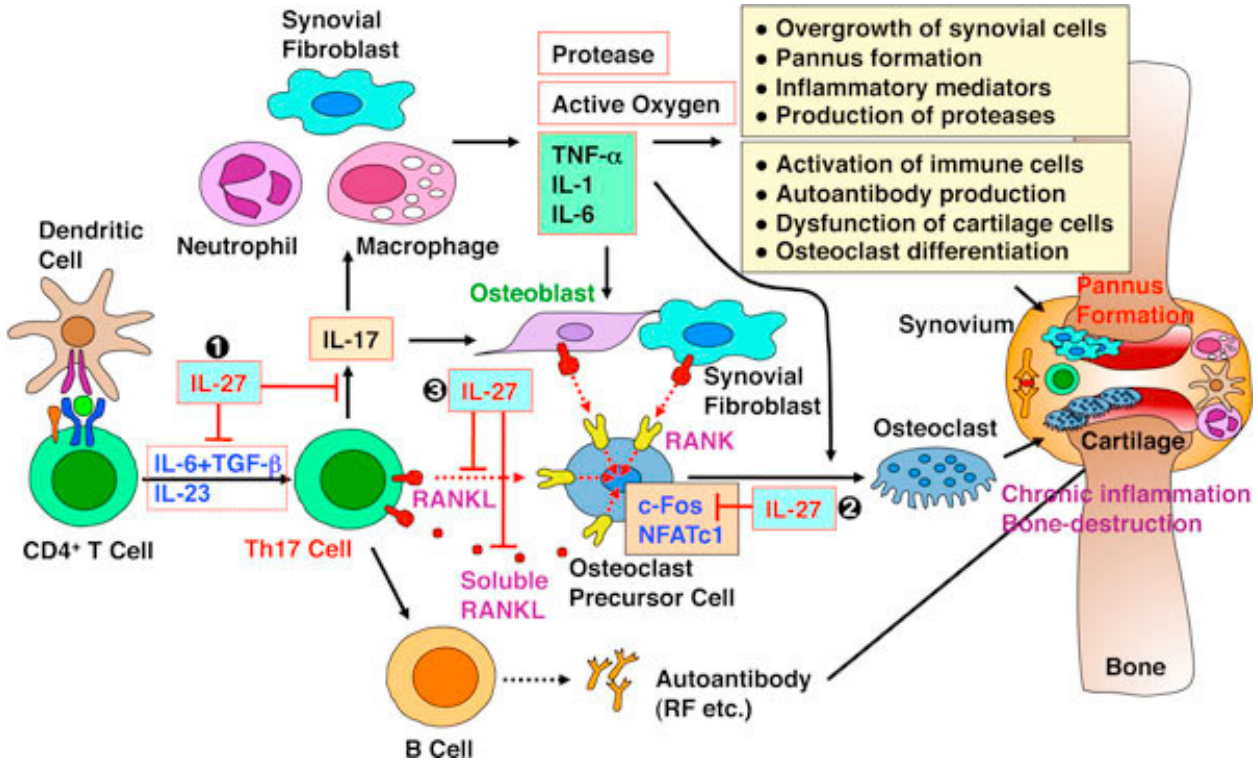

Figure 1(abstract O21) IL-27 regulates inflammatory immune responses leading to the development of bone destructive autoimmune disease RA through multiple mechanisms.

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O22

Postnatal *Syk* deletion in mice clarifies the function of Syk in an anti-collagen antibody-induced arthritis model

Naoko Ozaki[1,2*], Shinobu Suzuki[2], Hiromitsu Hara[1], Hiroki Yoshida[1]
[1]Division of Molecular and Cellular Immunoscience, Department of Biomolecular Sciences, Faculty of Medicine, Saga University, Nabeshima, Saga, 849-8501, Japan; [2]Department of Molecular & Cellular Biology, Kobe Pharma Research Institute, Nippon Boehringer Ingelheim Co Ltd, Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O22

Spleen tyrosine kinase (Syk) is a cytoplasmic protein expressed mainly in immune cells including macrophages and neutrophils and is associated with receptors containing an immunoreceptor tyrosine-based activation motif (ITAM), such as Fcγ receptors. As Syk-mediated signaling plays an important role in activation of immune responses, to investigate whether specific interruption of Syk-mediated signaling can affect the development of rheumatoid arthritis (RA), we used tamoxifen-induced conditional Syk-KO mice (iSyk KO) to evaluate the importance of Syk on disease development. Using a collagen antibody-induced arthritis model (CAIA), iSyk KO mice showed significantly attenuated disease severity compared to Syk non-deleted mice (Figure 1). Although iSyk KO mice contained reduced B cell numbers after deletion of Syk in adulthood, B cells are not required for arthritis development in CAIA, as demonstrated by using muMT mice which lack B cells. On the other hand, Syk-deficient macrophages produced less MCP-1 and IL-6 than Syk-sufficient cells after FcR ligation, which can account for the absence of a pronounced accumulation of neutrophils and macrophages in the joints of iSyk KO mice. Our results demonstrate that Syk in macrophages is likely a key player in antibody-induced arthritis, mediating the release of pro-inflammatory cytokines and chemokines after macrophages bind anti-collagen antibody, and indicate that Syk is a promising target for arthritis therapy.

O23

Synoviolin meets metabolic disorders

Naoko Yagishita[1*], Satoko Aratani[2], Daisuke Hasegawa[1], Yoshihisa Yamano[1], Kusuki Nishioka[2], Toshihiro Nakajima[2,3]
[1]Institute of Medical Science, St Marianna University School of Medicine, Kawasaki, Kanagawa 216-8512, Japan; [2]Institute of Medical Science, Tokyo Medical University, Shinjuku-ku, Tokyo 160-8402, Japan; [3]Bayside Misato Medical Center, Kochi 781-0112, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O23

Rheumatoid arthritis (RA) is consists of multiple processes such as chronic inflammation, overgrowth of synovial cells, joint destruction and fibrosis. To clarify the mechanism of outgrowth of synovial cells, we carried out immunoscreening using anti-rheumatoid synovial cell antibody, and cloned 'Synoviolin' [1]. Synoviolin is endoplasmic reticulum (ER)-resident E3 ubiquitin ligases, and is involved in ER-associated degradation (ERAD). Synoviolin is highly expressed in synoviocytes of patients with RA. Overexpression of synoviolin in transgenic mice leads to advanced arthropathy caused by reduced apoptosis of synoviocytes [1]. We postulate that the hyperactivation of the ERAD pathway by overexpression of synoviolin results in prevention of ER-stress-induced apoptosis leading to synovial hyperplasia [2]. In addition, Synoviolin ubiquitinates and sequesters the tumor suppressor p53 in the cytoplasm, thereby negatively regulating

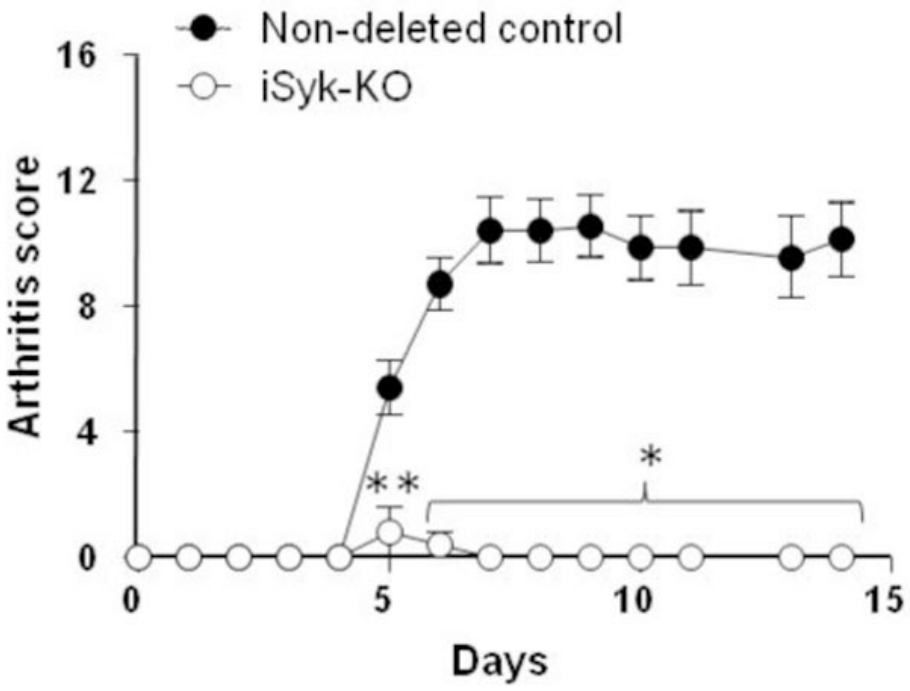

Figure 1(abstract O22) Arthritis development in iSyk KO mice. Arthritis was induced by i.p. administration of anti-collagen Ab followed by LPS. Arthritis score was monitored. *; $P < 0.001$, **; $P < 0.01$.

its biological functions [3]. Therefore Synoviolin regulates, not only apoptosis in response to ER stress, but also a p53-dependent apoptotic pathway. These studies indicate that Synoviolin is involved in overgrowth of synovial cells through its anti-apoptotic effects. Further analysis showed that Synoviolin is also involved in fibrosis among the multiple processes [4]. Therefore, it was suggested that Synoviolin is thought to be a candidate for pathogenic factor for arthropathy through its involvement of multiple processes.

As for the treatment of RA, biological agents are approved for clinical use, and these drugs have dramatically changed the treatment of RA during the past decade. However, in some cases patients fail to respond to the biologic treatment or adverse effects develop such as; an increased risk of infections. It was reported that elevated Synoviolin levels were identified in circulating monocytes and were associated with nonresponse to infliximab treatment. Moreover, these agents are associated with high costs and discomfort arising from subcutaneous or intravenous administration. Thus, there is a clear need for the development of cheaper, orally administrated therapies with fewer side effects. Then, we successfully discovered Synoviolin inhibitors. We are now proceeding with the optimization of small compounds, and we hope our research will lead to the development of a new therapy for RA and serve as an example of the therapeutic benefit of developing E3 ligase inhibitors.

In addition, to clarify the physiological function of Synoviolin in adult, we recently generate synoviolin conditional knockout mice using tamoxifen inducible Cre transgenic mice under CAG promoter. In today's session, I'd like to introduce the preliminary data of synoviolin conditional knockout mice.

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O24

IL-17, synoviolin and rheumatoid arthritis chronicity

Pierre Miossec
Department of Immunology and Rheumatology, Hôpital Edouard Herriot, University of Lyon, France

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Background: The use of cytokine inhibitors has been a major progress in the treatment of chronic inflammation. However, not all patients respond and response will be often lost when treatment is stopped. These clinical aspects indicate that other cytokines might be involved and we focus here on the role of IL-17. In addition, the chronic nature of joint inflammation may contribute to reduced response and enhanced chronicity. We had previously observed that patients not responding well to TNF inhibition had higher blood expression of synoviolin, an E3 ubiquitin ligase previously shown to be implicated in synovial hyperplasia in human and mouse rheumatoid arthritis (RA). Therefore we studied the capacity of IL-17 to regulate synoviolin in human RA synoviocytes and in chronic reactivated streptococcal cell wall (SCW)-induced arthritis.

Materials and methods: Chronic reactivated SCW-induced arthritis was examined in IL-17R deficient and wild-type mice. Synoviolin expression was analysed by real-time RT-PCR, Western Blot or immunostaining in RA synoviocytes and tissue, and p53 assessed by Western Blot. Apoptosis was detected by annexin V/ propidium iodide staining, SS DNA apoptosis ELISA kit or TUNEL staining and proliferation by PCNA staining. IL-17 receptor A (IL-17RA), IL-17 receptor C (IL-17-RC) or synoviolin inhibition were achieved by small interfering RNA (siRNA) or neutralizing antibodies.

Results: IL-17 induced sustained synoviolin expression in RA synoviocytes. Sodium nitroprusside (SNP)-induced RA synoviocyte apoptosis was associated with reduced synoviolin expression and was rescued by IL-17 treatment with a corresponding increase in synoviolin expression. IL-17RC or IL-17RA RNA interference increased SNP-induced apoptosis, and decreased IL-17-induced synoviolin. IL-17 rescued RA synoviocytes from apoptosis

induced by synoviolin knockdown. IL-17 and TNF had additive effects on synoviolin expression and protection against apoptosis induced by synoviolin knowndown. In IL-17R deficient mice, a decrease in arthritis severity was characterized by increased synovial apoptosis, reduced proliferation and a marked reduction in synoviolin expression. A distinct absence of synoviolin expressing germinal centres in IL-17R deficient mice contrasted with synoviolin positive B cells and Th17 cells in synovial germinal centre-like structures.

Conclusions: IL-17 induction of synoviolin may contribute in part to RA chronicity by prolonging the survival of RA synoviocytes and immune cells in germinal centre reactions. These results extend the role of IL-17 to synovial hyperplasia.

O25

Implication of microRNA-140 in osteoarthritis

Johanne Martel-Pelletier
University of Montreal and Osteoarthritis Research Unit, Notre-Dame Hospital, CRCHUM, Montreal, Quebec, Canada
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In osteoarthritis (OA), despite major progress regarding the identification and roles of catabolic mediators, further knowledge about factors regulating their expression is needed. In this line of thought, one recently identified class of molecules, the microRNA (miRNA), has been found to add another level of regulation to gene expression by down-regulating its target genes. miRNAs are 20-23 nucleotides (nt)-long single-stranded non-coding RNA molecules that act as transcriptional repressors by binding to the 3' untranslated region (UTR) of the target messenger RNA. Recently, miR-140 has emerged as being implicated in OA by modulating genes involved in the pathogenesis of this disease. The miRNA-140 gene is located between exons 16 and 17 in one intron of the WW domain containing the E3 ubiquitin protein ligase 2 (WWP2) gene [1]. The miR-140, originally found in cartilage [2], has recently been linked more specifically to the OA process [3,4]. The miRNA-140 decreases the expression of some genes known to play detrimental roles in OA cartilage. Those genes include histone deacetylase 4 (HDAC4) [2,5], ADAMTS-5 [6,7], Smad3 [8,9], and IGFBP5 [3]. On human chondrocytes, the expression level of miR-140 was found to be significantly decreased in OA compared to normal [3,4], thus favouring an increased expression of its target genes and consequently a role in OA progression. Interestingly, further investigation of the transcriptional regulation of miR-140 showed that in human OA chondrocytes miR-140 also has a WWP2-independent regulation. This occurs through the miR-140 intronic regulatory sequence in which the transcription factor NFAT3 acts directly and NFAT5 indirectly through the growth factor TGF-β1/Smad3. These data are of importance as they can provide a new basis for the rationalization of a therapeutic strategy for this disease.

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O26

Osteoclastic bone resorption directly activates osteoblast function

Nobuyuki Udagawa
Matsumoto Dental University, Shiojiri, Nagano, 399-0781, Japan
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Osteoclasts, the multinucleated cells that resorb bone, originate from cell cycle-arrested quiescent osteoclast precursors [1]. Mesenchymal osteoblastic cells are involved in osteoclast differentiation. Osteoclast precursors express RANK (a receptor of RANKL), recognize RANKL expressed by osteoblasts through cell-cell interaction and differentiate into osteoclasts in the presence of M-CSF. OPG, produced mainly by osteoblasts, is a soluble decoy receptor for RANKL. Deficiency of OPG in mice induces osteoporosis caused enhanced bone resorption. Elevated osteoblastic activity was suppressed by bisphosphonate administration in OPG-deficient mice. These results suggest that bone formation is accurately coupled with bone resorption. Collagen sponge disks containing BMP-2 were implanted into the dorsal muscle pouches in OPG-deficient mice. TRAP-positive osteoclasts and ALP-positive osteoblasts were observed in BMP-2-disks preceding the onset of calcification for one week. OPG and soluble RANK inhibited BMP-2-induced osteoclast formation but not the appearance of ALP-positive cells in OPG-deficient mice. We then examined how osteoblasts are involved in osteoclastogenesis other than RANKL expression, using RANKL-deficient mice. RANKL-deficient mice showed severe osteopetrosis due to loss of osteoclasts. Injection of RANKL into RANKL-deficient mice induced many osteoclasts in bone but not soft tissues [2]. These results suggest that osteoblasts determine the place of osteoclastogenesis from haemopoietic stem cells in bone. We next explored roles of osteoclasts in ectopic bone formation induced by BMP using op/op and c-fos-deficient osteopetrotic mice. The ectopic bones formed in op/op mice showed extremely rough surfaces, whereas those in wild-type mice showed smooth ones. Bone mineral density of BMP-induced ectopic bone in op/op mice was about 2-times higher than that in wild-type mice. TRAP-positive osteoclasts exhibit in outer of the ectopic bone in the wild-type mice. In op/op mice, although osteoclasts strongly exhibit in inside of the BMP-induced ectopic bone, TRAP-positive osteoclasts did not exhibit in outer of the BMP-induced ectopic bone. Furthermore, the accentuation of the BMP-induced ectopic bone formation did not exist in osteopetrotic c-Fos-deficient mice. In c-Fos-deficient mice, which are completely osteoclasts deficiency, the accentuation of the BMP-induced ectopic bone formation did not exist. Furthermore, there is no RANK-positive osteoclast progenitors in bone derived from c-Fos-deficient mice. These results suggest that RANK-positive osteoclast progenitors are positively regulate the signal of bone formation. In summary, osteoclastic bone resorption directly activates osteoblast function and osteoclasts are involved in normal bone morphogenesis.

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O27

Directed induction of chondrogenic cells from mouse dermal fibroblast culture

Noriyuki Tsumaki
Center for iPS Cell Research and Application, Kyoto University, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O27

Repair of cartilage injury with hyaline cartilage has been a challenging clinical problem. Articular cartilage damage sometimes heals with fibrocartilage, which is different from hyaline cartilage. Fibrocartilage is a type of scar tissue that expresses types I and II collagen. In contrast, hyaline cartilage does not express type I collagen. When aiming to induce hyaline chondrogenic cells directly from dermal fibroblasts, in addition to activation of cartilage-specific matrix genes, elimination of expression of type I collagen is needed for generation of hyaline cartilage. Otherwise, the presence of type I collagen impairs cartilage extracellular matrix architecture, which leads to formation of fibrocartilage. The generation of induced pluripotent stem cells has provided a tool for reprogramming dermal fibroblasts to an undifferentiated state by ectopic expression of reprogramming factors. We found that retroviral expression of two reprogramming factors (c-Myc and Klf4) and one chondrogenic factor (SOX9) induces polygonal chondrogenic cells directly from adult dermal fibroblast cultures. Induced cells expressed marker genes for chondrocytes but not fibroblasts; the promoters of type I collagen genes were extensively methylated. Transduction of c-Myc, Klf4, and SOX9 produced two types of cells: chondrogenically reprogrammed cells and partially reprogrammed intermediate cells. Chondrogenically reprogrammed cells generated stable homogenous hyaline cartilage-like tissue without tumor formation when subcutaneously injected into nude mice. Hyaline cartilage-like tissue expressed type II collagen but not type I collagen. On the other hand, partially reprogrammed intermediate cells expressed type I

collagen and produced tumor when injected into nude mice. Induced chondrogenic cells did not undergo pluripotent state during induction from dermal fibroblast culture, as time-lapse observation did not detect GFP reporter expression during induction from dermal fibroblasts prepared from transgenic mice in which GFP is inserted into the *Nanog* locus. These results suggest that chondrogenic cells induced by this approach are free from a risk of teratoma formation which associates with cells prepared through generation of iPS cells followed by redifferentiation into the target cell type. The dox-inducible induction system demonstrated that induced cells are able to respond to chondrogenic medium by expressing endogenous *Sox9* and maintain chondrogenic potential after substantial reduction of transgene expression. This approach could lead to the preparation of hyaline cartilage directly from skin, without going through pluripotent stem cells, in future regenerative medicine.

O28

A systems approach reveals that the musculoskeletal tissues development and homeostasis network

Hiroshi Asahara[1,2,3,4]
[1]Department of Systems BioMedicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan; [2]Department of Systems BioMedicine, National Research Institute for Child Health and Development, Setagaya-ku, Tokyo, 157-8535, Japan; [3]The Scripps Research Institute, La Jolla, CA, 92037, USA; [4]JST, CREST, Chiyoda-ku, Tokyo, 102-0075, Japan
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Materials and methods: We created a whole-mount in situ hybridization database, termed EMBRYS http://embrys.jp/embrys/html/MainMenu.html, containing expression data of 1520 transcription factors and cofactors expressed in E9.5, E10.5, and E11.5 mouse embryos –a highly dynamic stage of skeletal myogenesis. This approach implicated 43 genes in regulation of embryonic myogenesis, including a transcriptional repressor, the zinc-finger protein RP58 (also known as Zfp238) [1].
Results: Knockout and knockdown approaches confirmed an essential role for RP58 in skeletal myogenesis. Cell-based high-throughput transfection screening revealed that RP58 is a direct MyoD target. Microarray analysis identified two inhibitors of skeletal myogenesis, Id2 and Id3, as targets for RP58-mediated repression. Consistently, MyoD-dependent activation of the myogenic program is impaired in RP58 null fibroblasts and downregulation of Id2 and Id3 rescues MyoD's ability to promote myogenesis in these cells.
Conclusions: Our combined, multi-system approach reveals a MyoD-activated regulatory loop relying on RP58-mediated repression of muscle regulatory factor inhibitors. We applied our systems approaches to other locomotive tissues research including cartilage and tendon, and revealed novel molecular network regulating joint cartilage development and homeostasis via microRNA-140 [2,3] and tendon development by Mkx [4].

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O29

Angiogenesis in rheumatoid arthritis: the role of fut 1

Alisa Erika Koch
Department of Veteran's Affairs, Ann Arbor, MI USA and University of Michigan, MI, USA
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In rheumatoid arthritis (RA), targeting the vasculature may be beneficial to control the disease. Endothelial cells lining blood vessels are involved in a variety of functions in inflammation, including recruitment of leukocytes and cellular adhesion, antigen presentation, coagulation, cytokine production and angiogenesis. Angiogenesis, the growth of new vessels, is important for the proliferation of the rheumatoid synovial tissue pannus where these vessels also serve as a conduit for cells entering the inflamed synovium from the blood.
We have shown before that the endothelial adhesion molecule E-selectin, in soluble form, mediates angiogenesis via its endothelial receptor sialyl Lewis^x on adjacent endothelium [1]. We have used human RA synovial tissues to produce an antibody detecting related molecules, Lewis^y/H-5-2, which are mainly known as blood group antigens but are also found on endothelium in select organs such as skin, lymph node and synovium, but not most other endothelium. This antigen is rapidly upregulated on endothelium *in vitro* in response to stimuli such as tumor necrosis factor-alpha, that is present in the RA joint. Additionally, this antigen is upregulated on RA vs. normal synovial endothelial cells, and in soluble form is upregulated in RA synovial fluid vs. osteoarthritic synovial fluid. In soluble form, Lewis^y/H-5-2 mediates angiogenesis, cell adhesion via intercellular adhesion molecule-1, and monocyte recruitment.
Fucosyl transferases (fut1 and fut2) are enzymes that control the synthesis of Lewis^y/H-5-2. We have examined fut1 deficient mice to determine if fucosylation is important in angiogenesis and arthritis. Fut1 gene deficient mouse endothelial cells did not form endothelial sprouts on Matrigel *in vitro* to the same extent as wild type mouse endothelial cells. Moreover, the fut1 gene deficient mice were resistant to the development of angiogenesis in the Matrigel plug and sponge granuloma angiogenesis models *in vivo*. In terms of arthritis development, the Lewis^y/H-5-2 gene deficient mice were resistant to development of K/BxN arthritis. Moreover, the harvested joints of these mice had decreased monocyte chemoattractant protein-1/CCL2 and interleukin-1 compared to wild type littermates, indicating that some inflammatory mediators were downregulated when fut1 was absent. These experiments suggest that futs may be important in the development of angiogenesis and inflammatory arthritis and that they may serve as novel targets in RA therapy.

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O30

Citrullination of fibrinogen: generation of neoepitopes and enhancement of immunostimulatory properties

William H Robinson*, Jeremy Sokolove
VA Palo Alto Health Care System, Palo Alto, CA 94304 and the Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA 94305, USA
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Rheumatoid arthritis (RA) affects approximately 0.5% of the world population, yet the mechanisms underlying the development and progression of RA remain poorly understood. We are investigating the role of citrullinated fibrinogen as a pathogenic antigen in RA. Using arthritis antigen arrays we demonstrate that citrullinated fibrinogen is one of the earliest targets of the autoantibody response in RA, with autoantibodies against citrullinated fibrinogen appearing up to 10 years prior to the development of clinical arthritis. We further demonstrate that approximately 50% of CCP+ RA patients possess circulating immune complexes containing citrullinated fibrinogen, and that citrullinated fibrinogen containing immune complexes are deposited in human RA synovial tissues. To determine whether citrullinated fibrinogen can induce inflammatory arthritis in mice, we immunized mice with citrullinated fibrinogen and demonstrated that an inflammatory arthritis results and that both T cells and serum can transfer arthritis to naïve mice. Fibrinogen is an endogenous ligand for the innate immune receptor TLR4, and to determine whether citrullination might alter the ability of fibrinogen to bind TLR4 we performed in vitro macrophage stimulation assays with native and citrullinated fibrinogen. We found that citrullinated fibrinogen was ten-fold more potent than native fibrinogen at stimulating macrophage TNF release. Further, macrophage derived from mice deficient for TLR4 or MyD88 did not produce TNF in response to citrullinated fibrinogen. Thus, our results demonstrate a novel mechanism by which anti-citrullinated protein antibodies (ACPA) specifically targeting citrullinated fibrinogen may directly stimulate macrophage TNF production, via co-ligation of TLR4 and Fc-gamma-R. Our findings demonstrate a role for

citrullination both in creating neoantigens targeted by the adaptive immune response in RA as well as by increasing the potency of fibrinogen as an endogenous innate immune ligand. These results provide insights into the mechanisms by which anti-citrulline autoimmunity, and specifically the citrullination of fibrinogen, may contribute to both the onset and propagation of inflammation in RA.

O31

Novel regulatory T cells controlling antibody production and systemic autoimmunity

Kazuhiko Yamamoto*, Tomohisa Okamura, Keishi Fujio
Department of Allergy and Rheumatology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan
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Regulatory T cells (Tregs) are engaged in the maintenance of immunological self-tolerance and immune homeostasis. IL-10 has an important role in maintaining the normal immune state. We showed that IL-10-secreting Tregs can be delineated in normal mice as $CD4^+CD25^-Foxp3^-$ T cells that express lymphocyte activation gene-3 (LAG-3), an MHC class II-binding CD4 homolog. $CD4^+CD25^-LAG3^+$ Tregs characteristically express early growth response gene-2 (Egr-2), a key molecule for anergy induction. Retroviral gene transfer of Egr-2 converts naïve $CD4^+$ T cells into IL-10-secreting and LAG-3-expressing Tregs. Moreover, $CD4^+CD25^-LAG3^+$ Tregs show B cell-dependent development. $CD4^+CD25^-LAG3^+$ Tregs, but not $CD4^+CD25^+$ Tregs, strongly suppressed the antibody production in B cells co-cultured with helper T cells. Thus, IL-10-secreting $Egr\text{-}2^+LAG3^+CD4^+$ Tregs are closely related to B cells and can be exploited for the treat ment of autoimmune diseases.

Systemic lupus erythematosus (SLE) is a multisystem chronic inflammatory disease that affects many organs, and the immunological disorders are accompanied by autoantibody production. Recent case-control association study revealed that polymorphisms in the Egr-2 influence SLE susceptibility in humans. Interestingly, adoptive transfer of $CD4^+CD25^-LAG3^+$ Tregs from MRL/+ mice suppressed autoantibody production and the progression of nephritis in MRL/*lpr* lupus prone mice. In contrast, $CD4^+CD25^+$ Tregs from MRL/+ mice exhibited no significant therapeutic effect upon transfer to MRL/*lpr* mice. These results indicate that $CD4^+CD25^-LAG3^+$ Tregs play key roles in the regulation of humoral immunity by the strong suppressive activity for B cell antibody production.

O32

Innate and adaptive immune responses to dead and dying cells

Keith B Elkon[1,2*], Yueng Peng[1]
[1]Division of Rheumatology, University of Washington, Seattle, WA 98195, USA; [2]Department of Immunology, University of Washington, Seattle, WA 98195, USA
Arthritis Research & Therapy 2012, **14(Suppl 1):**O32

Under steady state conditions, billions of dead and dying cells are removed by extrusion from epithelial surfaces as well as by phagocytosis. Cells such as macrophages and dendritic cells have specialized receptors that directly recognize altered protein or lipids on apoptotic cells or opsonins that bind to the dying cell. Once engulfed, phagosomes containing apoptotic cells are rapidly acidified and the contents degraded by proteases and nucleases in lysozymes. During necrosis, cellular material is released prior to engulfment and extracellular nucleases as well as intracellular sensors dictate the inflammatory potential of the cellular debris. The outcome may be release of TNF-a, IL-1-b or interferon (IFN)-a depending upon the type of phagocyte, molecular nature of the cellular particle and the intracellular sensor engaged.

In addition to responses by cells of the innate immune system, we have recently defined a link between processing of apoptotic cells and their debris to T cell activation [1]. MFG-E8 is an opsonin (bridging protein) that binds to phosphatidylserine on apoptotic cells and facilitates their removal through interaction with integrins on phagocytes. Mice deficient in MFG-E8 develop lupus like autoimmunity associated with accumulation of apoptotic cells in vivo. We observed that older MFG-8-/- mice spontaneously developed a dermatitis associated with CD8 T cell infiltration and striking activation of effector memory CD8 T cells. T cell responses to both exogenous and endogenous apoptotic cell associated antigens were enhanced in MFG-E8 deficient mice and transfer of ovalbumin (OVA) reactive OT-I CD8 T cells caused accelerated diabetes in MFG-E8-/- RIP-mOVA mice and skin disease in kmOVA transgenic mice. The enhanced CD8 T cell response was attributed to increased cross-presentation by dendritic cells (DCs) associated with increased detection of antigen peptide MHCI complexes. Investigation of intracellular trafficking revealed that, whereas intact apoptotic cells ingested by wild type DC rapidly fused with lysosomes, in the absence of MFG-E8, smaller apoptotic cell fragments persisted in endosomal compartments and failed to fuse with lysosomes.

These observations suggest that in addition to altering the rate of clearance of apoptotic cells, MFG-E8 deficiency promotes immune responses to self antigens by altered intracellular processing leading to enhanced antigen presentation. Thus, handling of dead and dying cells impacts both innate and adaptive immune responses to self antigens.

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O33

From discovery of RANKL to clinical application of anti-human RANKL antibody

Hisataka Yasuda[1*], Yuriko Furuya[2], Kohji Uchida[2]
[1]Bioindustry Division, Oriental Yeast Co Ltd, Tokyo, Japan; [2]Nagahama Institute for Biochemical Science, Oriental Yeast Co Ltd, Shiga, Japan
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Osteoporosis is a common bone disease characterized by reduced bone and increased risk of fracture. In postmenopausal women osteoporosis results from bone loss attributable to estrogen deficiency. Receptor activator of nuclear factor-B ligand (RANKL) is a pivotal osteoclast differentiation factor [1]. Discovery of RANKL has opened a new era in the understanding of mechanisms in osteoclast differentiation over the last decade. The discovery also results in the development of a fully human anti-RANKL neutralizing monoclonal antibody (called denosumab) and denosumab has been approved for the treatment of osteoporosis in Europe and the US.

Here I report a novel rapid bone loss model with GST-RANKL as the first topic [2]. Pharmacologic studies of candidates for the treatment of osteoporosis with this model can be done in short periods such as 3 days and a couple of weeks although it took several months in the conventional methods with ovariectomized(OVX)-rats. This model also is useful for the rapid analyses in the functions of osteoclasts in vivo. The RANKL-induced bone loss model is the simplest, fastest, and easiest of all osteoporosis models and could be a gold standard in the evaluation of novel drug candidates for osteoporosis as well as OVX.

Osteopetrosis is generally caused by failure of osteoclast-mediated resorption of skeleton. There are a numerous mouse models of osteopetrosis without osteoclasts, including c-fos deficient mice, op/op mice, RANKL-deficient mice and RANK-deficient mice. As the second topic I report a mouse model of osteopetrosis induced by a denosumab-like anti-mouse neutralizing monoclonal RANKL antibody [3]. One injection of the antibody increased bone mass markedly with remarkable decrease in osteoclast surface and number after two weeks. In addition, osteoblast surface, mineral apposition rate, and bone formation rate were also reduced markedly. These results are consistent with the recent report treating human RANKL-knock in mice with denosumab [4]. These inducible models of osteoporosis and osteopetrosis using normal mice exhibit exactly mirror images in terms of change in bone mass and are quite useful to accelerate research on osteoclast biology as well as bone metabolism in vivo.

In conclusion, the discovery of OPG/RANKL/RANK system guided us to reveal the mechanism regulating osteoclast differentiation and activation. The past decade has witnessed significant progress in the development of the RANKL antibody as a pharmaceutical agent. This is a story from a discovery of RANKL to clinical application of anti-human RANKL antibody.

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O34

Microparticles as antigenic targets in human and murine SLE

Anirudh Ullal[1*], David S Pisetsky[1,2]
[1]Duke University Medical Center, Durham, NC, USA; [2]Durham VAMC, Durham, NC, USA

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Microparticles are small membrane-bound vesicles that are released from activated and dying cells by a blebbing process. These particles circulate in the blood and display potent pro-inflammatory and pro-thrombotic activities. In addition, particles are an important source of extracellular DNA and RNA and may participate in the transfer of informational nucleic acids. Because microparticles contain DNA as well as other nuclear antigens, we have investigated their ability to bind to anti-DNA and other anti-nuclesome antibodies that characterize the prototypic autoimmune disease systemic lupus erythematosus (SLE). For this purpose, we generated microparticles from HL-60, Jurkat and THP-1 cells induced to undergo apoptosis *in vitro*. Using FACS analysis to assess antibody binding, we showed that particles can bind some but not all monoclonal anti-DNA and anti-nucleosome antibodies from MRL-*lpr/lpr* and NZB/NZWF1 lupus mice. For the monoclonal anti-DNA, DNase treatment reduced binding. Like the monoclonal antibodies, patient plasma also bound to the particles although this activity was not directly correlated with levels of anti-DNA antibodies as measured by an ELISA. To determine whether particles circulating in the blood of patients can represent immune complexes, FACS analysis was performed on particles isolated from patient plasma. These studies indicated that, while the total levels of microparticles in the blood of patients with SLE did not differ significantly from those of normal controls, the number of IgG-positive particles was significantly elevated using a R-phycoerythrin-labeled anti-human IgG (γ-chain specific) reagent. In this study, the number of IgG-positive particles was correlated with levels of anti-DNA. In similar studies with plasma from MRL-*lpr/lpr* and NZB/NZWF1 mice, we showed that the total levels of particles were increased compared to those of BALB/c control mice and that the number of particles that stained with an anti-IgG reagent was also increased. Furthermore, plasma of mice could bind to particles generated *in vitro* from apoptotic cells. Together, these findings indicate that microparticles can express antigenically active DNA in an accessible form, either because of a surface location or particle permeability. Furthermore, they demonstrate that microparticles can form immune complexes and that at least some of the immune complexes in the blood in SLE contain particles. Current studies are characterizing the immune properties of these complexes and their potential role in pathogenicity.

O35

New mechanisms of action and signaling by TNF-α

Lionel B Ivashkiv[1,2]
[1]Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, NY 10021, USA; [2]Department of Medicine and Immunology and Microbial Pathogenesis Program, Weill Cornell Medical College, New York, NY 10065, USA

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TNF-α is a key pathogenic factor in inflammatory arthritis. Rapid and transient signaling and functional responses of cells to TNF-α, such as activation of NF-γB and MAPKs, are well known. These signaling mechanisms are widely assumed to be functional in cells chronically exposed to TNF-α and to mediate the pathogenic effects of TNF-α in chronic inflammation. We investigated the responses of primary macrophages to TNF-α over the course of several days and compared patterns of signaling and gene expression to RA synovial macrophages. The acute inflammatory response to TNF-α subsided after several hours and was followed by an IFN response characterized by sustained expression of STAT1 and downstream target genes. TNF-α-mediated induction of an IFN response was mediated by IFN-β and was sensitive to inhibition by Jak inhibitors. Concomitantly TNF-α induced a state of macrophage resistance to the homeostatic cytokines IL-10 and IL-27. Microarray analysis demonstrated that sustained TNF-α signaling induced expression of novel genes not appreciated to be 'TNF-inducible', but are highly expressed in RA synovial macrophages. Induction of an IFN response and abrogation of homeostatic cytokine signaling was also observed in RA synovial macrophages and likely contributes to the pathogenic actions of TNF-α during arthritis.

Subsequently and surprisingly, TNF-α induced a tolerant state in macrophages, with diminished cytokine production on lipopolysaccharide (LPS) challenge and protection from LPS-induced lethality. TNF-α-induced cross-tolerization was mediated by coordinate action of two inhibitory mechanisms, suppression of LPS-induced signaling and chromatin remodeling. Mechanistically, TNF-α-induced cross-tolerance was distinguished from TLR-induced tolerance by strong dependence on the nuclear kinase GSK3, which suppressed chromatin accessibility and promoted rapid termination of NF-γB signaling by augmenting negative feedback by A20 and IγBα. These results reveal an unexpected homeostatic function of TNF-α and provide a GSK3-mediated mechanism for preventing prolonged and excessive inflammation. This homeostatic mechanism may be compromised during RA synovitis, possibly by hypomorphic alleles of *TNFAIP3* (encodes A20) or by cytokines that suppress A20 expression or antagonize its function. These data suggest that augmenting homeostatic functions and signals and thereby rebalancing the pro- *versus* anti-inflammatory profile of TNF-α may represent an efficacious alternative therapeutic approach to suppress chronic inflammation.

Overall, the data reveal novel signals and functions of TNF-α and that are likely operative during chronic inflammation and RA synovitis. Targeted inhibition of these non-traditional functional components of the TNF-α response may be efficacious in alleviating chronic inflammation while preserving acute TNF-α responses and host defense against infections.

O36

Synovial fibroblasts display an uncontrolled inflammatory and tissue destructive response to TNF-α

George D Kalliolias[1,2*], Janice Chen[1], Galina Grigoriev[1], Lionel B Ivashkiv[1,3]
[1]Research Department, Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, NY, 10021, USA; [2]Medicine, Weill Cornell Medical College, New York, NY, 10021, USA; [3]Graduate Program in Immunology and Microbial Pathogenesis, Weill Cornell Graduate School of Medical Sciences, New York, NY, 10021, USA

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Background: Synovial fibroblasts are key players in the pathogenesis of Rheumatoid Arthritis (RA) and potentially attractive treatment targets. Upon activation within the joint's inflammatory milieu, they gain a transformed phenotype and produce pro-inflammatory cytokines (mainly IL-6) and tissue destructive enzymes [1].

Materials and methods: Synovial fibroblasts were isolated via enzymatic processing from synovial tissues obtained from patients with RA or Osteoarthritis (OA). Synovial fibroblasts (passages 2-4) were stimulated with TNF-α (10 ng/ml) only on day 1. The expression of TNF-α-target genes was measured by qPCR in time course experiments (1, 3, 6, 24, 48, 72, 96 and 120 hours after TNF-α stimulation).

Human macrophages (Mφ) generated in vitro (blood derived CD14+ cells stimulated for 48 h with M-CSF) were used in similar time course experiments as controls.

Results: In Mφ it was observed a rapid (within 1-3 hours) induction of TNF-α-target genes (including *TNF-a*, *IL-1β*, *IL-6* and *IL-8*) that was restrained back to the baseline within a few hours (3-24 hours depending on the gene). In stark contrast, synovial fibroblasts displayed a remarkably more sustained response to TNF-α. *IL-6* mRNA expression was induced within a few hours by TNF-α, and induction increased continuously for 72-96 h despite the absence of any further exogenous TNF-α stimulation. The levels of *IL-6* mRNA induced by TNF-α in synovial fibroblasts were substantially higher compared to human Mφ, suggesting that within the

joint microenvironment, synovial fibroblasts and not Mφ are the main source of IL-6. By adding the supernatants from 96 h TNF-α-stimulated fibroblast cultures on unstimulated synovial fibroblasts, a similar robust induction of *IL-6 mRNA* was observed, suggesting that there is a TNF-α-induced soluble factor that mediates the sustained response. A similar pattern of sustained expression was observed for other TNF-α-target genes including *IL-1β*, *IL-8* and *MMPs*. Interestingly, there was no difference between OA- and RA-derived synovial fibroblasts in their response to TNF-α.

Conclusions: In contrast to human Mφ, synovial fibroblasts display a sustained inflammatory and tissue destructive response to TNF-α. Our observations suggest that synovial fibroblasts may lack the homeostatic mechanisms that control and terminate the effects of TNF-α on human Mφ [2]. To support this hypothesis, further investigation is needed at the level of proximal and distal TNF-α signaling events and at the level of epigenetic regulation of TNF-α-target genes in synovial fibroblasts.

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O37

Interleukin-6 as a therapeutic target in locomotor disorders

Norihiro Nishimoto[1*], Miho Murakami[1], Takaji Matsutani[1], Jun Hashimoto[2], Nobuhiro Takagi[3]
[1]Laboratory of Immune Regulation, Wakayama Medical University, Ibaraki, 567-0085, Japan; [2]Department of Orthopaedic Surgery, NHO Osaka-Minami Medical Center, Kawachinagano, 586-8521, Japan; [3]Chugai Pharmaceutical Co Ltd, Tokyo, Japan

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Interleukin-6 (IL-6) is a multifunctional cytokine that regulates immune response, inflammation, and hematopoiesis. Although IL-6 plays several important physiological roles, deregulated overproduction of IL-6 causes various clinical symptoms and laboratory abnormalities. In the locomotor disorders such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA), IL-6 overproduction has been shown to be involved in inflammatory manifestations as well as joint destruction. Thus the blocking IL-6 signaling may be a therapeutic approach in those diseases. Various therapeutic antibodies targeting IL-6 have been developed, and tocilizumab (TCZ), an anti-IL-6 receptor antibody, precedes the others in clinical use.

TCZ, even in monotherapy, has been demonstrated to induce DAS28 remission frequently in patients with RA and suppress the radiographic progression of joint damage. TCZ more significantly reduced radiological progression in patients with risk factors for rapid progression (i.e. high urinary C-terminal crosslinking telopeptide, high urinary pyridinoline/deoxypyridinoline ratio, low body mass index, and presence of joint space narrowing at baseline) than those without the risk factors. Furthermore, early decreases in serum type IIA procollagen amino terminal propeptide, CRP, and/or matrix metalloproteinase 3 (MMP-3) within 12 weeks can predict for the preventive effects of TCZ on one year progression of joint destruction in RA.

Although long-term treatment with TCZ is well tolerated, it goes without saying that it is beneficial not only for the patients but also for medical economy. To test the possibility of drug free remission introduced by TCZ, Drug free REmission after cessation of Actemra Monotherapy (DREAM) study was conducted. A total of 187 patients, who had received TCZ in the previous clinical trials (the mean treatment duration was 4.3 years), were enrolled, and discontinued TCZ. Remission, defined as DAS28 less than 2.6, was maintained in 10% of the patients without any drug over 52 weeks. Furthermore, low serum IL-6 (<35 pg/mL) and normalization of MMP-3 levels at cessation of TCZ were identified as independent predictive markers for the longer duration of drug free remission. In addition, retreatment with TCZ in the patients, who responded to initial TCZ monotherapy, and experienced loss of efficacy after cessation of TCZ, was well tolerated and showed excellent efficacy equivalent to that observed at the initial treatment with TCZ.

In the near future, tailor made therapy for individual patients will be developed on the basis of genome wide association study results, gene expression profile in peripheral blood cells and/or various biomarkers.

O38

Signal transduction inhibitors for the treatment of rheumatoid arthritis

Yoshiya Tanaka*, Kunihiro Yamaoka
The First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan

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Rheumatoid arthritis (RA) is a representative autoimmune disease characterized by chronic and destructive inflammatory synovitis. The multiple cytokinesand cell surface molecules play a pivotal role in the pathogenesis of RA and binding of these molecules to their ligands on the cell surfaceinduce various signal intracellular transduction including phosphorylation of kinase proteins. The tyrosine kinase is the first intracellular signals to be phosphorylated and 14 tyrosine kinases are known to be involved in RA. Among them, members of Janus kinase (Jak) familyare essential for the signaling pathways of various cytokines and are implicated in the pathogenesis of RA. An orally available Jak3 inhibitor tofacitinib is currently in clinical trials for RA with satisfactory effects and acceptable safety [1,2]. A phase 2 double-blinded study wascarried out to investigate the efficacy and safety of tofacitinib in Japanese patients with active RA andinadequate responseto methotrexate (MTX). A total of 140 patients were randomized to tofacitinib 1, 3, 5, 10 mg, or placebotwice daily and ACR20 response rates at week 12, a primary endpoint, was significant for all tofacitinib treatment groups [3]. Thus, tofacitinib in combination with MTX was efficacious and had a manageable safety profile and tofacitinib 5 and 10 mg twice a day appear suitable for further evaluation to optimize their potential for the treatment of RA. Although the mode of action of tofacitinib has remain unclear, we clarified thatthe inhibitory effects of tofacitinib could be mediated through the suppression of IL-17 and IFN-γ production and proliferation of $CD4^+$ T cells, presumably Th1 and Th17 cells by in vitro experiments. We next conducted a treatment study in the SCID-HuRAg mice, an RA animal model utilizing SCID mice implanted with synovium and cartilage from patients with RA and tofacitinib was administered via an osmotic mini-pump. Tofacitinib decreased serum levels of human IL-6 and IL-8 in the mice and reduced invasion of the synovial tissue into the implanted cartilage as well as accumulation of immune cells in the synovium.Taken together, orally available low molecular weight products such as tofacitinibtargeting intracellular signaling molecules, would provide enormous power and flexibility in the treatment of RA.

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O39

Adult stem cell-based therapy for degenerative joint diseases

Peter G Alexander*, Rocky S Tuan
Center for Cellular and Molecular Engineering, University of Pittsburgh, Pittsburgh, PA 15219, USA

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Cell-based therapy for regenerative medicine is a major field of biomedical research including its use in the treatment of degenerative joint disease. The goal of regenerative medicine is to develop methods to repair, replace, and regenerate diseased, injured, or non-functional tissues. Towards this goal, stem or progenitor cells have been considered a highly desirable candidate cell type, because of their expandability and potential to be induced toward specific cell differentiation lineages. A key requirement in musculoskeletal

tissue engineering and regeneration is that ultimately the "regenerate tissue" needs to be a three-dimensional structure. This may be accomplished through the use of engineered constructs derived by cell seeding into natural or synthetic biomaterial scaffolds. While direct cell injection is the most convenient means of cell delivery, a scaffold-based approach is capable of producing three-dimensional engineered tissues with mechanical properties compatible with those of various musculoskeletal tissues. Of the 40-50 million Americans with osteoarthritis (OA), an estimated 10-12% suffer from post-traumatic OA. We have developed an impact model for the development of post-traumatic OA. Data on the characteristics of this model in vitro and in vivo will be presented. Focal lesions developed in vivo resulting from these traumatic impacts will be repaired using stem cell-laden hydrogel or nanofiber constructs. Concurrently, cell-hydrogel and cell-nanofibrous constructs are currently being developed for the engineering of cartilaginous tissues, and information on the fabrication and biological attributes of these various tissue-engineered composites will be presented. In conclusion, tissue engineering and regenerative medicine presents an exciting, emerging inter-disciplinary research field that is a natural platform for life scientists, engineers, and clinicians working together to develop therapeutic solutions for diseased or injured tissue and organs.

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O40

Peptide therapy in sepsis and inflammation: a novel strategy to suppress inflammation

Hidechika Okada*, Alan Okada
Research Institute for Protein Science, Nakayama-cho, Mizuho-ku, Nagoya, Japan
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Antisense homology box (AHB): In 1984, Blalock proposed the possible role of antisense peptides for molecular interaction among proteins (BBRC, *121*: 203, 1984).

We speculated that interactions between sense- and antisense-peptides should play a role in formation of the tertiary structure of proteins. We developed a novel computer program named ANTIS to find antisense peptide sequences between proteins to be compared (Nature Med. 1:894,1995). ANTIS revealed the presence of an appreciable number of sense and antisense peptide pairs within any protein molecule and those portions were designated as antisense homology boxes (AHB).

Complementary peptide: Each peptide should have specific structure determined by its amino acid sequence which may react with its antisense peptide. To generate candidates of complementary peptide (C-pep) reactive to a target amino acid sequence based upon the sense-antisense amino acid relationship. We invented an evolutionary computer program (MIMETIC) that generatesC-pep sequences that have a potential to interact with a target peptide (Microbiol. Imm.46:211, 2002).

C5a inhibitory peptides: C5a anaphylatoxin is considered to be an effective target for treatment of hyperinflammation since C5a stimulates generation of tumor necrosis factor alpha (TNFα and other inflammatory cytokines. Amino acids 37 to 53 of C5a (RAARISLGPRCIKAFTE) is an antisense peptide to AHBpeptides of the C5a receptor (C5aR), and this has been designated PL37. This region of C5a is presumed to be a potential site for C5aR stimulation. Using the computer program MIMETIC, we generated 19 C-peps to PL37. One of the 7 inhibitory C-peps to PL37 which interfered with C5a function was termed PepA(ASGAPAPGPAGP-LRPMF). To improve stability, we modified PepA by acetylation of its *N*-terminal alanine generating acetylated PepA (AcPepA).

AcPepA rescued Cynomolgusmonkyes at lethal shock induced by bacterial LPS (4 mg/kg).The excellent therapeutic effect of AcPepA is due to restriction of high mobility group box 1 (HMGB1) surge induced by the effect of C5a on C5L2, which is the second C5a receptor, since the released HMGB1 has the capacity to stimulate TLR4 as an endogeneous ligand resulting in further activation of inflammatory cells to release inflammatory cytokines forming positive feedback circuit of inflammation.

O41

Overview of biotherapy in rheumatoid arthritis (RA)

Tsutomu Takeuchi*, Hideto Kameda
Division of Rheumatology, Department of Internal Medicine, School of Medicine, Keio University, Shinjyuku,Tokyo, Japan
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Biological agents targeting a specific molecule provide an effective means for therapeutic management of rheumatoid arthritis (RA) due to their specificity and powerful functional capabilities, which has resulted in a paradigm shift in the treatment strategy of this disease. The dramatic improvement of the sign and symptoms of a patient with RA first came from the report with chimeric anti-TNF alpha monoclonal, infliximab in 1993. The observation was confirmed in the double-blind randomized controlled study comparing this biological agent and placebo in 1994. The first approved biologics for RA was TNF Receptor 1-Ig fusion protein, etanercept in the United States in 1998. Until now, nine biological agents are approved in RA worldwide. Revolutionary change of RA management with biological therapies obtained in western countries and Japan has been reviewed [1].

Atreatment strategy that uses tightly controlled dosesof administered biologics, targeting clinical remission or low disease activity, and followed by discontinuation of the biologics may be advantageous from botha health and economical point of view. This strategy is now being examinedin several clinical studies and trials in Japan for several biologics, including infliximab, etanercept, tocilizumab, and abatacept [1].

It is ideal to personalize medical treatment for individual RA patients by predicting efficacy and safety of a given biologic. In order to identify predictive factors, enormous amounts of efforts have put forth. Although several clinical variables have been associated with efficacy and safety, they are often unrealistic in clinical practice. We found that the baseline circulating TNF levels [2] and Fc gamma 3B polymorphism [3] are important predicting factors for response to infliximab in RA patients, and discuss the role of these markers in real world. Further clinical studies using biomarkers and molecular expression pattern [4,5] should provide a clue to find the appropriate predicting markers or even new therapeutic targets. In the near future, the information accumulated from these studies may allow selecting the best biological agents in individual patient.

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O42

Anti-TNF antibody therapy induces IL-17 suppressing regulatory T cells in patients with rheumatoid arthritis

Jenny McGovern*, Clare A Notley, Dao Nguyen, Claudia Mauri, David A Isenberg, Michael R Ehrenstein
Division of Medicine, University College London, London WC1E 6JF, USA
Arthritis Research & Therapy 2012, **14(Suppl 1):**O42

Biologic therapies not only offer the prospect of improved patient outcomes in a variety of autoimmune diseases, but also the opportunity to explore the specific target's role in the underlying mechanisms of disease. Over recent years we have studied the role of regulatory T cells (Treg) in patients with rheumatoid arthritis before and after anti-TNF therapy. We have shown that Treg from patients with rheumatoid arthritis have defective suppressor function. This Treg defect is linked with abnormalities in the expression and function of CTLA-4. Anti-TNF antibody therapy did not reverse CTLA-4 dysfunction but instead induced the differentiation of a distinct and potent Treg population. These induced Treg were able to inhibit IL-17 production, in contrast to Treg from healthy individuals, patients with active RA or RA patients treated with etanercept, a modified

TNF receptor. These results may provide mechanistic insight into the therapeutic benefit of switching between different anti-TNF agents and the differing incidence of tuberculosis between adalimumab and etanercept.

O43

Combined effects of the hedgehog pathway inhibitor LDE225 and nilotinib in a random mutagenesis screen

Tetsuzo Tauchi
First Department of Internal Medicine, Tokyo Medical University, Shinjuku-ku, Tokyo 160-0023, Japan
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Recent studies have demonstrated that hedgehog pathway is activated in chronic myeloid leukemia (CML) stem cells via up-regulation of Smoothened (Smo), a seven transmembrane domain receptor protein. LDE225 is a small molecule Smo antagonist which has entered Phase I clinical evaluation in patients with solid tumors. We performed a comprehensive drug combination experiment using a broader range of concentrations for LDE225 and nilotinib. Compared with single agents, the combination of LDE225 and nilotinib was more effective at reducing the outgrowth of resistant cell clones. No outgrowth was observed in the presence of 2 μM nilotinib plus 20 μM LDE225. Also co-treatment with LDE225 and nilotinib resulted in significantly more inhibition of growth than treatment with either agent alone in BaF3 cells expressing wt-BCR-ABL and BCR-ABL mutants (M244V, G250E, Q252H, Y253F, E255K, T315A, T315I, F317L, F317V, M351T, H396P). The observed data from the isobologram indicated the synergistic effect of simultaneous exposure to LDE225 and nilotinib even in BaF3 cells expressing T315I. To assess the in vivo efficacy of LDE225 and nilotinib, athymic nude mice were injected s.c. with BaF3 cells expressing random mutagenesis for BCR-ABL mutation. 7 days after injection (average tumor volume, 100 mm^3), the mice were randomised into four groups (5 mice per group), with each group receiving either vehicle, LDE225 (20 mg/kg; p.o. once every day), nilotinib (30 mg/kg; p.o. once every day), LDE225 (20 mg/kg; p.o. once every day) + nilotinib (30 mg/kg; p.o. once every day). The LDE225 and nilotinib combination more effectively inhibited tumor growth in mice compared to either vehicle- or nilotinib- or LDE225-treated mice. Histopathologic analysis of tumor tissue from LDE225 plus nilotinib-treated mice demonstrated an increased number of apoptotic cells detected by TUNEL staining. To investigate combined effects of LDE225 and nilotinib on primary Ph-positive acute lymphocytic leukemia (ALL) cells, NOD/SCID mice were injected i.v. with bone marrow mononuclear cells from a Ph positive ALL patient. Treatment with LDE225 and nilotinib demonstrated a marked segregation of apoptotic cells in both the central bone-marrow cavity and the endosteal surface. These results suggest that the combination with a Smo inhibitor and ABL TKIs may help to eliminate the Ph positive ALL cells. Taken together, the present study shows that the combination of LDE225 and nilotinib exhibits a desirable therapeutic index that can reduce the in vivo growth of mutant forms of BCR-ABL-expressing cells.

O44

Molecular mechanism of unloading-mediated muscle atrophy and development of its countermeasures

Takeshi Nikawa
Department of Nutritional Physiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan
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The ubiquitin ligase Cbl-b plays a major role in skeletal muscle atrophy induced by unloading [1]. The mechanism of Cbl-b-induced muscle atrophy is unique in that it does not appear to involve the degradation of structural components of the muscle, but rather it impairs muscular trophic signals in response to unloading conditions. Recent studies on the molecular mechanisms of muscle atrophy have focused on the role of IGF-1/PI3K/Akt-1 signaling cascade as a vital pathway in the regulation of the balance between hypertrophy and atrophy [2,3]. These studies indicate that under muscle wasting conditions, such as disuse, diabetes and fasting, decreased IGF-1/PI3K/Akt-1 signaling augments the expression of atrogin-1, resulting in muscle atrophy. However, these studies did not address the mechanisms of unloading-induced impairment of growth factor signaling. In the present study, we found that under both *in vitro* and *in vivo* experimental conditions, Cbl-b ubiquitinated and induced specific degradation of IRS-1, a key intermediate of skeletal muscle growth regulated by IGF-1/insulin and growth hormone, resulting in inactivation of Akt-1. Inactivation of Akt-1 led to upregulation of atrogin-1 through dephosphorylation (activation) of FOXO3, as well as reduced mitogen response, in skeletal muscle. Thus, activation of Cbl-b may be an important mechanism underlying the failure of atrophic muscle to respond to growth factor-based treatments such as IGF-1 (Figure 1).

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O45

Regulation of immune cell responses by semaphorins and their receptors

Atsushi Kumanogoh[1,2*], Hyota Takamatsu[1,2]
[1]Department of Respiratory Medicine, Allergy and Rheumatic Diseases, Graduate School of Medicine Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan; [2]World Premier International Immunology Frontier Research Center, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**O45

Background: Semaphorins were originally identified as axon guidance factors involved in the development of the neuronal system. However, accumulating evidence indicates that several members of semaphorins, so-called 'immune semaphorins', are crucially involved in various phases of immune responses (Figure 1) [1-3]. In addition, semaphorins and their receptors have been shown to be crucial for the pathogenesis of immunological disorders such as atopic dermatitis, multiple sclerosis, systemic sclerosis, systemic lupus erythematosus and rheumatoid arthritis, These semaphorins regulate immune cell interactions during physiological and pathological immune responses. However, conventional static analysis could not determine definitively whether they regulate immune cell movement.

Materials and methods: Plexin-A1-/- mice were previously established [4]. Combinational studies, including imaging technique for visualizing single-cell dynamics and conventional immunological assays were performed.

Results and discussion: We find that plexin-A1-mediated semaphorin signals are crucially involved in the transmigration of DCs across the lymphatics to exit the periphery to induce antigen-specific T-cell priming using plexin-A1-/- mice. In addition, adoptive transfer experiments identify that Sema3A produced in the lymphatics functions as a ligand for the plexin-A1/NP-1 receptor complex expressed in DCs. Interestingly, plexin-A1 is localized at the trailing edge but not the leading edge of DCs during migration. Sema3A induces phosphorylation of the myosin light chain to promote actomyosin contraction, resulting in increased DC velocity in the constricted area (Figure 2). Collectively, these findings not only demonstrate the involvement of semaphorins in immune cell trafficking but also indicate that semaphorins are therapeutic targets to treat immunological disorders.

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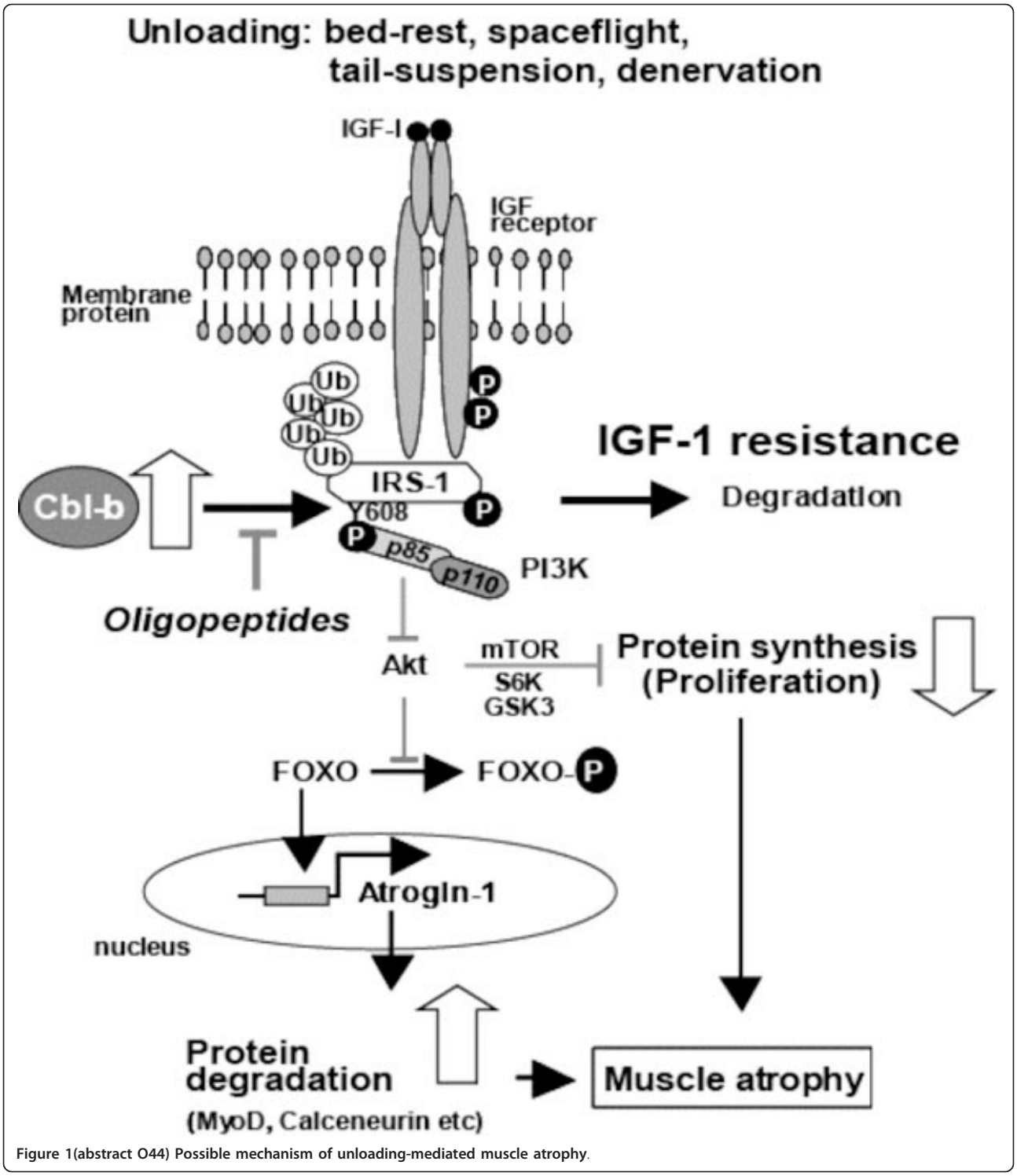


Figure 1(abstract O44) Possible mechanism of unloading-mediated muscle atrophy.

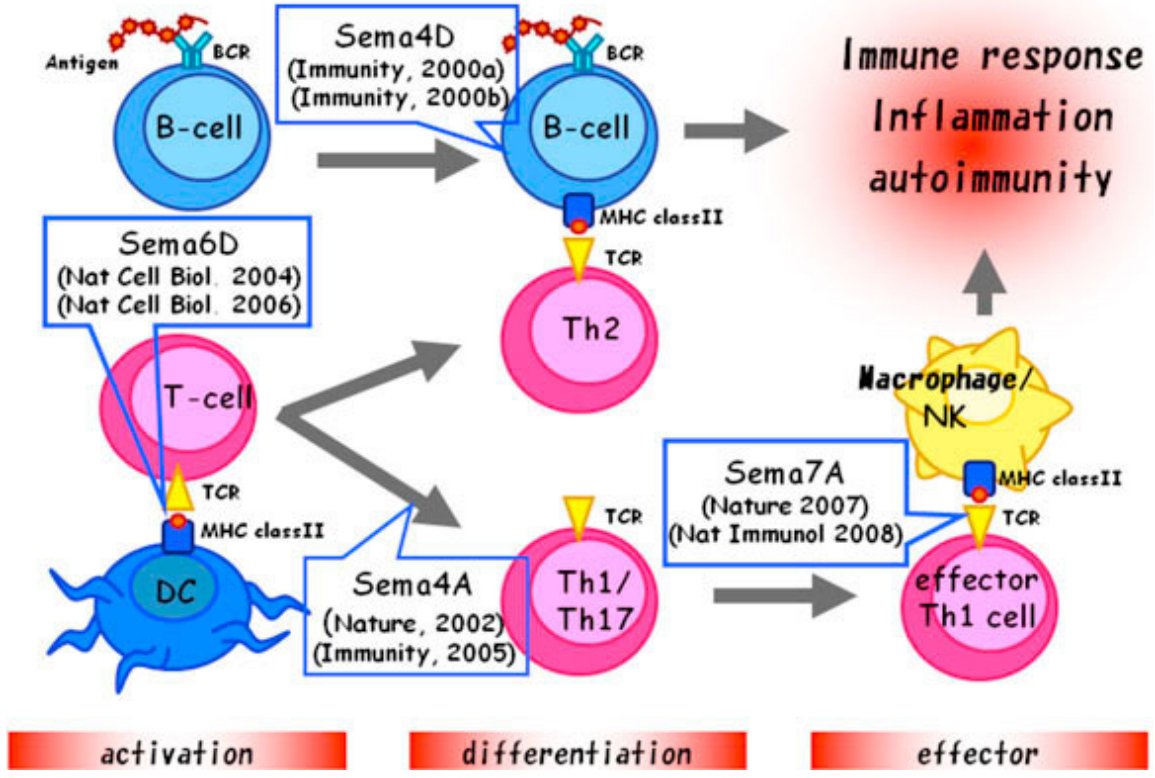
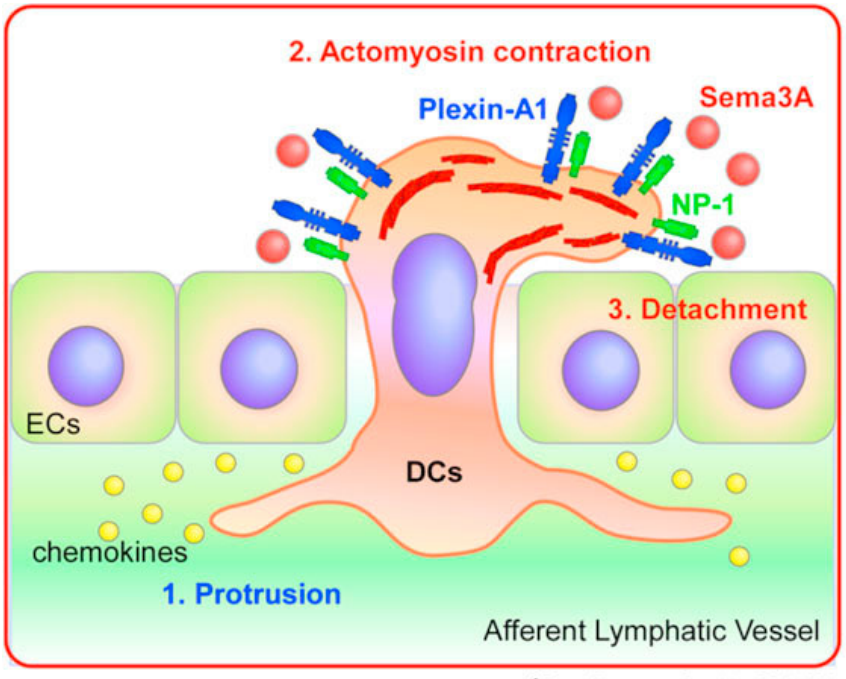


Figure 1(abstract O45) Semaphorins are involved in physiological and pathological immune responses.



(Nat Immunol. 11: 594-600, 2010)

Figure 2(abstract O45) Sema3A produced by the lymphatic induces actomyosin contraction during transmigration.

O46

A novel NEDD8-binding protein modulates NF-κB signaling pathway

Osamu Takashima*, Fuminori Tsuruta, Manato Ebina, Yu Kigoshi, Shingo Nakamura, Tomoki Chiba
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki 305-8577, Japan

Arthritis Research & Therapy 2012, **14(Suppl 1):**O46

In canonical NF-κB signaling pathway, a ubiquitin ligase called SCF (Skp1, Cul1, F-box protein) complex is essential for I-κB degradation. The activity of the SCF complex is positively regulated by a post-translational modification of Cul1 subunit with a ubiquitin-like protein NEDD8. Like ubiquitin, NEDD8 possesses evolutionary conserved Lys residues on its surface, and forms poly-NEDD8 chain *in vivo* and *in vitro* [1,2]. Despite the importance of the NEDD8 modification in all eukaryotic cells, little is known about the function of poly-NEDD8 chain. To elucidate the function of the poly-NEDD8 chain *in vivo*, we screened poly-NEDD8 chain binding proteins (PNBPs) using a yeast two-hybrid system. Of the identified PNBPs, PNBP1 was identical to a gene present in non-HLA celiac disease and rheumatoid arthritis risk loci [3].

PNBP1 interacted with NEDD8, NEDD8-conjugating enzyme Ubc12 and Cul1. PNBP1 strongly associated with wild-type Cul1, but not its NEDDylation defective Cul1(K720R) mutant, suggesting that the interaction is mediated in part through NEDD8. Furthermore, PNBP1 promoted NEDDylation of Cul1 in an *in vitro* reconstitution assay. These activities were dependent on RING-finger domain of PNBP1. Finally, knockdown of PNBP1 led to reduction of the NF-κB activation, suggesting that PNBP1 is an important modulator of the NF-κB signaling pathway.

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POSTER PRESENTATIONS

P1

Reconstruction of injured spinal cord by epigenetic regulation of transplanted neural stem cells

Masahiko Abematsu[1,2,3*], Keita Tsujimura[3], Mariko Yamano[5], Michiko Saito[4], Kenji Kohno[4], Takao Setoguchi[1,2], Kazunori Yone[1], Kinichi Nakashima[3], Setsuro Komiya[1,2]
[1]Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan; [2]The Near-Future Locomotor Organ Medicine Creation Course (Kusunoki Kai), Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan; [3]Laboratory of Molecular Neuroscience, Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma 631-0192, Japan; [4]Laboratory of Molecular and Cell Genetics, Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma 631-0192, Japan; [5]Department of Comprehensive Rehabilitation, Osaka Prefecture University, Habikino 583-8555, Japan

Arthritis Research & Therapy 2012, **14(Suppl 1):**P1

Background: Neural stem cells (NSCs) possess the ability to self-renew and to differentiate into the three major cell types found in the central nervous system (CNS). Recent studies have shown that epigenetic gene regulation events such as DNA methylation and histone modification play important roles in regulating NSC fate specification. In this context, we have previously shown that the histone deacetylase inhibitor valproic acid (VPA) enhances neuronal differentiation of NSCs. Perhaps because these patterns of NSC differentiation are exquisitely controlled during normal embryonic development, restoration of damaged neural networks in the injured adult CNS is severely limited. Here, using a mouse model of spinal cord injury(SCI), we examined the effectiveness of NSC transplantation and differentiation control by VPA administration.

Materials and methods: NSCs were transplanted into the SCI epicenter 7 days after injury. Non-transplanted control and transplanted mice were then intraperitoneally administered VPA or saline daily, for 7 days, whereafter we monitored their hindlimb motor function using the open field locomotor scale for 6 weeks. We next analyzed the migration, morphology, neuronal marker expression and viability of these cells after co-administration with VPA. We examined extensively the roles of the neurons responsible for reconstruction of broken neuronal networks using two neuronal tracers, immunoelectron microscopy, and two cell-ablation methods.

Results: We show that transplanting NSCs and administering VPA enhances the functional recovery of their hindlimbs. Neuronal differentiation of transplanted NSCs was promoted in VPA-treated mice. Anterograde corticospinal tract tracing revealed that transplant-derived neurons partially reconstructed the broken neuronal circuits, most likely in a 'relay' manner. Ablation of the transplanted cells abolished the recovery of hindlimb motor function, indicating that transplanted cells contributed directly to the improvement of motor function.

Conclusions: These data raise the possibility that epigenetic regulation in transplanted neural stem cells can be exploited to provide treatment for SCI.

P2

Fukushimura Brain Bank (FBB) -Based in a private geriatric hospital-

Hiroyasu Akatsu[1,2*], Akira Hori[1,2], Hidechika Okada[1], Yoshio Hashizume[1,2], Takayuki Yamamoto[1,2]
[1]Choju Medical Institute, Fukushimura Hospital, Japan; [2]Institute of Neuropathology, Fukushimura Hospital, Japan

Arthritis Research & Therapy 2012, **14(Suppl 1):**P2

Fukushimura Brain Bank (FBB) was established under the auspices of Fukushimura Hospital, a legally incorporated medical institution. It is managed completely within the private sector.

"Fukushi" is a Japanese word that means welfare and "mura" is a village. We have several buildings for the aged and disabled, and about 800 elderly people reside within the complex.

The Fukushimura Hospital was established in 1982 and is managed by the Sawarabi MedicalCooperative. It currently has 487 beds. Our patients mainly have dementia and cerebrovascular problems. The hospital plays a pivotal role within the village and acts as the central facility.

FBB was established in 1990. We have a long history of collecting samples, not only from patients but also from residents of our care houses and nursing homes within the Fukushimura complex. This allows us as medical doctors and researchers to obtain clinical information or blood samples, sometimes even before the onset of illness. In our institute, all clinical and pathological dataare held in the office of individual data management.

In collecting FBB samples, we always keep in mind future biochemical and molecular analyses and collaborations. The brains are separated into two hemispheres. One hemisphere is fixed in formalin for neuropathological analysis and the other is precisely subdivided into coronary sections and small blocks which are saved in Eppendorf tubes. After samples are photographed, they are frozen on dry ice (slices) and in liquid nitrogen (tubes). Finally, all material is stored at -80 degrees in 9 refrigerators for later use in research.

Although our bank has gone unrecognized in the past, our farsighted efforts have been gaining considerable attention in recent years in Japan. We now have over 20 collaborators and supply more than 30 research institutes with our samples. In addition, our research institute was approved in 2004 by the Japanese Ministry of Education, Culture, Sports, Science and Technology, as one of the non-governmental institutes which is permitted to apply for governmental grants and we became a member of the Comprehensive Brain Science Network in 2010. FBB at the Choju Medical Institute, Fukushimura Hospitalis a unique facility and one of the most active brain banks in the world.

P3

IL-17-producing ©™T cells are important for the development of arthritis in a rheumatoid arthritis model

Aoi Akitsu[1,2*], Harumichi Ishigame[1], Shigeru Kakuta[1], Shinobu Saijo[1], Yoichiro Iwakura[1,2]
[1]Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Japan; [2]Japan Society for the Promotion of Science (JSPS) 3Core Research for Evolutional Science and Technology (CREST), Japan

Arthritis Research & Therapy 2012, **14(Suppl 1):**P3

Background: IL-1 receptor antagonist deficient (*Il1rn*-/-) mice spontaneously develop arthritis. We previously demonstrated that IL-17 plays a crucial role in the development of arthritis in *Il1rn*-/- mice. Furthermore we showed that IL-1 Ra-deficiency in T cells is important for the development of arthritis. It is not known, however, which IL-17-producing cells are involved in the pathogenesis of arthritis in this model.
Results: To identify the source of IL-17 in *Il1rn*-/- mice, we analyzed IL-17-producing cells. We found that IL-17 production from both CD4+ T cells and ©™T cells was increased in the draining lymph nodes. To clarify the roles of CD4+ T cells and ©™T cells in the development of arthritis, ©™T cells or CD4+ T cells were depleted in *Il1rn*-/- mice using antibodies. The development of disease was suppressed in both cases, suggesting both Th17 cells and IL-17-producing ©™T cells were involved in the pathogenesis. Then, the pathogenic role of IL-17-producing ©™T cells in the absence of Th17 cells was examined.
We generated mice with IL-17 producing ©™T cells, but without Th17 cells, by adoptively transferring *Il17-/-Il1rn*-/–T cells into nude mice in which IL-17-producing ©™T cells are present. We found that these mice still developed arthritis and that only ©™T cells produced IL-17. Finally, to corroborate that the development of arthritis in this transfer system is dependent on IL-17, we adoptively transferred *Il17-/-Il1rn*-/--T cells into *Il17-/-nu/nu* mice. The development of arthritis was significantly suppressed in *Il17-/-Il1rn*-/--T cell-transferred *Il17-/-nu/nu* mice compared with *Il-17+/+nu/nu* mice transferred with *Il17-/-Il1rn*-/--T cells, suggesting that ©™T -cell-derived IL-17 is important for the develop arthritis.
Conclusion: These results indicate that ©™T cell-derived IL-17 plays an important role in the pathogenesis of arthritis in *Il1rn*-/- mice.

P4

Osteoporosis in Iraqi patients with thalassemia

Salim M AL Jadir[1*], Mohamed Z Jalal[1], Median F AL Ghreer[2], Mozahem S AL Hamdani[2], Wamid R AL Omaree[3]
[1]Al Salaam General Teaching Hospital in Mosul City, Iraq; [2]Ibn Al Atheer Teaching Hospital, Thalassemia center, Mosul City, Iraq; [3]Medical College of Mosul UNV, Mosul, Iraq
Arthritis Research & Therapy 2012, **14(Suppl 1):**P4

Background: Thalassemia is defined as a complete absence of one or more of the four globins in the red blood cells due to the deletion of or nonfunctioning of one or more genes.
Osteoporosis is a universal medical problem, affecting both genders.
Materials and methods: 74 thalassemic patients 36 male and 38 female below the age of 25 years.
The study was a clinical cross-sectional for both genders with thalassemia major, Investigation done included a chest × ray, serum iron, total iron binding capacity (TIBC), transferrin saturation, serum calcium, serum phosphorus, serum alkaline phosphatase, blood urea, serum creatinine, and a DXA bone scan.
Statistical analysis:-P-value–S.P.S.S.–chi-square.
Results: We found that the bony disorder in thalassemic patients increased with age (bone pain, carpopedal spasm, osteoporosis), and with low serum iron and low T.I.B.C. and with increased transferrin saturation. The compliance of patients with treatment was rated as in 24 good, in 36 fair and in 14 bad.
The prevalence of osteoporosis in thalassemic Iraqi patients DXA scans was found to be 67.5% while osteopenia was found in 9.4% and normal BMD in 22.9%.
Discussion: During the last decade, the presence of osteopenia and osteoporosis in well-treated thalassaemics has been described in different studies with high prevalence up to 50%.
Several factors are implicated in reduction of bone mass in thalassaemia major. Delayed sexual maturation, growth hormone (GH) and insulin growth factor-(IGF)-1 deficiency, parathyroid gland dysfunction, diabetes, hypothyroidism, ineffective haemopoiesis with progressive marrow expansion, direct iron toxicity on osteoblasts, as well as liver disease have been indicated as possible etiological factors for thalassaemia-induced osteoporosis. Furthermore, iron chelating has correlated with growth failure and bone abnormalities, and high desferrioxamine dosage has been associated with cartilage alterations.
Conclusions: Osteoporosis in thalassemic Iraqi patient was too high and even more in those patients with bad compliance regard attendance to the Thalassemia centre.
Recommendations: We need to inform the thalassemic patients about the risk of osteoporosis and the need for their awareness regard such complication and the importance of their compliance with therapy.

P5

A novel role for monosodium urate monohydrate crystals and gouty synovial fluids in monocyte migration in gout

M Asif Amin[1*], Qiang Shu[1], Jonathon W Vargo[1], Jeffrey H Ruth[1], Takeo Isozaki[1], Solhee Lee[1], Alisa E Koch[2]
[1]University of Michigan Medical School, Ann Arbor, MI, USA; [2]Department of Veteran's Affairs and University of Michigan, Ann Arbor, MI, 48109, USA
Arthritis Research & Therapy 2012, **14(Suppl 1):**P5

Background: Gout is characterized by intra-articular deposition of monosodium urate monohydrate (MSU) crystals. The role of neutrophil influx in acute gouty arthritis is well established, while the contribution of monocytes (MNs) and their secreted inflammatory mediators is not. Here we demonstrate the role of MSU in MN migration.
Materials and methods: To examine the role of MSU crystals in normal human peripheral blood (PB) MN migration, we performed MN chemotaxis in a modified Boyden chamber *in vitro* using either MSU crystals or gouty synovial fluids (SFs) as stimuli. To examine mechanisms of MN migration, we performed MN chemotaxis with MSU in the presence or absence of chemical signaling inhibitors. We determined the *in vivo* role of MSU crystals or gouty SFs in homing of dye-tagged MNs using normal human synovial tissue (ST)-severe combined immunodeficient (SCID) mouse chimeras. To investigate the contribution of MSU to production of leukocyte chemoattractants macrophage migration inhibitory factor (MIF) and epithelial neutrophil activating factor-78 (ENA-78/CXCL5), and the signaling molecules involved in secretion of these cytokines, we stimulated MNs with MSU crystals with or without chemical signaling inhibitors, and performed ELISAs on conditioned medium. We also assayed for MIF in gouty SF by ELISA.
Results: We found a significant two fold increase in *in vitro* MN migration in response to MSU crystals, while gouty SFs increased MN migration five fold compared to negative control ($p < 0.05$). MSU crystal induced MN migration was significantly decreased by inhibitors of p38 MAPK, Src, and NFκB, suggesting that crystal induced MN migration occurs via these pathways. After engrafting SCID mice for 4 weeks, we injected dye-tagged human PB MNs via tail vein. Simultaneously, we injected MSU crystals or gouty SFs into ST grafts. After 48 hours, we harvested the STs and found an increase in MN homing to the grafts injected with MSU crystals or SFs ($p < 0.05$), indicating that either of these stimuli could recruit MNs *in vivo*. Human MNs stimulated with MSU for 24 hours released significantly higher quantities of the potent leukocyte chemoattractants MIF and ENA-78/CXCL5. MIF was six fold higher in gouty SFs compared to osteoarthritic fluids, suggesting the importance of MIF in gouty arthritis. MIF or ENA-78/CXCL5 secretion depended on the p38 MAPK pathway.
Conclusions: This data suggests an intriguing role for MSU crystals and gouty SFs in MN migration and provides evidence that MNs and their secreted products may be potential therapeutic targets for treating gout.

P6

Intermittent cold stress-induced experimental fibromyalgia model in mice - pharmacology and neurobiology

Kohei Araki[*], Michiko Nishiyori, Hiroshi Ueda
Division of Molecular Pharmacology and Neurosciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8521, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P6

Stress-induced pain, as in Fibromyalgia (FM), is considered to be caused by intense events involving physical and psychological injury and is reinforced by successive stress. Previously, we have established a novel mice model of FM, using intermittent cold stress (ICS) exposure. Mice given ICS caused abnormal pain, including mechanical allodynia and hyperalgesia to nociceptive thermal and chemical stimuli, which lasted for more than 2 weeks. In contrast, those given constant cold stress (CCS) did not. The abnormal pain was generalized, female-predominant and specific for A-delta and A-beta, but not C-fiber-stimuli in the electrical stimulation-induced nociceptive test. The mechanical allodynia induced by ICS was effectively suppressed by intraperitoneal or intracerebroventricular injection of gabapentin. The potency and duration of anti-allodynia effects were much

higher and longer, respectively, than the neuropathic pain induced by sciatic nerve injury. Taken together, these findings indicate that mice given ICS manifest most of characteristics observed in fibromyalgia patients in terms of pharmacology and pain physiology.

Acknowledgements: The research described in this article was supported in part by MEXT KAKENHI (17109015 to Hiroshi Ueda) and Health Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan (to Hiroshi Ueda): "Research on Allergic disease and Immunology" also supported this work.

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P7

Frequency of appearance of anemias at rheumatoid arthritis - a disease of autoimmune genesis (on the data of retrospective study)

Abdumalik N Aripov[1*], Marif SH Karimov[2], Aida A Eshmurzaeva[2]
[1]Tashkent Institute of Postgraduate Medical Education, Tashkent, Uzbekistan; [2]Tashkent Medical Academy, Tashkent, Uzbekistan

Arthritis Research & Therapy 2012, **14(Suppl 1):**P7

The purpose of research is study of offenses of appearance of anemia among rheumatoid arthritis (RA) patients, revealing of their etiologic reasons, as well as the estimation of character of used anti anemia means of medicine on the basis of retrospective analysis of history of disease.

Coming out of above stated histories of illness of RA patients were analyzed to presence of established as accompanying disease of anemia. Results of this analysis are represented on picture as it seen on the presented data, 33,3% of patients with RA anemia is verified as accompanying pathology. Therefore at 1/3 patients with P anemia takes place. The study of etiologic causes of anemia at these patients shows that in 76,6% cases anemia bears ferrous deficit character, 20%- anemia of chronic diseases and only in 3,4% cases - auto immune anemia. Therefore, the majority of patients of RA anemia bears ferrous deficit character.

The high frequency of appearance of ferrous deficit anemia among RA patients, probably is explained by that in conditions of this disease changes of pH happen among gastro duodenal area. Besides, wide use of non steroidal anti inflammatory medicine (NAIM) at RA also may effect to pH of stomach. And in cases of destroyed reaction of ambience change of ferrous assimilation. That fact of ferrous deficit anemia may has independent character at analyzed RA patients is excluded. But on their history of illness it is impossible to determine this fact.

Study of offenses of appearance of anemia at RA patients depending on age categories is evidencing on that 83,4% of patients with anemia comes to patients from 31 to 60 years old, and among patients of 31 to 40 years old appears 25% patients, from 41 to 50 years old - 26,7% and from 51 to 60 years old - 31,7%, accordingly.

Results of these analysis showed that if at patients with debut RA anemia appears at 1,5% cases, than among RA patients with prolongation of anamnesis from 1 to 5 years old, from 5 to 10 years old appears in 33,3%, 28,7% and in 34,8% cases accordingly. Therefore as far as increasing of prolongation of current of RA, specific gravity of patients with anemia increases.

P8

The bacterial effector protein YopM reduces rheumatoid arthritis (RA) outcome by inhibiting inflammation and bone destruction

J Bertrand[1*], C Rueter[2], C Cromme[3], J Scharnert[2], A Schmidt[2], T Pap[3]
[1]Experimental Medicine and Rheumatology, William Harvey Research Institute, London, UK; [2]Institute of Infectiology, ZMBE, Muenster, Germany; [3]Institute of experimental musculoskeletal medicine, University hospital Muenster, Muenster, Germany

Arthritis Research & Therapy 2012, **14(Suppl 1):**P8

Osteoclasts mediate the degradation of bone during RA and are derived from macrophages. The yersinia outer protein M (YopM) is an effector protein of *Yersinia* species that is able to enter host cells by membrane penetration. In the cell YopM mediates down-regulation of inflammatory responses. We investigated whether YopM has the potential to act as a "selfdelivering" immune therapeutic agent by reducing the inflammation and joint destruction linked to RA.

Using confocal laser scanning we analysed the penetration of recombinant YopM into bone marrow macrophages (BMMs). Furthermore we studied the effects of YopM on osteoclastogenesis using *in vitro* osteoclast formation assay. To unravel the signaling pathways of YopM, we tested for phosphorylation of MAP-kinases (ERK, AKT and p-38) and activation of NF-KB signaling by Western Blot analysis. With respect to a potential *in vivo* application of YopM, we injected YopM intra articular and intravenous in mice and monitored the distribution by fluorescence reflection imaging (FRI). We treated hTNFtg mice, as animal model for RA, with YopM and recorded clinical parameters (weight, grip strength and paw swelling). Finally we analysed the destruction of bone and cartilage histologically compared to untreated hTNFtg mice and wildtype mice.

As seen in confocal scanning microscopy, YopM penetrated the cell membrane of BMMs and accumulated near the nucleus. Studying the signaling pathways affected by YopM, we found that YopM reduced the TNFa induced activation of NF-kB via reducing the phosphorylation of IkBa. TNFa mediated phosphorylation of MAP kinases were not altered by YopM. Most interestingly, we found a strong reduction of osteoclast formation by YopM. Incubation of BMMs with YopM led to a 90% reduction in osteoclasts precursors and osteoclasts. YopM-Cy5 injected into the hind paws of hTNFtg mice was detectable in the joint without a systemic distribution for 48 hours and elimination mediated through renal clearance.

Analysing the clinical parameters of RA in hTNFtg mice, we observed a delay of onset of paw swelling in mice treated with YopM. At histological analysis of the hind paws, we found reduced bone destruction and decreased osteoclast formation, as well as less inflammation in YopM treated hTNFtg mice in comparison to untreated hTNFtg mice.

These results suggest that YopM has the potential to reduce inflammation and bone destruction in vivo. For this reason YopM may constitute a novel therapeutic agent for the treatment of RA.

P9

PTEN in antigen presenting cells is a master regulator for Th17-mediated autoimmune pathology

Stephan Blüml[1*], Gernot Schabbauer[2*], Eva Hainzl[2], Birgit Niederreiter[1], Anastasia Hladik[1], Tobias Lohmeyer[2], Michael Bonelli[1], Elisabeth Zinser[3], Marije Koenders[4], Wim van den Berg[4], Giulio Superti-Furga[5], Josef S Smolen[1], Kurt Redlich[1]
[1]Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Austria; [2]Institute for Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University Vienna, A-1090 Vienna, Austria; [3]Department of Dermatology, Hartmannstasse 14, University Hospital Erlangen, 91052 Erlangen, Germany; [4]Rheumatology Research and Advanced Therapeutics, Department of Rheumatology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [5]CeMM - Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna 1090, Austria

Arthritis Research & Therapy 2012, **14(Suppl 1):**P9

Autoreactive T cells are a central element in many systemic autoimmune diseases. The generation of these pathogenic T cells is instructed by antigen presenting cells. However, signalling pathways in APC that drive autoimmunity are not completely understood. Here we show that that conditional deletion of PTEN in myeloid cells are almost completely protected from the development of two prototypic model autoimmune diseases, collagen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE). Myeloid specific deletion of PTEN lead to a significant reduction of cytokines pivotal for the induction of systemic autoimmunity such as IL-23 and IL-6 *in vitro* and *in vivo*. In addition, PTEN deficient dendritic cells showed reduced activation of p38 MAP-kinase and increased inhibitory phosphorylation of GSK3β in vitro. Dendritic cell and macrophage phenotypic maturation and migration to lymph nodes as well as collagen specific T and B cell activation was comparable in wt and myeloid specific $PTEN^{-/-}$. However, analysing the impact of myeloid specific PTEN deficiency on T cell polarization, we found a significant reduction of a Th17 type of immune response characterized by reduced production of

IL-17 and IL-22. Moreover, there was an increase in IL-4 production and higher numbers of regulatory T cells myeloid specific PTEN$^{-/-}$. In contrast, myeloid specific PTEN deficiency did not affect serum transfer arthritis, which is independent of the adaptive immune system and solely depends on innate effector functions. These data demonstrate that the presence of PTEN in myeloid cells is required for the development of systemic autoimmunity. Deletion of PTEN in myeloid cells inhibits the development of CIA and EAE by preventing the generation of a pathogenic Th17 type of immune response.

P10

Acute Serum Amyloid A induces cell migration cytoskeletal rearrangement and Notch signalling in rheumatoid arthritis

Mary Connolly*, Peadar Rooney, Wei Gao, Douglas Veale, Ursula Fearon
Translational Research Group, Dublin Academic Medical Centre, St. Vincent's University Hospital, Dublin, Ireland
Arthritis Research & Therapy 2012, **14(Suppl 1):**P10

Background: Acute Serum Amyloid A (A-SAA) is an acute phase protein strongly expressed in rheumatoid arthritis (RA) synovial tissue (ST) critically involved in regulating cell migration and angiogenesis. These processes are dependent on downstream interactions between extracellular matrix and cytoskeletal components. Additionally the Notch signalling pathway has been show to regulate endothelial cell (EC) morphogenesis and is critically involved in vessel formation, branching and morphogenesis. The aim of this study was to examine if A-SAA-induced angiogenesis, cell migration and invasion are mediated by the NOTCH signalling pathways.
Materials and methods: Immunohistology was used to examine Notch1, DLL-4 and HRT-1 in RA synovial tissue (RAST). $\alpha v\beta 3$ and $\beta 1$-integrins, filamentous actin (F-actin) and focal adhesion expression in RAST and rheumatoid arthritis synovial fibroblast cells (RASFC) was assessed by immunofluorescence. NOTCH1 IC, its ligands DLL-4, JAGGED 1 and downstream signaling components HRT1, HRT2 were quantified by Real-time PCR. NOTCH1 IC protein was assessed by western blot. A-SAA-induced angiogenesis cell migration and invasion were assessed by Matrigel tube formation, scratch and invasion assay. A-SAA modulation of filamentous actin (F-actin) and focal adhesions (vinculin) was examined by dual immunofluorescence. Finally, A-SAA-induced angiogenesis, invasion, altered cell shape and migration were performed in the presence or absence of siRNA against NOTCH 1.
Results: Notch1 and its ligands DLL-4 and HRT-1 were expressed in RAST both in the lining layer and perivascular regions. Additionally $\alpha v\beta 3$, $\beta 1$-integrin and F-actin predominantly localised to vascular endothelium and lining cells in RAST, compared with osteoarthritis and normal control synovial tissue. A-SAA significantly upregulated levels of Notch1 mRNA and protein in ECs. Differential effects were observed on Notch ligands HRT-1 and Jagged 1 mRNA in response to A-SAA stimulation. In contrast, A-SAA inhibited DLL-4 mRNA ($p < 0.05$), consistent with a negative feedback loop controlling interactions between NOTCH1 IC and DLL-4 in the regulation of EC tip vs. stalk cells development. A-SAA induced disassembly of endothelial cell F-actin cytoskeleton and loss of focal adhesions as demonstrated by a reduction in vinculin staining. Finally, A-SAA-induced angiogenesis, cell migration and invasion were inhibited in the presence of NOTCH 1 siRNA ($p < 0.05$).
Conclusion: A-SAA induces the NOTCH signalling pathway and cytoskeletal rearrangement which allows temporal and spatial reorganization of cells during cell migratory events and EC morphology. Together these results suggest a critical role for A-SAA in driving cell shape, migration and invasion in the inflamed joint.

P11

Cigarette smoke downregulates HDAC2 in rheumatoid arthritis synovial fibroblasts

Anna Engler[1*], Astrid Jüngel[1], Christoph Kolling[2], Beat A Michel[1], Renate Gay[1], Steffen Gay[1], Caroline Ospelt[1]
[1]Center of Experimental Rheumatology, University Hospital Zurich and Zurich Center of Integrative Human Physiology (ZIHP), Zurich, Switzerland;
[2]Schulthess Clinic, Zurich, Switzerland
Arthritis Research & Therapy 2012, **14(Suppl 1):**P11

Background: Cigarette smoking has been shown as major environmental risk factor for rheumatoid arthritis (RA). Epidemiological studies indicate an association of cigarette smoking with development of RA [1,2], although molecular mechanisms remain unknown. The aim of this study is to analyze the influence of cigarette smoke on the gene expression regulated by histone deacetylases (HDACs) in RA synovial fibroblasts (RASF).
Methods: RASF obtained from patients undergoing joint replacement surgery were stimulated with freshly prepared cigarette smoke extract (CSE) for 24 hours. Expression of HDACs was measured at the mRNA level by Real-time TaqMan and SYBR green PCR and at the protein level by immunoblot analysis. Global histone 3 (H3) acetylation was analyzed by immunoblot.
Results: Stimulation of RASF (n = 8-10) with CSE significantly enhanced the expression of HDAC1 (x-fold: 2.0 ± 0.4; p = 0.04), HDAC2 (1.9 ± 0.3; p = 0.02) and HDAC3 (2.4 ± 0.4; p = 0.01) at the mRNA level while the expression of HDAC 4-11 remained unchanged. On the protein level, expression of HDAC1 and HDAC3 were not altered, whereas the expression of HDAC2 protein was decreased in CSE stimulated RASF. No measurable changes in global acetylation of H3 were induced by CSE in RASF (n = 6).
Conclusion: CSE specifically downregulates the expression of HDAC2 in RASF. Differential regulation of HDAC2 at the mRNA and protein level points to post-transcriptional degradation mechanisms induced by smoking. Even though global H3 acetylation was not changed by CSE, decreased HDAC2 levels might be associated with hyper-acetylation and thus increased expression of specific HDAC2 regulated genes.

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P12

Egr-1 mediates the suppressive effect of IL-1 on PPARg expression in human OA chondrocytes

Sarah S Nebbaki*, Fatima Ezzahra El Mansouri, Mohamed Benderdour, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi
Osteoarthritis Research Unit, Montreal, H2L 4M1, Canada
Arthritis Research & Therapy 2012, **14(Suppl 1):**P12

Background: Peroxisome proliferator-activated receptor gamma (PPARg) is a ligand activated transcription factor and member the nuclear hormone receptor superfamily. Several lines of evidence indicate that PPARg have protective effects in osteoarthritis (OA). Indeed, PPARg has been shown to down-regulate several inflammatory and catabolic responses in articular joint cells and to be protective in animal models of OA. We have previously shown that IL-1 down-regulated PPARg expression in OA chondrocytes. In the present study we will investigate the mechanisms underlying this effect of IL-1.
Materials and methods: Chondrocytes were stimulated with IL-1, and the level of PPARg and Egr-1 protein and mRNA were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction, respectively. The PPARg promoter activity was analyzed in transient transfection experiments. Egr-1 recruitment to the PPARg promoter was evaluated using chromatin immunoprecipitation (ChIP) assays.
Results: We demonstrated that the suppressive effect of IL-1 on PPARg expression requires de novo protein synthesis and was concomitant with the induction of the transcription factor Egr-1. ChIP analyses revealed that IL-1 induced Egr-1 recruitment at the PPARg promoter. IL-1 inhibited the activity of PPARg promoter and overexpression of Egr-1 potentiated the inhibitory effect of IL-1, suggesting that Egr-1 may mediate the suppressive effect of IL-1.
Conclusions: These results indicate that Egr-1 contributes to IL-1-mediated down-regulation of PPARg expression in OA chondrocytes and suggest that this pathway could be a potential target for pharmacologic intervention in the treatment of OA and possibly other arthritic diseases.

P13

Prevalence of interstitial lung disease among patients with systemic sclerosis in Iraqi Kurdistan

Taha Ahmad Qaradakhy[1*], Kosar Mohamed Ali[2], Omer Hama Karim[1]
[1]Department of Rheumatology, Sulaimani Internal Medicine Teaching Hospital, Sulaimani, Iraq; [2]Respiratory/General Medical Department, College of Medicine, Sulaimani, Iraq
Arthritis Research & Therapy 2012, **14(Suppl 1):**P13

Background: Systemic sclerosis (SSc) associated interstitial lung disease (ILD) is the leading cause of morbidity and mortality in SSc patients.
Aim of the study: To detect and determine the prevalence of ILD in patients with SSc in Sulaimani Governorate.
Patients and methods: A sample of thirty patients with SSc (whom fulfilled the American Rheumatism Association preliminary criteria for the diagnosis of SSc), were collected from Sulaimani internal Medicine teaching hospital from July 2009 to July 2010.
All patients were evaluated in a cross sectional study for the evidence of ILD, almost all patients were submitted to chest radiographs (CXR), pulmonary function tests (PFT) and oxygen saturation by pulse oximetry (Spo_2) and high-resolution computed tomography (HRCT) scan.
Results: Patients ages ranged from 23-68 years with mean (**45.57)** years, with female predominance 27(90%) compare to 3(10%) male.
Majority of patients had limited type of systemic sclerosis 21(70%), and 15 (50%) cases had restirictive ventilatory defect. Out of the thirty patients in the study 16(53.3%) patients had evidence of ILD on HRCT.
Conclusion: 1. ILD is common among patients with SSc (dcSSc type).
2. PFT & HRCT are sensitive tools for diagnosis ILD among patients with SSc.

Table 1(abstract P13) Results of pulse oximetry both during rest and exertion, chest x-ray finding, pulmonary function test

	Frequency	Percent
O_2 Saturation (rest)		
Above 92	20	66.7
Below 92	10	33.3
O_2 Saturation (exertion)		
Above 92	13	43.3
Below 92	17	56.7
CXR		
Normal	19	63.3
Basal reticular shadowing	11	36.7
Pulmonary function test		
Normal	15	50.0
Restrictive	15	50.0
Obstructive	0	00.0

Table 2(abstract P13) Distribution of HRCT scans abnormalities

Variables	Frequency	Percent
CT chest		
Normal	14	46.7
Abnormal	16	53.3
Fibrosis		
No	19	63.3
Yes	11	36.7
Traction bronchiactetic changes		
No	19	63.3
Yes	11	36.7
Ground glass		
No	22	73.3
Yes	8	26.7
Honey comb		
No	26	86.7
Yes	4	13.3

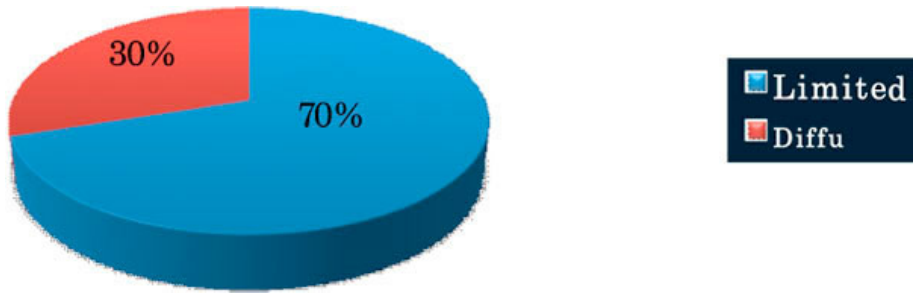

Figure 1(abstract P13) Subsets of Systemic sclerosis.

P14

MiRs in RA: possible biomarkers and therapeutic targets

Maria Filkova[1*], Caroline Ospelt[1], Joanna Stanczyk[1], Serena Vettori[1], Ladislav Senolt[2], Mojca Frank[1], Christoph Kolling[3], Beat A Michel[1], Renate E Gay[1], Steffen Gay[1], Astrid Jüngel[1]
[1]Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland; [2]Institute of Rheumatology, Department of Experimental Rheumatology of the 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic; [3]Schultess Clinic, Zurich, Switzerland
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Background and objective: New concepts of therapy highlight an early use of effective treatment to prevent further joint damage in RA. Altered expression of epigenetic marks like miRs offers us the possibility to develop new diagnostic tools and novel therapeutic targets.
We found miR-146, -155 and -203 to be upregulated in rheumatoid arthritis (RA) synovial fibroblasts (SF) compared to osteoarthritis (OA) SF [1,2]. Based on the comprehensive analysis of the expression of 260 miRs we found miR-196a to be one of the most downregulated miRs in RASF. In peripheral blood mononuclear cells, miR-132 and -223 are upregulated in established RA compared with healthy controls (HC) [3,4].
Our aim was to analyze miRs as potential systemic markers in early stages of the disease and to find new miRs locally at the site of inflammation that play a role in the pathogenesis of RA.
Methods: MiRs from sera of patients with treatment naïve early RA (ERA), with treated established RA and HC were isolated by phenol-chloroform extraction. TaqMan Low Density Array was used to analyze the expression of 260 miRs in RASF and OASF. MiR-196a expression was further analyzed in additional RASF and OASF, RA and OA synovial tissues. TaqMan RealTime-PCR was used for quantification of miRs and functional experiments (MTT, scratch assay, AnnexinV FACS) were performed following transfection with pre-miR or miR-196a inhibitor.
Results: In sera of patients with ERA, the expression of miR-146a was lower than in both HC ($p < 0.05$) and established RA sera ($p < 0.001$) while miR-155, 132, -203 and -223 showed no differences.
In RASF, the expression of miR-196a is significantly lower than in OASF ($p < 0.0001$) as well as in RA synovial tissues compared with OA ($p = 0.01$). RASF transfection with pre-miR/miR-196a inhibitor resulted in down/upregulation of predicted targets HOXC8 and ANXA1. Pre-miR-196a suppressed cell proliferation (27.5%) and migration (41.5%) and induced apoptosis (54.1%) while miR-196a inhibitor enhanced both proliferation (81.9%) and migration (231%) and reduced apoptosis (52.3%) in RASF.
Conclusion: In contrast to established RA synovial fibroblasts where an increased expression of miR-146a was reported, our data showed that in early arthritis sera miR-146a is significantly downregulated and might characterize an early clinical stage of the disease. The low expression of miR-196a in both RA synovial tissue and in isolated SF contributes to the aggressive and invasive phenotype of RASF by modifying proliferation, migration and apoptosis with an impact on the pathogenesis of RA.
Acknowledgements: This work was supported by IAR-EPALINGES, FP7 Masterswitch, MH CR- grant project No.10065-4 and ARTICULUM fellowship.

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P15

Immune cell - derived microparticles contribute to the resistance of rheumatoid arthritis synovial fibroblasts to death receptor-mediated apoptosis

Mojca Frank[1*], Meike Dahlhaus[1], Maria Filkova[1], Christoph Kolling[2], Beat A Michel[1], Diego Kyburz[1], Blaž Rozman[3], Renate E Gay[1], David Pisetsky[4], Steffen Gay[1], Astrid Jüngel[1]
[1]Center of Experimental Rheumatology, University Hospital Zürich, Zürich, Switzerland; [2]Schultess Clinic, Zürich, Switzerland; [3]Department of Rheumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia; [4]Medical Research Service, Durham Veterans Administration Medical Center, Durham, NC, USA
Arthritis Research & Therapy 2012, **14(Suppl 1):**P15

Background: Immune cell-derived microparticles (MPs) are present at increased amounts in synovial fluid of rheumatoid arthritis (RA) patients [1] and can activate disease-relevant signalling pathways in RA synovial fibroblasts (SF) [2,3]. Increased resistance to apoptosis is one of the main characteristics of aggressive phenotype of RASF [4,5] and MPs have been shown to mediate both pro- and anti- apoptotic effects in different target cells [6,7]. The aim of the present study was to investigate the functional role of immune cell-derived MPs in modulating the apoptosis of SF in RA.
Methods: MPs were isolated by the differential centrifugation from cell culture supernatants of U937 cells, untreated or stimulated with TNFα or poly(I:C) for 16 h. Flow cytometry was used to measure the counts and surface expression of CD4 and Fas on MP. Proinflammatory response of RASF induced by MPs was determined by measuring IL-6 protein levels by ELISA. Proliferation of OASF (n = 3) and RASF (n = 4) stimulated with MPs for 24 h was investigated by MTT Cell Proliferation Assay. Functional role of MPs (after 24 h treatment) in spontaneous apoptosis and apoptosis mediated by Fas Ligand (FasL) or TNFα-Related Apoptosis Inducing Ligand (TRAIL) was measured by flow cytometry using Annexin V/propidium iodide staining of RASF and OASF.
Results: Poly(I:C)-induced MPs but not MPs from unstimulated U937 cells increased the production of IL-6 in RASF (mean ± SE: 1873 ± 325

pg/mL, p = 0.002, n = 9 and 476 ± 182 pg/mL, n = 6, respectively) when compared to unstimulated RASF (304 ± 61 pg/mL, n = 9). No changes in proliferation or spontaneous rate of apoptosis were observed in RASF or OASF stimulated with MPs. Treatment of RASF (n = 5) and OASF (n = 5) with FasL or treatment of RASF (n = 7) with TRAIL for 24 h significantly increased apoptosis of SF (p = 0.010; p = 0.036 and p = 0.016, respectively). Poly(I:C)-induced MPs inhibit FasL-induced apoptosis of RASF (% decrease ± SE: 40.2 ± 7.0%; p = 0.001; n = 5) and OASF (41.1 ± 9.5%; p = 0.036, n = 5) and decreased TRAIL-induced apoptosis of RASF (29.9 ± 6.8%, p = 0.093). In contrast, TNFα-induced MPs had no effect on Fas-induced apoptosis in SF (n = 3). MPs from untreated U937 cells did not influence FasL- or TRAIL-induced apoptosis of RASF (n = 5) and OASF (n = 4). Fas was not expressed on the surface of MPs, indicating that Poly(I:C)-induced MP did not act as a decoy to decrease the effective concentration of FasL in cell culture supernatants.

Conclusions: Immune cells and SF can communicate via MPs. The impairment of the death receptor-induced apoptosis pathway mediated by immune cell-derived MPs may contribute to synovial hyperplasia and joint destruction in RA.

Acknowledgements: This work was supported by IAR-EPALINGES, FP7 Masterswitch, and ARTICULUM Fellowship.

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P16

Increased concentration of serum soluble LAG3 in systemic lupus erythematosus

Seri Yu*, Keishi Fujio, Kazuyoshi Ishigaki, Hirofumi Shoda, Tomohisa Okamura, Tanita Noor, Shuji Sumitomo, Kazuhiko Yamamoto
Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, 113-0033, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P16

Background: In systemic lupus erythematosus (SLE), type I interferon and plasmacytoid DCs (pDCs) are supposed to play important roles. However, there are few evidences for pDCs activation in SLE. Murine pDCs are reported to produce soluble LAG3 (sLAG3) upon activation and pDCs are responsible for most of sLAG3 in mice serum [1]. Therefore, serum sLAG3 concentration was examined in SLE and other autoimmune diseases.

Materials and methods: This study enrolled 45 SLE patients who met ACR criteiria. Disease activity was rated using a SLE disease activity index (SLEDAI). sLAG3 concentrations were measured by a quantitative sandwich enzyme immunoassay [2].

Results: The ratio of sLAG3 concentration in SLE to control was 3.10+/-1.05, PM/DM to control was 1.04+/-0.08, and RA to control was 0.77+/-0.14. In addition, sLAG3 concentrations showed a significant correlation with SLEDAI. Interestingly, elevation of sLAG3 was observed even in patients with SLEDAI = 0. These results suggested that sLAG3 could be a specific and novel marker for SLE.

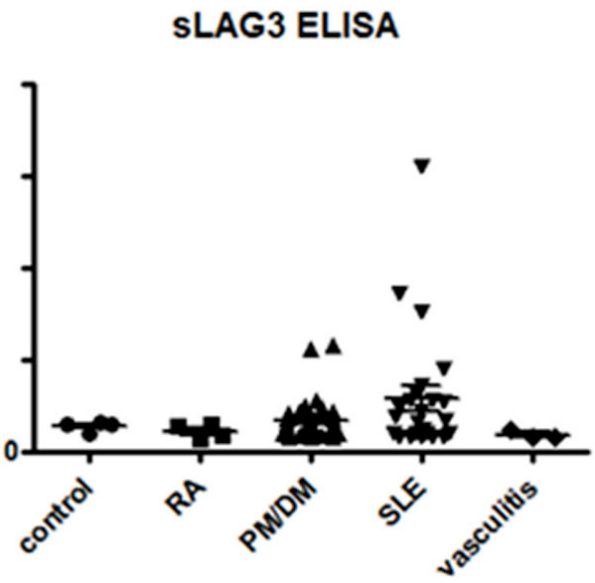

Figure 1(abstract P16) sLAG3 concentrations in SLE and other autoimmune diseases measured by ELISA.

Conclusions: sLAG3 can be a novel marker for SLE. sLAG3 in sera of SLE patient may reflect the activation of pDCs. Because sLAG3 shows adjuvant effect when combined with active immunization [3], sLAG3 may contribute to the exacerbation of lupus. The association between elevated sLAG3, type I interferon signature and activation of pDCs should be investigated further.

P17

GCIP, Id like HLH protein, negatively regulates cell proliferation of rheumatoid synovial cells via interaction with CBP

Hidetoshi Fujita[1,2*], Minako Nakazawa[1], Satoko Aratani[1,3], Kusuki Nishioka[3], Akiyoshi Fukamizu[4], Toshihiro Nakajima[1,3]
[1]Institute of Medical Science, St Marianna University School of Medicine, Kanagawa 216-8512, Japan; [2]Advanced Radiation Biology Research Program, and Hospital, Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba, 263-8555, Japan; [3]Institute of Medical Science, Tokyo Medical University, 6-1-1 Shinjyuku, Shinjyuku-ku, Tokyo, 160-8402, Japan; [4]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
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Background: Rheumatoid arthritis (RA) is one of the most common articular diseases with a prevalence of 1% worldwide [1,2]. The clinical features of RA include chronic inflammation of systemic joints associated with synovial hyperplasia followed by impairment of quality of life [3,4]. Recently, we have shown that Synoviolin/Hrd1, an E3 ubiquitin ligase, is a novel causative factor for arthropathy [5]. However, the mechanism that regulates synovial cell outgrowth is not fully understood.

Materials and methods: Human embryonic kidney (HEK)-293 cells, HEK-293T cells, NIH3T3 cells and synovial cells were cultured in DMEM medium. Transient transfection assays were performed in HEK-293 cells and HEK-293T cells. HEK-293 cells transfected with NF-κB-Luc were treated with 100 ng/ml of phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), or 10 ng/ml of TNF-α for 24 h, and luciferase activities were measured. siRNAs with 21 nucleotides for human GCIP were chemically synthesized. Transfection with siRNAs and cell survival assay were carried out.

Results: Grap2 cyclin D interacting protein (GCIP), Id like HLH protein, was down-regulated in the rheumatoid synovial cells. Introduction of GCIP into mouse fibroblast NIH3T3 cells resulted in growth suppression, whereas knockdown with siRNAs in synovial cells enhanced cell growth. GCIP associated with CBP and repressed transcription of CREB-target genes such as cyclin D1 by inhibition of interaction between CBP and RNA polymerase II complexes. Binding assays revealed that GCIP bound to CBP via acidic region, not HLH domain, and this interaction was regulated by phosphorylation of GCIP in a cell cycle-dependent manner. Therefore, GCIP has inhibitory effect on cell proliferation via interference with CBP-mediated transcription.

Conclusions: We propose the novel inhibitory mechanisms of Id protein family; the coactivator CBP is a functional target. Furthermore, down-regulation of GCIP may be a key factor in rheumatoid synovial cell outgrowth.

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P18

Unc93 homolog B1 restricts systemic lethal inflammation by orchestrating TLR7 and TLR9 response

Ryutaro Fukui[1*], Shin-Ichiroh Saitoh[1], Atsuo Kanno[1], Masahiro Onji[1], Takuma Shibata[1,2], Akihiko Ito[4], Morikazu Onji[5], Mitsuru Matsumoto[6], Shizuo Akira[7,8], Nobuaki Yoshida[3], Kensuke Miyake[1,2]

[1]Division of Infectious Genetics, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan; [2]Laboratory of Innate Immunity, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan; [3]Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan; [4]Department of Pathology, Faculty of Medicine, Kinki University, Osaka 589-8511, Japan; [5]Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Ehime 791-0295, Japan; [6]Division of Molecular Immunology, Institute for Enzyme Research, University of Tokushima, Tokushima 770-8504, Japan; [7]Laboratory of Host Defense, World Premier International Immunology Frontier Research Center, Osaka 565-0871, Japan; [8]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

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Nucleotide sensing-TLRs (Toll-like receptors) recognize pathogen derived-nucleic acids and trigger immune response [1]. Because of the highly conserved structure of nucleic acids, these TLRs have risk to recognize host derived-nucleic acids and induce autoimmune disease, therefore it is important to clarify the mechanisms and control the response.

We found that the responses of TLR7 and TLR9 are balanced reciprocally, and Unc93 homolog B1 (Unc93B1) is a key molecule for this balancing system [2]. Unc93B1 is known as an essential molecule for TLR3, TLR7, and TLR9 responses, and the function depends on its C-terminal region [3]. The balancing function of Unc93B1 is located on 34th aspartic acids from N-terminal, and alanine mutant (D34A) Unc93B1 up-regulates TLR7 response and down-regulates TLR9 response (Figure 1) [2].

It is reported that TLR7 or TLR9 response contributes to some kinds of autoimmune disease and TLR7 overexpressed mice develop SLE like autoimmune disease [4-8]. To investigate the significance of reciprocal TLR7/TLR9 balance in vivo, we generated *Unc93b1*$^{D34A/D34A}$ mice and observed the phenotypes.

As results, *Unc93b1*$^{D34A/D34A}$ mice were born according to Mendelian rule but started to die spontaneously at 10 weeks old and over half of *Unc93b1*$^{D34A/D34A}$ mice died within 1 year (Figure 2A) [9]. *Unc93b1*$^{D34A/D34A}$ mice developed various phenotypes, for example, splenomegaly, hepatitis, glomerulonephritis, thrombocytopenia, myeloproliferative disorder (Figure 2B-2E). Especially, lethal acute hepatitis was observed in moribund mice and infiltrated myeloid cells in liver were expanded in spleen. These phenotypes are vanished by TLR7 deficient Unc93B1$^{D34A/D34A}$ mice, thus TLR7 hyper-response caused by TLR7/TLR9 balance disruption is factor of phenotypes in *Unc93b1*$^{D34A/D34A}$ mice (Figure 2).

Not only innate immune system, acquired immune system is also affected by D34A mutation. Expanded memory T cells, up-regulation of ICOS and CD69 on T cells were observed by TLR7 dependent manner and some classes of serum immunoglobulin level is increased in *Unc93b1*$^{D34A/D34A}$ mice. In addition, Th1 and Th17 cells were expanded and activated in *Unc93b1*$^{D34A/D34A}$ mice. The activation of T cells were TLR7 dependent, and

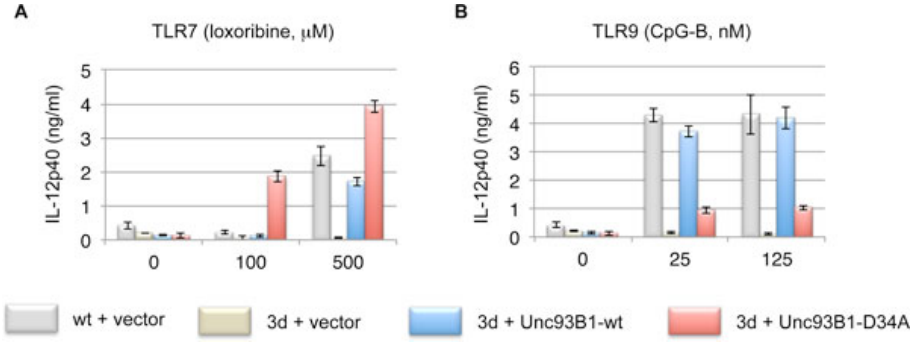

Figure 1(abstract P18) The D34A mutation of Unc93B1 up-regulates TLR7 response and down-regulates TLR9 response. (A and B). Empty vector was transfected to bone marrow derived stem cells (BMSCs) from wild tipe mice (gray bars). Empty vector (yellow bars), wild type Unc93B1 expressing vector (blue bars), or D34A Unc93B1 expressing vector (red bars) were transfected to BMSCs from 3d mice. Transfected BMSCs were cultured with puromycin and GM-CSF to differentiate to dendritic cells (DCs). After differentiation, DCs were harvested and stimulated by TLR7 ligands (**A**, loxoribine, μg/ml) or TLR9 ligands (**B**, CpG-B, nM). Culture supernatant was corrected and subjected to ELISA for measurement of IL-12p40 (ng/ml).

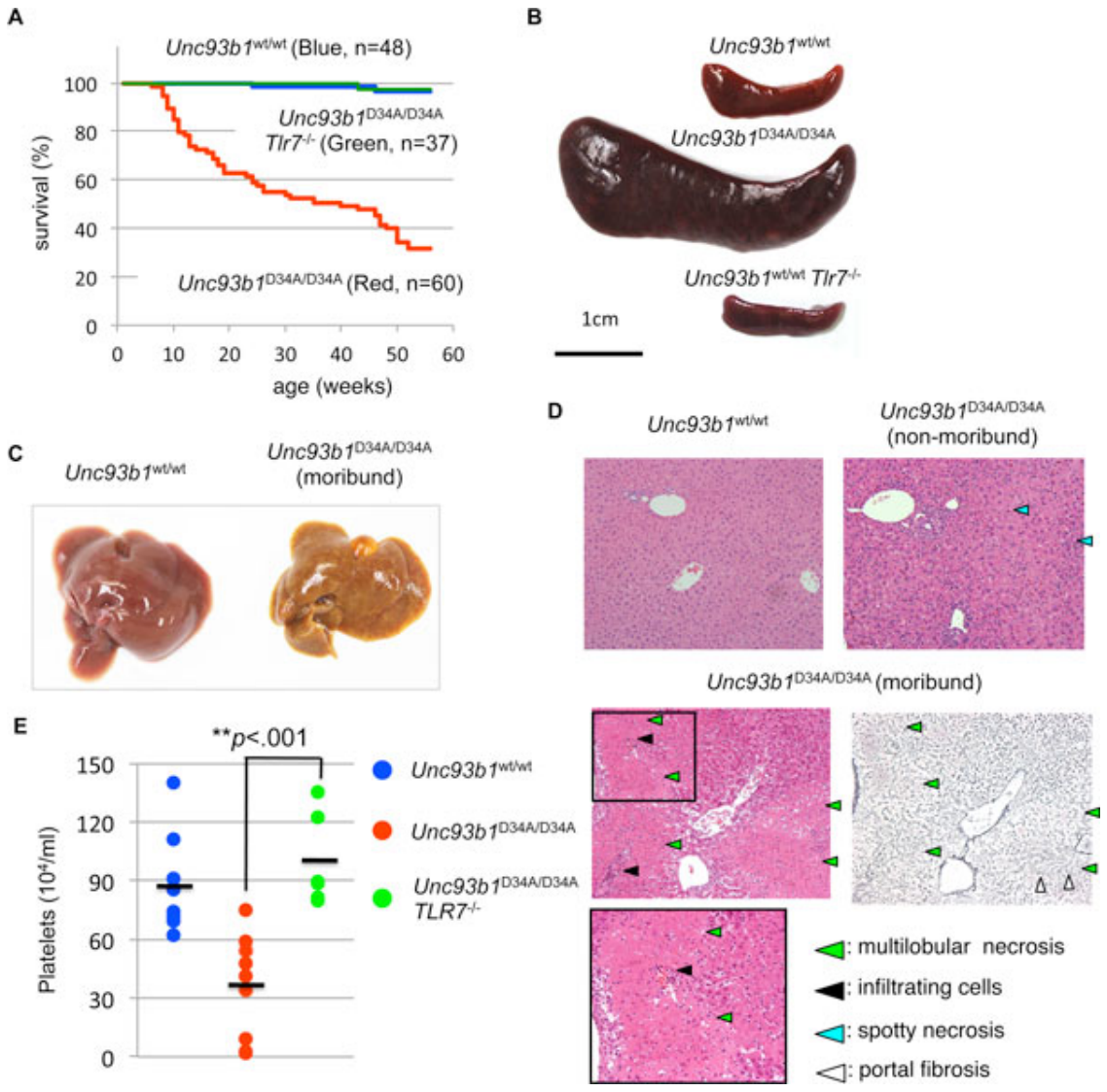

Figure 2(abstract P18) *Unc93b1*$^{D34A/D34A}$ mice develop systemic lethal inflammation spontaneously. **(A)** Survival curves of *Unc93b1*$^{WT/WT}$, *Unc93b1*$^{D34A/D34A}$, or *Unc93b1*$^{D34A/D34A}$*Tlr7*$^{-/-}$ mice (blue, red, or green line, respectively). **(B and C)** Macroscopic images of spleen **(B)** and liver **(C)**. **(D)** Microscopic analyses of liver. Histological samples were stained by Hematoxyline and Eosine (H&E) or silver impregnation. **(E)** Platelet counts in peripheral blood from indicated genotypes of mice. Bars in the graph indicate averages.

mature B cell depleted *Ighm*$^{-/-}$*Unc93b1*$^{D34A/D34A}$ mice did not induce T cell activation and moderated phenotypes (Figure 3D and 3E). It suggests that B cells are activated by TLR7 hyper-response, and the B cells activate T cells to generate phenotypes of *Unc93b1*$^{D34A/D34A}$ mice.However, thrombocytopenia was not completely recovered in *Ighm*$^{-/-}$*Unc93b1*$^{D34A/D34A}$ mice but completely recovered in *Rag2*$^{-/-}$*Unc93b1*$^{D34A/D34A}$ mice. Interaction between cell types and phenotypes should be confirmed as a future plan.

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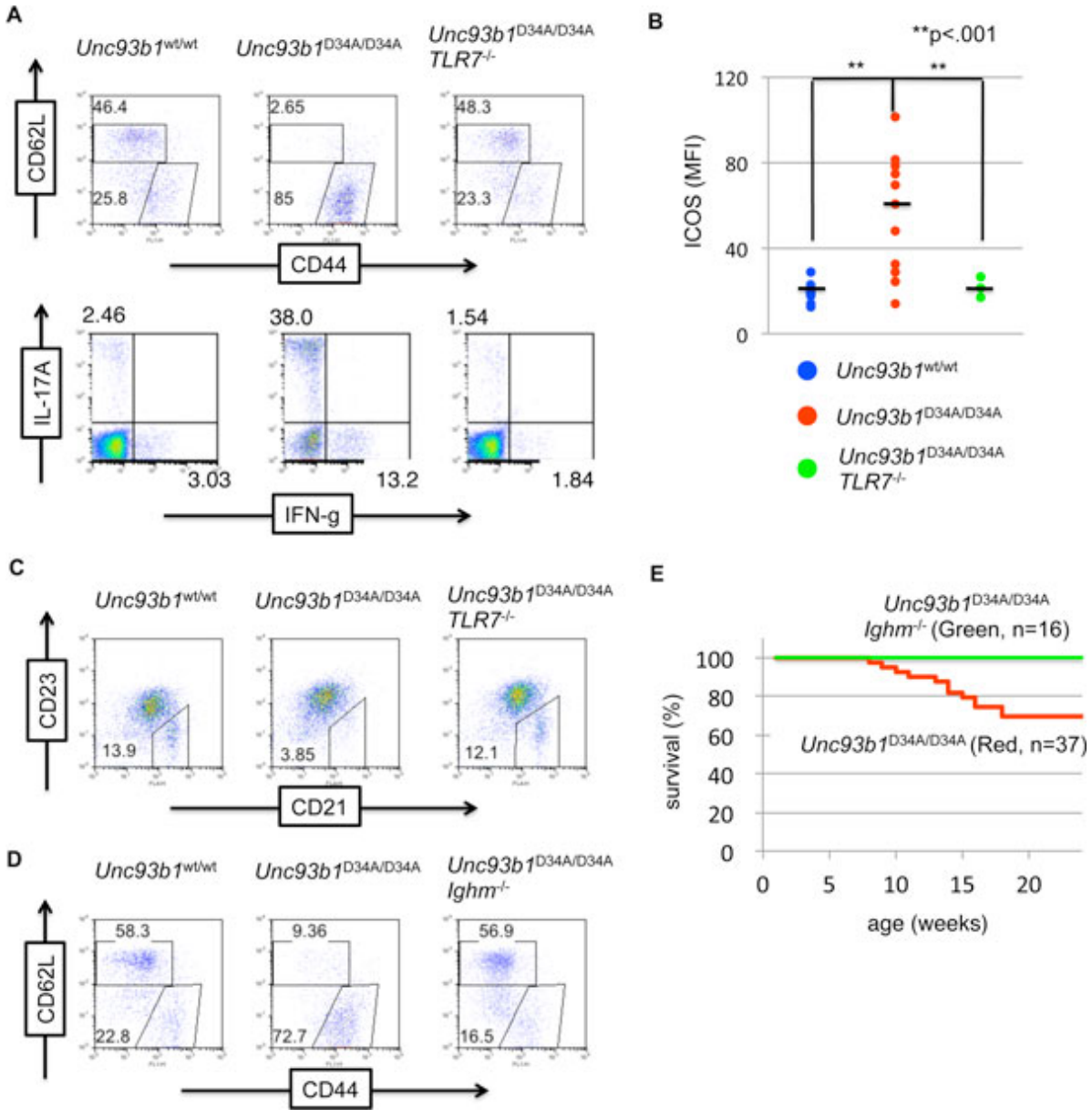

Figure 3(abstract P18) T cells and B cells are activated in *Unc93b1*$^{D34A/D34A}$ mice. **(A or D)** Flow cytometry analysis for memory T cells (**A** upper, or **D**) or Th1/Th17 cells (**A** lower). **(B)** Expression of ICOS was measured by cell surface staining of CD4+ T cells. Mean fluorescent intensity (MFI) was calculated and indicated by dots. Black bars in the graph indicate average of MFI. **(C)** Flow cytometry analysis for matrginal zone B cells. **(E)** Survival curves of *Unc93b1*$^{D34A/D34A}$ (Red) or *Unc93b1*$^{D34A/D34A}$*Ighm*$^{-/-}$ (Green) mice.

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P19

Balb/c FasKO mice develop allergic blepharitis associated with hyper-production of IgE

Ayumi Fukuoka[1*], Shizue Yumikura-Futatsugi[2], Suzuka Takahashi[1,3], Hirotaka Kazama[1], Kenji Nakanishi[2], Shin Yonehara[1]
[1]Guraduate School of Biostudies, Kyoto University, Japan; [2]Department of Immunology and Medical Zoology, Hyogo College of Medicine, Japan; [3]Institute of Genome Reserch, The University of Tokushima, Japan
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Fas is a member of the TNF receptor family and crucial for induction of apoptosis. MRL- *lpr/lpr* mice, which carry a mutation of Fas, spontaneously develop systemic autoimmune disease including arthropathy, indicating that Fas plays an important role in elimination of self-reactive immunocytes by apoptosis. In addition to autoimmune diseases, we found a novel phenotype of FasKO mice exclusively in Balb/c genetic background that is allergic blepharitis. Allergic blepharitis is revealed in Balb/c FasKO mice from 15 week-old and about 85% of the mice suffered from allergic blepharitis at 35 week-old. Serum concentrations of both IgG1 and IgE Abs were about 100-times higher in 20-week old FasKO mice than in WT mice; however, there was no significant difference between WT and FasKO mice in the ability of B cells to produce IgG1 and IgE Abs in the presence of IL-4 and anti-CD40 Ab inducing co-stimulatory signals. Additionally, the production of IL-4 by T cells was same. These results suggested that other type of cells

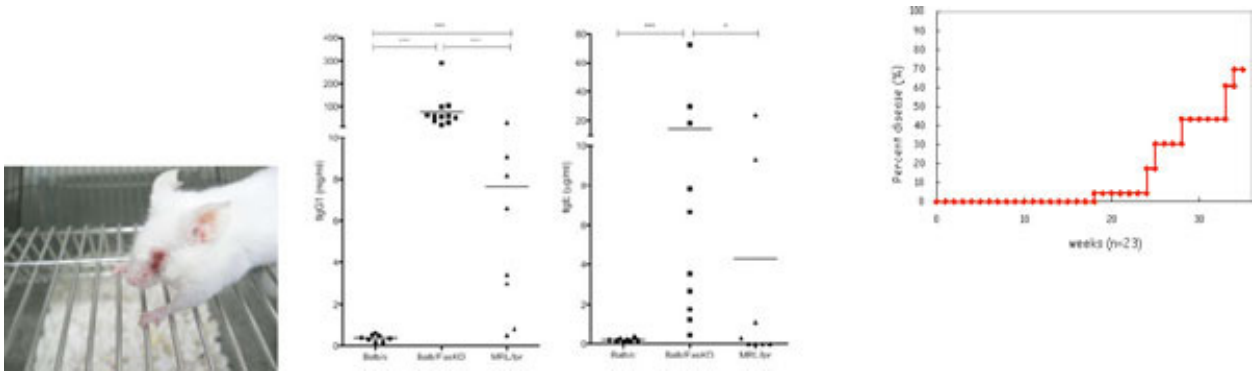

Figure 1(abstract P19) Balb/c FasKO mice develope allergic blepharitis.

enhanced IgG1 and IgE Abs production from B cells in Balb/c FasKO mice. To identify the cells enhancing IgG1 and IgE Abs production, we cultured B cells *in vitro* in the presence of IL-4 and anti-CD40 Ab together with various types of cells from Balb/c FasKO mice. In the result, we found FasKO non-T non-B cells upregulated the production of both IgG1 and IgE from B cells. Moreover, the number of these cells was specifically increased in Balb/c FasKO mice.All the results indicate that these cells enhance production of IgG1 and IgE from B cells in the presence of IL-4 and anti-CD40 Ab, and excessive accumulation of these cells may cause allergy *via* hyper-production of IgE.

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P20

Stimulation of bone formation in cortical bone of the mice treated with a novel bone anabolic peptide with osteoclastogenesis inhibitory activity

Yuriko Furuya[1*], Kohji Uchida[1], Hisataka Yasuda[2]
[1]Nagahama Institute for Biochemical Science, Oriental Yeast Co Ltd, Shiga, Japan; [2]Planning & Development Group Bioindustry Division, Oriental Yeast Co Ltd, Tokyo, Japan

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Background: Receptor activator of nuclear factor-κB ligand (RANKL), a member of tumor necrosis factor (TNF)-α, is produced by osteoblasts (Obs) and stimulates its receptor RANK on osteoclast (Oc) progenitors to differentiate them to osteoclasts. WP9QY peptide designed to mimics TNF receptor's contact site to TNF-α was known to abrogate osteoclastogenesis in vitro by blocking RANKL-RANK signaling. WP9QY ameliorated collagen-induced arthritis and osteoporosis in mouse models. Here we report that the peptide surprisingly exhibited bone anabolic effect in vitro and in vivo.
Materials and methods: WP9QY was administered subcutaneously to mice three times per day for 5 days at a dose of 10 mg/kg in normal mice, followed by peripheral quantitative computed tomography (pQCT) and histomorphometrical analyses. To clarify the mechanism by which the peptide exerted the bone anabolic effect, we examined the effects of the peptide on osteoblast (Ob) differentiation/mineralization with mouse MC3T3-E1 (E1) cells and human mesenchymal stem (MSC) cells, and those on osteoclast (Oc) differentiation with RAW264 cells in the presence of sRANKL.
Results: WP9QY augmented bone mineral density (BMD) significantly in cortical bone not in trabecular bone. Histomorphometrical analysis showed that the peptide had little effect on osteoclasts in distal femoral metaphysis, but markedly increased bone formation rate in femoral diaphysis. The peptide markedly increased alkaline phosphatase (ALP, a marker for Ob) activity in E1 and MSC cell cultures and decreased tartrate-resistant acid phosphatase (TRAP, a marker for Oc) activity in RAW264 cell culture in a dose-dependent manner, respectively. In addition, the peptide stimulated mineralization evaluated by alizarin red staining in E1 and MSC cell cultures. The anabolic effect of WP9QY peptide was enhanced markedly by addition of BMP2.
Increases in mRNA expression of IGF1, collagen type I, and osteocalcin were observed in E1 cells treated with the peptide for 12 and 96 h in GeneChip analysis. Addition of p38 MAP kinase inhibitor reduced ALP activity in E1 cells treated with the peptide, suggesting a signal through p38 was involved in the mechanisms.
Conclusions: Taken together, the peptide abrogated osteoclastogenesis by blocking RANKL-RANK signaling and stimulated Ob differentiation/mineralization with unknown mechanism in vitro. However, in our experimental conditions the peptide exhibited bone anabolic effect dominantly in vivo. Since the peptide is known to bind RANKL, we hypothesize that the peptide shows the bone anabolic activity with reverse signaling through RANKL on Obs.

P21

T-regs/Th17 function defect in systemic autoimmunity as a result of "recent thymic emigrants" maturation defect

Mark Goloviznin[1*], Natalia Lakhonina[1], Alexander Yarilin[2], Yulia Buldakova[1], Vitaly Timofeev[3], Tatiana Kremenchugskaya[1], Marina Struchkova[1]
[1]Department of Internal Diseases of Dental Faculty, Moscow State University of Medicine and Dentistry, Russia; [2]Laboratory of Cell Immunology, Research Center Institute of Immunology, Moscow, Russia; [3]Department of Faculty Therapy of Russian State Medical University, Moscow, Russia

Arthritis Research & Therapy 2012, **14(Suppl 1):**P21

T-regs and Th17 cells are the new generation of CD4+T-cells which play crucial role in autoimmunity. Both of subsets can influence each other and probably have common precursor. A key question for understanding the mechanism of autoimmunity is to recognize how T-regs and Th17 cells turn from self-protection to autoreactivity. Based on literature data and own observations, we have constructed a conception of age-dependent thymic T-cells maturation "peripherialisation" as cause of errors in Th17-T-reg cells interrelations. The connection of T-regs with thymus is determined currently. Connection of Th17 cells with thymus remains to be determined properly. Main, there may be naturally occurring Tregs of thymic origin that are resistant to cell death and serve as "reserve pool" for autoimmunity protective suppressors. This mechanism could be affected by external factors producing profound lymphopenia [1]. Previously we found that RA patients with numerous rheumatoid nodules (RN) and lymphopenia had statistically reliable decrease of CD3+T-cells level. We found definite negative correlation between CD3+PBL amount and RN number (p = 0,029). In all RA patients with and without RN we didn't found the decrease of CD4 receptor. Hereby we expected to find unusual CD3-4+ and CD3-8+ cells in RA. Otherwise the percentage of CD3+4+ and CD3+8+ cells was normal in general. But in 4 RA patients after magnetic separation of CD3+T-cells we detected reliable amount of CD3-4+ lymphocytes (25-28%) These cells were not detected before separation. One of possible explanation of this phenomenon is CD3 molecule modulation after the contact with anti-CD3 antibodies conjugated with magnetic particles. So the presence of T-cells with unusual phenotype in peripheral blood of RA patients doesn't give absolute evidence of T-cells maturation disorders. According to our viewpoint "recent thymic emigrants" (RTE) fraction presence among

T-regs and hypothetically among Th17-cells is the sign of normal Th17/T-regs function. Otherwise the absence of RTE among them leads to immunopathology. CD31 receptor and T cell receptor rearrangement excision circles (TREC) are now markers of RTE. We investigated the number of CD4+CD31+T-cells in RA patients. The preliminary results permit us to suggest the diminution of RTE in RA (less then 1%/ml) We also found the diminution of TREC amount in PBL of 22 rheumatoid arthritis patients, (Median 0,035539 units). FOXP3, RORγ, RORα and CD31 expression in RA will permit to establish role of RTE in autoimmunity.

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P22

Dendritic cell immunoreceptor (DCIR) is associated with anti-cyclic citrullinated peptides (anti-CCP) antibody - negative rheumatoid arthritis in Chinese Han population

Jianping Guo, Xinyu Wu, Xiaolan Lu, Yin Su, Ru Li, Jing He, Xu Liu, Zhanguo Li*
Department of Rheumatology and Immunology, Peking University People's Hospital, Beijing, 100044, China
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Background: The dendritic cell immunoreceptor (DCIR) is an important member of C-type lectin superfamily, which has been shown evidence for susceptibility to arthritis in multiple animal models. The human DCIR polymorphisms have been shown a nominal association with rheumatoid arthritis (RA) susceptibility, mainly with anti-cyclic citrullinated peptides (anti-CCP) antibody -negative RA in Swedish population. We aimed to investigate the possible association of *DCIR* with RA susceptibility in Chinese Han population.
Methods: A total of 1193 patients with RA and 1278 healthy controls were genotyped for single-nucleotide polymorphism (SNP) rs2377422 and rs10840759. Association analyses were performed on the whole data set and on RA subsets based on the status of anti-CCP antibody in RA patients. The interaction between rs2377422 and HLA-DRB1 shared epitope (SE) was also analyzed for RA susceptibility. Finally, we carried out association analysis of rs2377422 with DCIR mRNA expression in RA patients.
Results: The DCIR rs2377422 was found significantly associated with RA (allele analysis: OR 1.17, 95%CI 1.04-1.31, $p=3.67 \times 10^{-3}$; genotype analysis (recessive model C/C vs. T/T + T/C): OR 1.37, 95%CI 1.08-1.73, $p=9.04 \times 10^{-3}$). Following stratification for anti-CCP status, a suggestive association of rs2377422 with anti-CCP-positive RA was observed ($p = 0.058$, OR 1.34, 95%CI 0.99-1.82). In contrast, the CC genotype of rs2377422 was found specifically to confer susceptible risk for anti-CCP-negative RA (OR 1.92, 95%CI 1.27-2.90, $p=1.99 \times 10^{-3}$), despite loss of power in the analysis. The relative risk of RA was 3.0 (95%CI 1.33-6.91, $p=6.48 \times 10^{-3}$) in individuals carrying rs2377422 TT genotype with SE alleles, and 9.06 (95%CI 3.33-25.61, $p=2.08 \times 10^{-6}$) in individuals carrying rs2377422 CC genotype with SE genes. The interaction between rs2377422 and SE alleles was significant, as measured by the attributable proportion (AP) due to interaction (0.60). DCIR gene transcription quantification analysis further proved the dominant effect of rs2480256 CC genotype on DCIR expression levels in RA patients (C/C vs. T/T + T/C: 0.55 ± 0.09 vs. 0.24 ± 0.02, $p=1.67 \times 10^{-3}$).
Conclusions: Our study provides evidence for association between DCIR rs2377422 and RA, particularly with anti-CCP-negative RA in non-Caucasian populations.

P23

Association between serum level of Vitamin D with autoantibodies expression, disease activity (SLEDAI) and bone mineral density (BMD) in patients with Systemic Lupus Erythematosus (SLE)

Handono Kalim[1*], Singgih Wahono[1], Putra Suryana BP[1], Lenny Puspitasari[1], Fajar Hadi Wijayanto[1], Kusworini Handono[2]
[1]Rheumato-Immunology Division, Department of Internal Medicine, Brawijaya University, Malang, Indonesia; [2]Department of Clinical Pathology Faculty of Medicine, Brawijaya University, Malang, Indonesia
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Backround: Vitamin D defficiency has been reported to have negative association with clinical manifestation and disease activity of SLE [1,2]. Vit D has an important role in the pathogenesis of SLE [3] and it is necessary to give vit D supplementation to the patients [4]. The objective of our study was to determine the association between serum vitamin D level with auto antibodies expression, disease activity and bone mineral density in SLE patients.
Patients and methods: 55 female patients with SLE were recruited from Clinic of Rheumato-Immunology, Saiful Anwar Hospital, Malang, Indonesia. Mean age of the patients 31.12 years (12-64 yo) with duration of illness 18,4 months (2-54 mo). Serum vitamin (25 (OH)D3 level was assayed using ELISA method (Cusabio, normal value>30 ng/mL). Anti ds-DNA and Anti Cardiolipin antibodies were assayed using ELISA method (Diagnostic Automation, Inc, USA). Disease activity assessed by SLE disease activity index (SLEDAI) and BMD was assessed by bone densitometry using DEXA. Association between variables (serum vitamin D and autoantibodies level, BMD and SLEDAI) were analyzed using Spearman correlation.
Result: The mean of serum 25(OH)D3 level was 22.80 ± 16,23 ng/mL.14 patients (25.5%) had vitamin D deficiency (<10 ng/mL), 34 patients (61.8%) had vitamin D insufficiency (10-30 ng/mL), and 7 patients (14.7%) had normal vitamin D levels. There were significant difference level of anti-dsDNA antibodies (112.46 vs 267.13 U/ml; $p < 0.05$) and IgM ACA (16.40 vs 29.7 IU/ml; $p < 0,05$) in patients with vitamin D insufficiency and vitamin D defisiency. Serum level of 25(OH)D3 were negatively related with level of anti-dsDNA and IgM ACA (r: - 0, and r: - 0,72 respectively). The mean of SLEDAI was 15,0 + 10.46. Serum vitamin D levels were inversely correlated with SLEDAI ($\overline{R}$=-0.319, p<0.05). Normal BMD at lumbal spine found in 21 (38.2%) patients. 26 patients (47.3%) were osteopenia, and 8 (14.5%) patients were osteoporosis. At femoral neck, 25 (45.5%) patients had normal BMD, 23(41.8%) patients were osteopenia, 7 (12.7%) patients were osteoporosis. There were no significant correlation between vitamin D level and BMD at lumbal spine ($p = 0,531$) and at femoral neck ($p = 0,175$).
Conclusion: A large proportion ofSLE patients had low vitamin D levels. There were positive association between vit D level and autoantibodies expression in SLE and negative association between serum vitamin D levels with SLEDAI. No association was found between serum vit D level and BMD.

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P24

Uncoupling protein 3 attenuates generation of reactive oxygen species by interacting with thioredoxin 2 in the mitochondrial intermembrane space

Katsuya Hirasaka[1*], Edward M Mills[2], Shohei Kohno[1], Tomoki Abe[1], Chika Ikeda[1], Tasuku Maeda[1], Shigetada Kondo[1], Ayako Maita[1], Yuushi Okumura[1], Takeshi Nikawa[1]
[1]Department of Nutritional Physiology, Institute of Health Biosciences, University of Tokushima, Tokushima, 770-8503, Japan; [2]Division of Pharmacology/Toxicology, University of Texas at Austin, Austin, TX 78712, USA
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Uncoupling protein 3 (UCP3) is primarily expressed in the inner membrane of skeletal muscle mitochondria. It has been proposed that UCP3 reduces production of reactive oxygen species (ROS) and oxidative damage. However, the mechanisms by which UCP3 attenuates ROS production are not well understood. Here we report that UCP3 interacts with the non-processed form of thioredoxin 2 (Trx2), a redox protein that is localized in mitochondria, but not processed Trx2, which is involved in cellular responses to ROS. The hydrophilic sequences within the N-terminal tail of UCP3, which faces the intermembrane space, are necessary for binding to

Trx2. In addition, Trx2 directly associated with UCP3 through a mitochondrial targeting signaling sequence, was processed in the intermembrane space, and thereby allowing redox reactions. A bimolecular fluorescence complementation analysis demonstrated that the interaction of these proteins occurs in the mitochondrial intermembrane space. Furthermore, increased UCP3 expression significantly attenuated ROS production in isolated mitochondrial without effects on membrane potential, however this effect is lost by Trx2 knock down. These results suggest that UCP3 binds to Trx2 in the mitochondrial intermembrane space and attenuates ROS production.

P25

Conditional inactivation of the ectodomain shedding of pro-TNFα in monocytes prevents lethality from LPS-induced septic shock

Keisuke Horiuchi[1*], Tokuhiro Kimura[2], Yasunori Okada[2], Kazuhiro Chiba[1], Carl P Blobel[3], Yoshiaki Toyama[1]
[1]Department of Orthopedic Surgery, School of Medicine, Keio Univ. Tokyo, Japan; [2]Department of Pathology, School of Medicine, Keio Univ. Tokyo, Japan; [3]Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, USA
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Background: TNFα is synthesized as a membrane-bound precursor and proteolytically released from cells. Soluble TNFα is the primary mediator of pathologies such as rheumatoid arthritis, Crohn's disease, and endotoxin shock. Although several different enzymes have been implicated in this proteolytic activity, recent studies lean toward the TNFα converting enzyme (TACE/ADAM17) as the most relevant TNFαsheddase*in* vivo.In the present study, we asked whether the inactivation TACE could yield a protection from lipopolysaccharide(LPS)-induced septic shockin mice.
Materials and methods: To abrogate TNFα shedding activity in vivo, we generated conditional TACE-deficient mice using Cre-loxP system [1]. We mated these mice with *Mx1-Cre*tg mice and *LysM-Cre*tg mice to inactivate TACE in BM cells and macrophage/monocyte lineage cells, respectively. Endotoxin shock was induced by i.p. injection of 5 μg of LPS and 20 mg of D-galactosamine. All injected mice were closely monitored every hour for the first 16 h and every 3-6 h thereafter.
Results/conclusions: We found that temporal disruption of TACE under the control of *Mx1* transgene prevented lethality from endotoxin shock. Furthermore, inactivation of TACE in macrophage/monocyte lineage cells also rendered significant protection against LPS-induced septic shock. Consistent with these findings, serum TNFα levels in the TACE mutant mice were much lower than those in control mice. The present study thus shows that 1) TACE is indeed a principal enzyme responsible for the release of soluble TNFα in vivo, and that 2) inactivation of TACE in macrophage/monocyte lineage cells is sufficient to yield strong protection against LPS-induced endotoxin shock. Taken together, the present data indicate inhibition of TACE activity as a potential therapeutic target for TNFα-related disorders.

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P26

Community-based epidemiological study on hyperuricemia and gout over 5 years in Huang-pu district, Shanghai

Hui Du[1*], Shun-Le Chen[1], Chun-De Bao[1], Xiao-Dong Wang[1], Yuan Wang[1], Yue-Ying Gu[1], Kusuki Nishioka[2]
[1]Department of Rheumatology, Ren Ji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200001, China; [2]Institute of Medical Science, Tokyo Medical University, Tokyo 160-8402, Japan
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Background: A community-based survey on the prevalence of hyperuricemia and associated factors was carried out in 1996 and 2001.
Materials and methods: In the target community in1996, 2037 dwellers (age≥15 years old) were interviewed with relevant questionnaires from house to house. According to even house number, 807 blood samples (age≥ 40 years old) were taken for serum uric acid (SUA) levels measured with the uricase-peroxidase enzymatic method. In 2001, 830 residents ≥ 40 years of age were taken for SUA levels measured with the same enzymatic method. Cholesterol, triglyceride, blood urea nitrogen, glycosylated hemoglobin, ESR, rheumatoid factor etc were measured as possible risk factors to enter the multiple logistic regression analysis on hyperuricemia.
Results: The prevalence of hyperuricemia was 15.1% (51 cases/338, SUA>7 mg/dl) in men, 8.7% in women (41 cases/469, SUA>6 mg/dl) and seven gout male patients were found in 1996. The prevalence of hyperuricemia was 19.5% (52 cases/266, SUA>7 mg/dl) in men, 12.6% (71 cases/564, SUA>6 mg/dl) in women in 2001.The prevalence of gout in 2037 dwellers in Huangpu District was 0.77% in men and 0.34% in both sexes in 1996 [1].
Conclusions: The mean SUA level in each age group in 2001 was higher than that of in 1996 (see Table 1). The prevalence of hyperuricemia was increased rapidly (Male: 15.1% in 1996 to 19.5% in 2001; Female: 8.7% in 1996 to 12.6% in 2001 $p < 0.05$). Azotemia (≥23 vs. <23 mg/dl), hypertriglyceridemia (≥200 vs. <150 mg/dl, 150–200 vs. <150 mg/dl) were the associated risk factors by multiple logistic regression analyzing the independent effect of each variable on hyperuricemia.

Table 1(abstract P26) Comparison of SUA levels in different age group over 5 years

Year/Age	*40-49*	*50-59*	*60+*
Male 2001	**5.85 ± 1.02 (56)	*6.04 ± 1.14 (74)	6.20 ± 1.32 (136)
1996	5.38 ± 1.06 (100)	5.53 ± 1.30 (50)	5.90 ± 1.45 (188)
Female 2001	4.19 ± 0.88 (164)	4.72 ± 1.07 (146)	**5.14 ± 1.17 (254)
1996	4.13 ± 0.94 (118)	4.49 ± 1.05 (84)	4.74 ± 1.07 (267)

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P27

Adrenal function in rheumatoid arthritis: acorrelation with disease activity

Richard Imrich[1,2*], Miroslav Vlcek[1,2], Jana Kerlik[1], Michael Vogeser[3], Fabian Kirchhoff[3], Adela Penesova[1,2], Zofia Radikova[1], Jozef Lukac[4], Jozef Rovensky[4]
[1]Laboratory of Human Endocrinology, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, 833 06, Slovakia; [2]Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, 831 01, Slovakia; [3]Institute of Clinical Chemistry, Hospital of the University of Munich, Munich, 81377, Germany; [4]National Institute of Rheumatic Diseases, Piestany, 921 12, Slovakia
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Background: Hypothalamic-pituitary-adrenocortical dysfunction contributes to a complex pathogenesis of rheumatoid arthritis (RA). Decreased production of adrenal androgens and subtle changes in cortisol production has been observed in RA, particularly in femalepatients with premenopausal onset of the disease. Our study was aimed to investigate (1) adrenocortical function in relation to disease and inflammatory activity and to analyze cortisol bioavailability in RA females.
Materials and methods: Adrenal steroids including free plasma cortisol responses to the low-dose ACTH stimulation test (1 μg Synactheni.v.) were investigated in 23 premenopausal RA and in 15 age- and BMI-matched healthy females. Twelve (N = 12) out of 23 RA patients were on low-dose glucocorticoids (<8.5 mg/day of prednisone or equivalent). When patients were divided into low (disease activity score 28; DAS28≤ 3.2) and moderate to high disease activity (DAS28>3.2) subgroups, glucocorticoid-treated patients comprised 53% and 50% of patients in each of the subgroups. Plasma C-reactive protein, interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-17, interferon gamma and tumor necrosis factor alpha concentrations were measured at the baseline.
Results: RA patients had high C-reactive protein, IL-6, IL-8 and tumor necrosis factor alpha. Patients with DAS28>3.2 had lower ($p < 0.05$) total

plasma cortisol, 17-hydroxyprogesterone, dehydroepiandrosterone and androstenedione responses in the ACTH test compared to healthy controls. Patients with DAS28>3.2 had lower ($p < 0.05$) dehydroepiandrosterone response in the ACTH test compared to patients with DAS28≤ 3.2. C-reactive protein (CRP), DAS28, and interleukin (IL)-6 negatively correlated with androstenedione response to Synacthen. Responses of all measured adrenal steroids were lower ($p < 0.05$) in patients on low-dose glucocorticoids compared to healthy controls. RA patients not treated with glucocorticoids had lower total cortisol response ($p = 0.038$) compared to controls, however, these patients did not differ in free plasma cortisol in the ACTH test.
Conclusions: The present data indicate an association of increased disease activity with a decrease in adrenal androgen-producing *zonareticularis*in RA. A modest suppression of stimulated cortisol in glucocorticoid-untreated RA patients is not associated with decreased cortisol bioavailability.

P28

Rheumatoid arthritis fibroblast-like synoviocytes show the upregulation of myeloid cell specific transcription factor PU.1 and B cell specific transcriptional co-activator OBF-1, and express functional BCMA

Kenji Itoh[1*], Katsuya Nagatani[2]
[1]Department of Rheumatology, National Defense Medical College, Tokorozawa, Saitama, Japan; [2]Department of Rheumatology & Clinical Immunology, Jichi Medical University, Shimotsuke, Tochigi, Japan
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Objective: Fibroblast-like synoviocytes (FLS) are among the principal effector cells in the pathogenesis of rheumatoid arthritis (RA).This study shows the variety of stimulating effects of a proliferation-inducing ligand (APRIL), and its specific effect on the FLS in the affected RA synovium (RA-FLS).
Results: A significantly higher level of soluble APRIL was detected in RA serum compared with in normal serum. Among the three receptors of APRIL tested, RA-FLS expressed only the B cell maturation antigen (BCMA), whereas the FLS in the affected osteoarthritis synovium (OA-FLS) expressed none of the receptors. Moreover, RA-FLS expressed transcription factor PU.1 and B cell specific-transcriptional co-activator OBF.1, which were normally expressed during myeloid and B-lymphoid cell development. The expression levels of PU.1 and OBF-1 were correlated with those of BCMA in RA-FLS. APRIL stimulated RA-FLS but not OA-FLS to produce interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β and APRIL itself. APRIL also enhanced the receptor activator of nuclear factor kappa B ligand (RANKL) expression in RA-FLS. Moreover, APRIL enhanced the cell-cycle progression of RA-FLS. Neutralization of APRIL by BCMA-Fc fusion protein attenuated all these stimulating effects of APRIL on RA-FLS.
Conclusions: RA-FLS express BCMA, and are stimulated by APRIL. These results provide evidence that APRIL is one of the main regulators in the pathogenesis of RA.Epigenetic regulation of BCMA transcription in RA-FLS might contribute to the underlying mechanisms of this condition.

P29

Methyl glyoxal increase apoptosis (CASPASE-3 expression) in pre-osteoblast MC3T3E1 cell line via SOD activity

Izaak Zoelkarnain Akbar[1*], Handono Kalim[2], Djoko Wahono Soeatmadji[2], Mohammad Hidayat[3]
[1]Department of Orthopaedic, Ulin General Hospital, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia; [2]Department of Internal Medicine, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, Indonesia; [3]Department of Orthopaedic, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, Indonesia
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Background: Increased advanced glycation end (AGE) products have been reported to be an important cause of increased osteoblast apoptosis in osteoporosis [1,2]. Methylglyoxal (MG) is a reactive dicarbonyl compound endogenously produced mainly from glycolytic intermediates. The involvement of specific reactive oxygen spesies ($^{\bullet}OH$/ H_2O_2 / $^{\bullet}O_2^-$) in increased apoptosis (caspase-3 expression) caused by methyl glyoxal exposure in osteoblast still speculative [3,4]. The aim of our study is to assess the role of specific reactive oxygen species signalling on the effect of MG as an AGE on increased caspase-3 expression in pre-osteoblast.
Materials and methods: Pre-osteoblast MC3T3E1 cell line was obtained from American Type Culture Cell. Caspase-3 expression in the cells were assayed in basal condition and after the cells exposed with methyl glyoxal on dose 5 μM for 6 hours incubation. Diethylthiocarbamoic acid, mercaptosuccinate, or deferoxamine was added in the culture media to block specific reactive oxygen species signalling for the development of osteoblast apoptosis. The caspase 3 expression were assesses from each different groups of preosteoblast culture: preosteoblast exposed to nothing, preosteoblast exposed to methyl glyoxal, preosteoblast exposed to diethylthiocarbamoic (SOD blocker), exposed to mercaptosuccinate (glutathion peroxidase blocker) and exposed to deferoxamine (Fe^{++} blocker); and osteoblast exposed to methyl glyoxal and diethylthiocarbamoic, or mercaptosuccinate, or deferoxamine. The result were analyzed using Kruskall Wallis test with $p < 00.5$ significant.
Results: Our study showed that MG significantly increased caspase3 expression (apoptosis) of osteoblast. Expression of caspase3 in osteoblast were significantly highest when the cells exposed to SOD blocker compare with when the cells exposed to GSH and Fe^{++} blocker whether the cells exposed to MG. Hydroxyl radical increase caspase-3 expression higher than another reactive oxygen species ($^{\bullet}OH > H_2O_2 > {}^{\bullet}O_2^-$) in pre-osteoblast MC3T3E1 without exposed methyl glyoxal. The result showed that superoxide radical more dominant in increasing caspase-3 expression than another reactive oxygen species ($^{\bullet}O_2^- > {}^{\bullet}OH > H_2O_2$) in pre-osteoblast MC3T3E1 with MG exposure. There is no significant differences regarding the effecfts of GSH and Fe^{++}block on osteoblast caspase3 expression.
Conclusion: The increased osteoblast apoptosis caused by AGE (MG) is mediated by specific reactive oxygen signalling, SOD activation.

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P30

Discrepancy between patient and physician in assessment of global severity in early rheumatoid arthritis

Yuko Kaneko[*], Masataka Kuwana, Tsutomu Takeuchi
Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Japan
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Objective: To evaluate the discrepancy between patient and physician in assessment of global severity in early rheumatoid arthritis (RA) and to explore factors affecting the discrepancy at 1-year since the diagnosis of RA.
Methods: One hundred nine patients with RA with median disease duration of 4 months were enrolled in this study. The global assessment was performed using 100 mm-visual analog scale (VAS). The difference between patient's and physician's assessment wascalculated by subtracting physician's VAS from patient's VAS, and the difference more than 20 mm was defined as discordant.RA patients were stratified by concordance and discordance of VAS scoring at 1-year after the diagnosis. To clarify the factors affecting the discrepancy, clinical characteristics, disease activity using Disease Activity Score (DAS28) 3-variables, functional status by Health Assessment Questionnaire (HAQ) were compared between patients with concordance and discordance.
Results: The discordance between patient's and physician's VAS at 1-year was found in 41 patients (37%), consisting of 5 patients whose VAS was better than physicians and 36 patients whose VAS was worse than

physicians. Tender joint count, DAS28 3-variables, CRP andHAQ were significantly higher in patients with discordance group where patients rated themselves worse than physicians than in patients with concordance ($p < 0.05$). HAQ score was correlated with the degree of the difference ($R = 0.49$, $p < 0.05$).

Conclusions: Higher disease activity and higher HAQ score was associated the discordance between patient's and physician's VAS in early RA patients, indicating the possibility of physicians underestimating the patient's global disease severity at 1-year since diagnosis.

P31

Cartilage-specific deletion of prar-gamma in mice results in early endochondral ossification defects and accelerated aging-dependent development of osteoarthritis

Roxana Monemdjou[1*], Faezeh Vasheghani[1], Hassan Fahmi[1], Gemma Perez[1], Meryem Blati[1], Noboru Taniguchi[2], Martin Lotz[2], René St-Arnaud[3], Jean-Pierre Pelletier[1], Johanne Martel-Pelletier[1], Frank Beier[4], Mohit Kapoor[1]
[1]Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM) and Department of Medicine, University of Montreal, Montreal, Quebec, Canada, H2L 4M1; [2]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037, USA; [3]Genetics Unit, Shriners Hospital for Children, Montreal, Quebec, Canada, H3G 1A6; [4]Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1
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Background: Long bones develop through a strict coordinated process of endochondral ossification within the growth plate resulting in the replacement of cartilage by bone and defect in this coordinated process may result in skeletal abnormalities such as dwarfism, kyposis and also age-related defects such as osteoarthritis (OA). PPARγ, a transcription factor, plays a key role in lipid homeostasis but its in vivo role in cartilage/bone development is unknown. Therefore, we determined the specific *in vivo* role of PPARγ in endochondral bone ossification, cartilage/bone development and in OA using cartilage-specific PPARγ knockout (KO) mice.
Materials and methods: Cartilage-specific PPARγ KO mice were generated using LoxP/Cre system. Histomorphometric/immunohistochemical analysis was performed to account for ossification patterns, chondrocyte proliferation, differentiation, hypertrophy, skeletal organization, bone density, calcium deposition and mouse OA phenotypic changes during aging using OARSI scoring. Real-Time PCR and western blotting was performed to determine the expression of key markers involved in endochondral ossification and cartilage degradation.
Results: Histomorphometric analyses of embryonic and adult mutant mice demonstrate reduced long bone growth, calcium deposition, bone density, vascularity as well as delayed primary and secondary ossification. Mutant growth plates are disorganized with reduced cellularity, proliferation, differentiation, hypertrophy and loss of columnar organization. Isolated chondrocytes and cartilage explants from E16.5 and 3 weeks old mutant mice further show decreased expression of ECM production products, aggrecan and collagen II, and increased expression of catabolic enzyme, MMP-13. Furthermore, aged mutant mice exhibit accelerated OA-like phenotypes associated with enhanced cartilage degradation, synovial inflammation, and increased expression of MMP-13, and MMP-generated aggrecan and collagen II neoepitopes. Subsequently, we show that loss of PPARγ and subsequent downstream alterations in phosphatase and tensin homolog on chromosome ten (PTEN)/Akt pathway contribute towards increased expression of OA catabolic and inflammatory markers, thus enabling the articular cartilage of PPARγ-deficient mice to be more susceptible to degradation during aging.
Conclusions: For the first time, we demonstrate that loss of PPARγ in the cartilage results in endochondral bone defects and subsequently accelerated OA in mice. PPARγ is essential for normal development of cartilage and bone.

P32

Average findings of uric acid in blood in patients with gout with different categories of hyperglycemia

Ulugbek K Kayumov[1*], Marif Sh Karimov[2], Nargiza A Abdukhakimova[1]
[1]Tashkent Institute of Postgraduate Medical Education, Tashkent, Uzbekistan; [2]Tashkent Medical Academy, Tashkent, Uzbekistan
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Table 1(abstract P32) Average findings of uric acid in blood in patients with different categories of hyperglycemia

Categories of hyperglycemia	n	M	± m
Normal tolerance	13	531,56	0,38
Hyperglycemia on an empty stomach	11	658,18 *	0,27
Hyperglycemia after 1 hour	20	501,16	0,33
Hyperglycemia after 2 hours	76	656,22 *	0,34

* Note: in the table is shown the reliability of differences concerning an indicator in hyperglycemia group in 1 hour after loading a glucose.

Along with a huge amount of works about the importance of a metabolic syndrome in development of cardiovascular diseases, within last decade in the literature there was a series of reports on a pathogenetic role of this syndrome in formation and more serious current of some other diseases of an internal. In process of doctrine development about a metabolic syndrome [1], there was new data about existence at gout of various signs insulin resistance [2]. At the same time, there are insufficiently studied questions on a role of various categories of a hyperglycemia (a hyperglycemia on an empty stomach and a postloading hyperglycemia) in a pathogenesis and gout and hyperuricemia clinic.
Method of the inquiry: 120 males with gout at age 30-69 were examined to investigate the connection between different categories of hyperglycemia and level of uric acid in patients with gout. Gout was revealed on the basis of criteria of American Rheumatic Association. Glucose tolerance condition was revealed by carrying out standard test of glucose tolerance (TGT) with revealing of glycemia on an empty stomach, and also in one and two hours after taking 75 gr glucose by the examined patients.
The results: According to the revealed findings average levels of uric acid in patients with gout with normal glucose tolerance had 531,56 ± 0,38 mcmol/l. With damaged glucose tolerance on an empty stomach and in two hours after glucose loading, levels of uric acid were more higher(658,18 ± 0,27 mcmol/l and 656,22 ± 0,34 mcmol/l correspondingly). At the same time on damaged glucose tolerance in an hour after glucose loading average level of uric acid was 501,16 ± 0,33 mcmol/l. We should draw attention that the difference of average levels of uric acid among people with disorders glucose tolerance on an empty stomach and in two hours after glucose loading was more differ from level of uric acid among people with glucose tolerance disorder in an hour after glucose loading ($p^*<0,05$).
Conclusion: According to these results we can come to the conclusion that the level of hyperglycemia has connection with existence in patients with hyperglycemia on an empty stomach and two hours after glucose loading. At the same time the problem about connection of uric acid level with hyperglycemia in an hour after glucose loading should be examined farther. Perhaps, that rising of glycemia level in an hour after glucose loading is a compensator mechanism in patients with gout.

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P33

Effect of B cell depletion using peptide tetramers in collagen-induced arthritis

Kazuya Michishita*, Kimito Kawahata, Takeyuki Kanzaki, Lisa Akahira, Toshiki Eri, Kazuhiko Yamamoto
Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan
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Background: B cell depletion therapy is effective in the treatment of various autoimmune diseases. However, this therapy is shown to be associated with increased risk of adverse effects such as opportunistic infections. Therefore, in this study, we developed and analyzed the

selective depletion therapy of pathogenic B cells using peptide tetramers in collagen-induced arthritis model.

Methods: Since the antigenic targets of pathogenic antibodies are identified in collagen-induced arthritis (CIA) model, we developed toxin-conjugated peptide tetramers, which contained pathogenic epitope of mouse type II Collagen (CII). The male DBA/1J mice were immunized with bovine CII and injected with toxin-conjugated peptide tetramers on day 10 and day 20 after CIIimmunization.We analyzed the effect of toxin-conjugated peptide tetramers on the production of autoantibodies and clinical course of arthritis.

Results: The incidence of arthritis was significantly lower ($P < 0.05$) in the tetramer-treated group than in the control group. The mean serum antibody levels for CII did not differ significantly, but there were significant differences in the anti-peptide antibodies over time.

Conclusions: Peptide tetramer is effective in the selective depletion of antigen-specific B cells and decreased the incidence of arthritis in CIA model. Therefore, depletion of antigen-specific B cells using this strategy might be a new therapeutic intervention of autoimmune diseases.

P34

Peripheral tolerance induced by apoptotic cells and PD-1+ CD8 T cells

Hirotaka Kazama[1*], Tomonori Iyoda[1], Satoko Yokoyama[1], Kayo Inaba[1], Thomas A Ferguson[2], Shin Yonehara[1]

[1]Department of Biostudies, Kyoto University Graduate School, Kyoto 606-8501 Japan; [2]Department of Ophthalmology and Visual Science, Washington University School of Medicine, MO 63110 USA

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Self tolerization in peripheral is critical to prevent autoimmune diseases including arthritis and here we focus on the role of PD-1 in tolerance induction against the antigen associated with apoptotic cellsdelivered intravenously(i.v.). We accessed delayed type hypersensitivity (DTH) reaction against hapten (TNP) as antigen specific immune response, in which the injection of TNP-apoptotic cells i.v.suppressedDTH in wild type mice but we found not in PD-1 KO mice (Figure 1). Adaptive transfer of CD8 T cells into PD-1 KO mouse from wild type mice tolerated with TNP-apoptotic cells suppresses DTH. This result shows PD-1 functions on CD8 T cells for immune suppression.Additionally we neutralized the PD-1 with antibody to determine the phase when PD-1 functions for immune tolerance by apoptotic cells, and identified PD-1functionsparticularly at the initial phase of antigen specific immune response. We are further studying the mechanism of suppressive role of PD-1+ CD8 T cells that should be activated with apoptotic cells.

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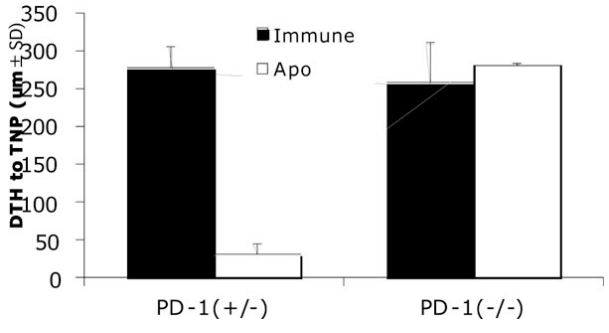

Figure 1(abstract P34) PD-1 is essential for tolerance induced by apoptotic cells. TNP-apoptotic cells were injected intravenously into PD-1 hetero- or homo- deficient mice. The mice were immunized with TNP (Filled bar) or preconditioned with apoptotic cells before immunization with TNP (open bar).

P35

Decreased plating efficiency, proliferation and osteogenic differentiation of synovial fluid mesenchymal progenitors as a marker of severity of juvenile idiopathic arthritis

Elvira Lazic Mosler[1*], Marija Jelusic-Drazic[2], Danka Grcevic[1,3], Ana Marusic[4], Natasa Kovacic[1,5]

[1]Laboratory for Molecular Immunology, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia; [2]Department of Pediatrics, Division of Pediatric Rheumatology and Immunology, University Hospital Centre Zagreb, Zagreb HR-10000, Croatia; [3]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia; [4]Department of Research in Biomedicine and Health, University of Split School of Medicine, Split HR-21000, Croatia; [5]Department of Anatomy, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia

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Background: Juvenile idiopathic arthritis (JIA) is a rheumatic pediatric disease characterized by synovial inflammation in one or more joints [1]. Inflammation results in hyperplastic changes of the synovium, destruction of articular cartilage and subchondral osteoresorption. Murine models of arthritis revealed impaired osteogenic/chondrogenic differentiation of synovial mesenchymal progenitors via inflammation-induced activation of NF-κB [2].

We aimed to explore frequency, plating efficiency and osteoblastogenic potential of synovial mesenchymal progenitors and correlate them with intensity of local and systemic inflammation in patients with JIA.

Materials and methods: Synovial fluid cells were collected from 19 patients with oligoarticular JIA (oJIA) and 8 patients with poliarticular JIA (pJIA), plated in density 1.5×10^6/mL in 24-well plates, and cultured in αMEM + 10% FCS. Osteoblastogenesis was stimulated by the addition of 50 μg/ml ascorbic acid and 5 mmol β-glycerophosphate. To exclude inflammatory and hematopoietic cells, adherent cells were passaged three times, and osteoblastogenesis again induced in fourth passage (P4). Osteoblastogenesis was assessed by intensity of alkaline phospatase (AP) histochemical staining. In addition, osteoblast (Runx2, AP, OPG, RANKL) and cytokine/chemokine (IL-1, IL-4, CCL2, CCL4 and MIP1α) gene expression were assessed in P4 osteoblastogenic cultures.

Results: Plating efficiency of synovial mesenchymal progenitors was decreased in patients with pJIA in comparison to patients with oJIA. Passage was successful only in 3 (37.5%) pJIA patients, and 18 (94.7%) oJIA patients. Plated at equal density, P4 synovial adherent cells from pJIA patients formed less fibroblastic colonies. Osteoblastogenesis was higher in children with oJIA than in children with pJIA, both from primary synovial cells (median 1119.08; IQR 476.57-1470.26 vs. 141.58; IQR 14.47-237.50, arbitrary units, $p < 0.005$, Mann-Whitney test), and P4 cells (median 1162.00; IQR 102.00 to 5484.50 vs. 12.00; IQR 6.00-307.37, arbitrary units, $p < 0.05$). Osteoblastogenesis from primary synoviocytes negatively correlated with erythrocyte sedimentation rate ($\rho = -0.4139$, $p = 0.03$), and synovial concentration of IL-17 ($\rho = -0.4174$, $p = 0.04$). Expression of osteoprotegerin and CCL2 was decreased in P4 osteoblastogenic cultures from pJIA in comparison with oJIA patients ($p < 0.05$).

Conclusions: Severe forms of JIA are characterized by decreased proliferation, osteogenic differentiation and immunoregulatory potential of synovial mesenchymal cells, correlating with inflammatory activity.

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P36

LC-MS/MS-based shotgun proteomics identified the targets of arthritis-related microRNA

Riho Kurata[1,2*], Tomo Yonezawa[1], Hideki Nakajima[3], Shyuji Takada[1], Hiroshi Asahara[1,4,5]

[1]Department of Systems BioMedicine, National Research Institute for Child Health and Development, Setagaya-ku, Tokyo 157-8535, Japan; [2]Department of Molecular Life Sciences, Basic Medical Science and Molecular Medicine,

Tokai University School of Medicine, Isehara, Kanagawa, Japan; [3]Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Setagaya-ku, Tokyo 157-8535, Japan; [4]Department of Systems BioMedicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8510, Japan; [5]Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Saitama 332-0012, Japan
E-mail: riho@hope.tokai-u.jp
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microRNAs (miRNAs), which are class of post-transcriptional regulators such as short 19 to 23-nucleotide non-coding RNAs, complementarily bind seed sequences in the 3'-untranslational region of multiple target mRNAs, resulting in their suppression of translation or degradation [1]. In the former case, since the mRNA expression of the targets does not any change, transcriptomics approach, such as expression array, cannot identify the targets.
Recent studies shed light on the fine-tuning mechanism of miRNAs in myriad biological processes including development [2], tumorigenesis [3] and inflammation [4]. We have identified enhancement of mir-146a expression in rheumatoid arthritis synoviocyte and macrophages [5], whilst suppression of them in osteoarthritis [6]. Another group also have identified the enhancement of mir-146a and mir-155 in response to bacterial pathogen such as lipopolysaccaride [7]. Recently, mice lacking of mir-155 are resistant to collagen-induced arthritis (CIA) [8], whilst administration of mir-146a complexed with aterocollagen into joint attenuates pathological condition of CIA [9]. These results indicate that mir-146a and mir-155 plays an important role for developing arthritis and inflammation. However, the targets of both two miRNAs and their molecular mechanisms are not still fully identified.
In this study, in order to identify the targets of them in translational level, we established gain of function models using adenovirus- and CMV promoter-mediated overexpression in several culture models and performed liquid chromatography-tandem mass spectrometry-based shotgun proteomics in these models.
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P37

T cell receptor rearrangement excision circles (TREC) study as an approach to "in vivo" thymus gland function investigation

Natalia Lakhonina[1*], Mark Goloviznin[1], Almira Donetskova[2], Margarita Nikonova[2], Alexander Yarilin[2], Yulia Buldakova[1], Anna Tektova[1]
[1]Department of Internal Diseases of Dental faculty, Moscow State University of Medicine and Dentistry, Russia; [2]Laboratory of cell immunology, Research center Institute of Immunology, Moscow, Russia
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Age-dependent changes in immune system such as thymus gland involution, T-cells amount decrease, are typical both for autoimmune diseases (Rheumatoid arthritis - RA), and progressive atherosclerosis characterized as "accelerated ageing". But till now processes of T-cell maturation were studied only by indirect methods. The introduction of T-cell receptor excision circle (TREC) PCR-assay seemed to enable direct detection of recent thymic emigrants in peripheral blood and therefore the quantification of thymic output [1]. High TREC levels were detected during childhood, and were decreasing with age, but TREC-expressing cells are not completely lost in the elderly. At the first stage of our investigation we studied TREC level in 3 groups of healthy donors: 16 people. 16 - 30 years old (group 1, TREC Median 0,156299 Units), 8 persons 30 - 45 years old (group 2, TREC Median 0,08782 Units) and 9 people over 45 years (group 3 TREC Median 0,051858 Units). Thereby we confirmed age-related decline of thymic output in healthy donors.
In RA patients we found age-dependent statistical definite difference of TREC expression. In the 1-st group (n-12, age range 40,4+2,8 y) TREC amount was following: Median 0,00766 I/U lower level 0,00045, upper level 0,01961. In the 2-nd group (n = 16, age range 57,5+1,32) TREC were diminished (Median 0,00065, lower level 0,000002, upper level 0,00095). Detected high TREC amount in some young RA patients is not entirely consistent with the data of literature.TREC level in patients with chronic forms of coronary heart disease (age 55 - 70 years old) was lower but comparable with donors group 3 (TREC Median 0,0200 Units). Unexpectedly high level of TREC comparable with donors group 2 we detected in patients with Acute Myocardial Infarction (AMI) (10 patients, age range 48 - 71 y) (TREC Median 0,089845 Units). According to our viewpoint, the content of TREC in peripheral blood lymphocytes depends both on thymic output and "peripheral" factors, such as survival time of "naive" T cells in periphery. Recent data give evidence that the up-regulation of Th1 cell-functions and interferon-γ hyperproduction existed in patients with AMI after the onset of symptoms. This may participate in the immune-mediated ventricular remodeling after AMI. The slowing of "naive"-T-cells turnover and Th1/Th2 imbalance could be the reason of TREC increase in AMI patients.
The work is done in framework of project 11-04-01670 sponsored by Russian Foundation of Basic Research. Project director Dr. Goloviznin M.V.
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P38

Fas deficiency attenuates bone loss during antigen induced arthritis in mice

Elvira Lazic Mosler[1*], Sania Kuzmac[1,2], Sanja Ivcevic[1,3], Danka Grcevic[1,3], Ana Marusic[4], Natasa Kovacic[1,2]
[1]Laboratory for Molecular Immunology, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia; [2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia; [3]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb HR-10000, Croatia; [4]Department of Research in Biomedicine and Health, University of Split School of Medicine, Split HR-21000, Croatia
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Background: Antigen induced arthritis (AIA) is an experimental model of rheumatoid arthritis induced by methylated bovine serum albumin (mBSA) [1]. Hyperplastic synovia in AIA contains fibroblast-like

synoviocytes (FLS) with reduced ability to differentiate into osteoblasts, chondroblasts or adipocytes [2]. Since Fas is shown to inhibit osteoblast differentiation [3], we were interested whether such inhibitory effect may contribute to the pathogenesis of AIA.

Materials and methods: AIA was induced in mice with a Fas gene knockout (Fas -/-). Three weeks after pre-immunization with mBSA in complete Freund's adjuvant, wild-type (C57BL/6, wt) and Fas -/- mice were injected with mBSA into each knee, whereas controls were injected with equal volume of phosphate buffered saline (PBS). Three weeks after injection we assessed joint diameters, histology, μCT scans, and differentiation of bone marrow- and synovia-derived osteoblasts.

Results: Knee diameters were increased in mBSA-injected wt mice compared to PBS-injected controls (3.21 ± 0.2 vs. 2.98 ± 0.1, $p < 0.05$, t-test), and this increase was not significant in Fas -/- mice (2.97 ± 0.2 vs. 2.87 ± 0.1). Histology revealed presence of synovial hyperplasia in both mBSA-injected groups, but mBSA-injected wt mice had decreased trabecular bone volume in distal femoral metaphyses (BV/TV) compared to controls (1.08 ± 0.57 vs. 2.55 ± 0.43; $p < 0.05$, t-test). There was no significant difference between mBSA-injected and control group in Fas -/- mice (2.34 ± 0.62 vs. 2.61 ± 0.65). μCT analysis showed that mBSA-injected wt mice had decreased BV/TV (2.99 ± 0.19 v. 1.96 ± 0.19; $p < 0.001$, t-test) and trabecular number (TbN) (1.03 ± 0.03 vs. 0.64 ± 0.02), as well as increased trabecular separation (TbSep) (256,89 ± 1395,12 vs. 312.40 ± 1323.91), compared to controls. mBSA injected Fas -/- mice had decreased TbN compared to controls (0.815 ± 0.01 vs. 0.64 ± 0.04; $p < 0.05$, t-test), with no significant difference in other trabecular parameters. Osteoblast differentiation was increased in both wt and Fas -/- mBSA-injected mice.

Conclusions: Our study demonstrated that Fas deficiency attenuated the development of clinical signs and bone loss in AIA. The mechanisms of this phenomenon need to be clarified.

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P39

Abnormal expressions of immune response-related genes in RA bone marrow cells

Hooi-Ming Lee[1*], Chieko Aoki[2], Yasunori Shimaoka[3], Kensuke Ochi[4], Takahiro Ochi[5], Norihiro Nishimoto[1,2]
[1]Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-Oka, Suita, Osaka 565-0871, Japan; [2]Laboratory of Immune Regulation, Wakayama Medical University, 105 Saito Bio Innovation Center, 7-7-20 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan; [3]Yukioka Hospital, 2-2-3 Ukita, Kita-ku, Osaka 530-0021, Japan; [4]Kawasaki Municipal Kawasaki Hospital, 12-1 Shinkawa-dori, Kawasaki-ku, Kawasaki, Kanagawa 210-0013, Japan; [5]Osaka Police Hospital, 10-31 Kitayama-chou, Tennoji-ku, Osaka 543-0035, Japan
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Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovitis that progresses to destruction of cartilage and bone. Bone marrow (BM) cells have been shown to contribute to this pathogenesis. In this study, we compared differentially expressed molecules in BM cells from RA and osteoarthritis (OA) patients and analyzed abnormal regulatory networks to identify the role of BM cells in RA.

Materials and methods: Gene expression profiles (GEPs) in BM-derived mononuclear cells from 9 RA and 10 OA patients were obtained by DNA microarray. Up- and down-regulated genes were identified by comparing the GEPs from the two patient groups. Bioinformatics was performed by Expression Analysis Systemic Explorer (EASE) 2.0 based on gene ontology, followed by network pathway analysis with Ingenuity Pathways Analysis (IPA) 7.5.

Results: The BM mononuclear cells showed 764 up-regulated and 1,910 down-regulated genes in RA patients relative to the OA group. EASE revealed that the gene category response to external stimulus, which included the gene category immune response, was overrepresented by the up-regulated genes. So too were the gene categories signal transduction and phosphate metabolism. Down-regulated genes were dominantly classified in three gene categories: cell proliferation, which included mitotic cell cycle, DNA replication and chromosome cycle, and DNA metabolism. Most genes in these categories overlapped with each other. IPA analysis showed that the up-regulated genes in immune response were highly relevant to the antigen presentation pathway and to interferon signaling. The major histocompatibility complex (MHC) class I molecules, HLA-E, HLA-F, and HLA-G, tapasin (TAP) and TAP binding protein, both of which are involved in peptide antigen binding and presentation via MHC class I molecules, are depicted in the immune response molecule networks. Interferon gamma and interleukin 8 were overexpressed and found to play central roles in these networks.

Conclusions: Abnormal regulatory networks in the immune response and cell cycle categories were identified in BM mononuclear cells from RA patients, indicating that the BM is pathologically involved in RA.

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Risk factors for latent tuberculosis infection in RA patients treated with anti-tumor necrosis factor

Shiang-Fen Huang[1*], Wei-Juin Su[1], Sheng-Yuan Ruan[2], Chong-Jen Yu[2], Song-Chou Hsieh[3], Yu-Chih Liu[4], Yeong-Jian Jan Wu[5], Hsiao-Yi Lin[6]
[1]Department of Chest Medicine, Taipei Veterans General Hospital, School of Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [3]Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [4]Department of Medicine, Division of Pulmonary Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan; [5]Department of Medicine, Division of Allergy, Immunology and Rheumatology, Chang Gung Memorial Hospital, Keelung, Taiwan; [6]Division of Allergy, Immunology and Rheumatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan
E-mail: syruan@ntu.edu.tw
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Background: To estimate the prevalence of latent tuberculosis (TB) infection according to the interferon-gamma release assay (IGRA, QuantiFERON®-TB Gold In-Tube, QFT) in patients with rheumatoid arthritis (RA), and assess the risk factors for incidence of active TB after TNF *alpha* blocking agents treatment.

Methods: A multicenter, prospective, and observational study was started in April, 2011 for patients with RA in Taiwan University Hospital, Taipei Veterans General Hospital, and Chang Gung Memorial Hospital in Keelung. Patients who take anti-TNFα regiments or not (defined as naïve or never take agent) were both enrolled in the study. The clinical history, DAS-28 score, chest film finding, sputum survey for active TB, and QFT screening results were collected.

Results: A total of 147 patients were enrolled in the study, in which five of them (3.4%) had history of anti-TB treatment and none had active TB at the beginning of the investigation. There were 75 patients undergoing anti-TNFα treatment before the study (42 patients (56%) took etanercepts and the other 33 (46%) ones took adalimumabs) and 72 patients had not (Table 1).

Based on QFT test, the frequency of latent TB infection (LTBI) were 12.5% (9/72) for naïve patients, and 10.7% (8/75) for biologics users ($p > 0.05$). Risk analysis showed no difference between different QFT results in study patients (Table 2).

The interval between starting etanercepts or adalimumabs treatment and screening for QFT test were 22.5 and 14.4 months ($p > 0.05$), respectively. Subgroup analysis showed possible risk factors for LTBI in patients who had history of adalimumabs or etanercept treatment were the history of anti-TB treatment and negative for BCG scar, respectively ($p < 0.05$). Other factors including DAS-28 score, presence of rheumatoid factor, white cell count, and previous immunosuppressant dosage (ie, prednisolone and methotrexate) were not related to the LTBI status (Table 3).

More patients had indeterminate QFT result after entracept treatment but negative QFT result after adalimumab therapy ($p<0.05$). In current study, none of patients with positive or indeterminate QFT result received preventive INH treatment and none of them had evidence of non-tuberculosis *mycobacterium* infection.

Conclusion: The overall frequency of LTBI in patients with RA was 11.6% in this study. Although history of anti-TB treatment and negative BCG scar were risk factors for LTBI, other factors still need to be considered due to limited sample size in current study. Further regular follow up should be done.

P41

TGF-β signaling induces SnoN to suppress BMP-induced hypertrophic maturation of chondrocytes

Shingo Maeda[1*], Ichiro Kawamura[1,2], Yasuhiro Ishidou[1], Katsuyuki Imamura[1,2], Masahiro Yokouchi[2], Setsuro Komiya[1,2]
[1]Department of Medical Joint Materials, Kagoshima University, Kagoshima, 890-8544, Japan; [2]Department of Orthopaedic Surgery, Kagoshima University, Kagoshima, 890-8544, Japan

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Background: Loss of TGF-β signaling in mice leads to promoted hypertrophic conversion of articular chondrocytes, which process is suggested to be linked to progression of osteoarthritis (OA). However, the molecular mechanisms by which TGF-β signaling inhibits chondrocyte maturation remain unclear. We screened for mediators downstream of TGF-β signaling to inhibit chondrocyte hypertrophy.

Materials and methods: We induced choncrocyte differentiation of ATDC5 cells with BMP-2. A TGF-β type I receptor inhibitor compound SB431542 was applied to inhibit endogenous TGF-β signaling. Expression of differentiation markers was evaluated by real-time RT-PCR and immunoblot. The function of SnoN was studied by stable overexpression and siRNA-knockdown approaches. Organ culture system using mouse embryo metatarsal bone was employed to study the roles of TGF-β signaling and SnoN in chondrocyte maturation.

Results: BMP-induced expression of *Col10a1* gene, a specific marker for hypertrophic chondrocytes, was further up-regulated dramatically, upon treatment with SB431542. In metatarsal bone organ culture, zone of calcified matured chondrocytes was expanded upon SB431542 application. Expression of *Id1* gene, the direct target of BMP Smads, was enhanced by SB431542, although the phosphorylation (activated status) of BMP-Smads-1/5/8 was not influenced by SB431542 application. Therefore, BMP signaling seemed to be blocked by TGF-β signaling at the level beneath the phosphorylation process of BMP-Smads. We evaluated expression profile of BMP signal-inhibitors, and found that *SnoN* was the only gene which expression was induced upon TGF-β treatment, while was inhibited by SB431542 application. Indeed, knockdown of SnoN resulted in enhanced hypertrophic maturation of ATDC5 cells, and overexpression of SnoN suppressed it. To evaluate *in vivo* contribution of SnoN in cartilage cell hypertrophy, we studied expression of SnoN protein by immunohistochemistry. In mouse growth plate, SnoN was present only in prehypertrophic chondrocytes, but excluded from hypertrophic zone. In human OA specimens, SnoN was positive around ectopic hypertrophic chondrocytes of moderate OA cartilages, whereas SnoN was not detected in severe-graded OA cartilages. These data support the idea that SnoN inhibits hypertrophic conversion of chondrocytes *in vivo*, as well as *in vitro*.

Conclusions: Our results suggest that SnoN suppresses hypertrophic transition of chondrocytes, as a mediator of TGF-β signaling, to prevent the progression of OA.

P42

Activation of TRPV4 promotes osteoclasts differentiation

Ritsuko Masuyama
Department of Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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Osteoclast differentiation is critically dependent on cellular calcium (Ca^{2+}) signaling. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is regulated by two flux pathways; Ca^{2+} oscillations evoked by the release of Ca^{2+} from the endoplasmic reticulum, and/or Ca^{2+} entry from the extracellular fluid. The latter is carried out by the plasmamembrane localized Ca^{2+} permeable channel such as "transient receptor potentials (Trps)". Trpv4-deficient mice show an increased bone mass due to impaired osteoclast maturation, because Trpv4 mediates Ca^{2+} influx at the late stage of osteoclast differentiation and hereby regulates Ca^{2+} signaling [1]. Furthermore, substitutions of amino acids R616Q/V620I of Trpv4 have been discovered as gain of function mutations resulting in increased Ca^{2+} transport [2]. Since the region of these substitutions at the trans-membrane pore domain is perfectly conserved between species, we created a mutant of the mouse Trpv4 ($Trpv4^{R616Q/V620I}$) and characterized it on Ca^{2+} signaling especially in the occurrences of oscillations at the initial step of osteoclast differentiation. Intact Trpv4 and $Trpv4^{R616Q/V620I}$ were equally transduced by retroviral infection into bone marrow derived hematopoietic cells isolated from WT mice, and mock-transfection was used as control. The resorptive activity was significantly increased in $Trpv4^{R616Q/V620I}$-expressing osteoclasts when treated with RANKL for 7 days, associating increased NFATc1 and calcitonin receptor mRNA expression. Noteworthy, the expression of these differentiation markers was already elevated in $Trpv4^{R616Q/V620I}$ cells before RANKL treatment, suggesting that the activation of Trpv4 advances osteoclast differentiation through Ca^{2+}-NFATc1 pathway. Accordingly, basal $[Ca^{2+}]_i$, analyzed in progenitor cells treated with RANKL for 24 hr, increased 2 fold in intact Trpv4 ($p < 0.05$) and 3 fold in $Trpv4^{R616Q/V620I}$ ($p < 0.01$) compared to controls. Although spontaneous Ca^{2+} oscillations were absent in control progenitor cells, $Trpv4^{R616Q/V620I}$ progenitor cells already displayed irregular oscillatory pattern.

In summary, our findings provide evidences that the activation of Ca^{2+} permeable channel supports Ca^{2+} oscillations in progenitor cells and therefore promotes the potential of osteoclast differentiation.

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P43

STAT3 is critical to promote inflammatory cytokines and RANKL expression in inflammatory arthritis

Takeshi Miyamoto[1*], Tomoaki Mori[1], Akihiko Yoshimura[2], Toshiaki Toyama[1]
[1]Department of Orthopedic Surgery, Keio University School of Medicine, Shinjuku, Tokyo, 160-8582, Japan; [2]Department of Immunology, Keio University School of Medicine, Shinjuku, Tokyo, 160-8582, Japan

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Rheumatoid arthritis (RA) causes sever joint damage and significant disability of daily living. The symptoms of RA patients are mainly from chronic inflammation and continuous joint destruction, however, the mechanisms underlying how inflammation and joint destruction in RA develop and are sustained chronically remain largely unclear. In this study, we show that signal transducer and activator of transcription 3 (STAT3) plays a critical role in both chronic inflammation and joint destruction in RA. We found that inflammatory cytokines, such as IL-1β, TNFα and IL-6, activated STAT3 either directly or indirectly and induced expression of inflammatory cytokines, further activating STAT3. STAT3 activation also induced expression of receptor activator of nuclear factor kappa B ligand (RANKL), an essential cytokine for osteoclast differentiation. STAT3 knockout or pharmacological inhibition resulted in significant reduction of the expression of both inflammatory cytokines and RANKL *in vitro*. STAT3 inhibition was also effective in treating an RA model, collagen induced arthritis (CIA), *in vivo* through significant reduction in expression of inflammatory cytokines and RANKL, inhibiting both inflammation and joint destruction. Thus our data provide new insight into pathogenesis of RA and provide evidence that inflammatory cytokines induce a cytokine amplification loop via STAT3 that promotes sustained inflammation and joint destruction.

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Combined depletion of interleukin-1 and interleukin-6 does not exceed single depletion of interleukin -1 in TNF-mediated arthritis

Silvia Hayer*, B Niederreiter, J Smolen, K Redlich
Department of Internal Medicine III, Division of Rheumatology, Medical University of Vienna, Vienna, Austria
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Background: Previous studies demonstrated a regulatory role of interleukin 1 (IL-1) in inflammatory cartilage damage and bone destruction in human tumor necrosis factor transgenic (hTNFtg) mice, an animal model for Rheumatoid Arthritis (RA). Moreover, blocking of IL-6 has been shown to reduce local bone erosions in this model. Therefore we wanted to investigate the effect of a combined depletion of IL-1 and IL-6 on the development and severity of inflammatory, erosive arthritis.
Methods: We first crossed IL1α and ß deficient (IL1-/-) mice with IL6-/- mice to generate IL1-/-IL6-/- double knockout mice. We next intercrossed these animals with arthritogenic hTNFtg mice to receive IL1-/-IL6-/-hTNFtg mice. We weekly assessed clinical signs of arthritis in hTNFtg, IL1-/-hTNFtg mice, IL6-/-hTNFtg mice and IL1-/-IL6-/-hTNFtg mice starting from week 4 after birth until week 16. We stained decalcified paw sections from all 4 genotypes with hematoxylin&eosin to determine the amount of inflammatory synovial pannus formation, with tartrate-resistant acid phosphatase (TRAP) to evaluate the number of synovial osteoclasts and the occurrence of subchondral bone erosions, with toluidine-blue to assess articular cartilage damage. Quantitative analysis of histopathological changes were performed using the Osteomeasure Software System.
Results: We found a significant reduction in the clinical signs of arthritis, indicated by an increase of paw swelling and a decrease in grip strength, in IL1-/-IL6-/-hTNFtg mice when compared to their hTNFtg littermates. In line with these findings we observed a significant decrease in synovial inflammation in IL1-/-IL6-/-hTNFtg mice when compared to hTNFtg animals. Moreover, the number of synovial TRAP+ osteoclasts was markedly diminished in IL1-/-IL6-/-hTNFtg mice and reduced osteoclast formation, was accompanied by significantly less subchondral bone erosions. Additionally, we found a conserved articular cartilage structure showing almost no cartilage degradation in IL1-/-IL6-/-hTNFtg mice compared to their hTNFtg littermates. In IL1-/-IL6-/-hTNFtg mice clinical, as well as, histological signs of disease, including joint inflammation, bone destruction and cartilage damage were also significantly diminished when compared to IL6-/-hTNFtg mice. However, by comparing IL1-/-IL6-/-hTNFtg mice with IL1-/- hTNFtg mice we found a similar reduction on synovial inflammation, as well as subchondral bone erosions and articular cartilage destruction.
Conclusion: The phenotype of IL1-/-IL6-/-hTNFtg mice does not differ from IL1-/-hTNFtg animals indicating no synergistic effects when IL-1 and IL-6 is simultaneously blocked in TNF-mediated arthritis.

P45

The functions of the post-translational modifications in rheumatoid arthritis

Satoko Aratani[1*], Naoko Yagishita[2], Teruhisa Kanazawa[1], Fukami Nakajima[1], Yoshihisa Yamano[2], Kusuki Nishioka[1], Toshihiro Nakajima[1]
[1]Institute of Medical Science, Tokyo Medical University, Shinjuku-ku, Tokyo, 160-8402, Japan; [2]Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki, Kanagawa, 216-8512, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P45

Rheumatoid Arthritis (RA) is a chronic inflammatory joint disease and characterized by synovial hyperplasia. We previously cloned an E3 ubiquitin ligase, Synoviolin, as a regulatory factor of cell proliferation [1]. It suggested that endoplasmic reticulum (ER) associated degradation system (ERAD) via Synoviolin has important roles for overgrowth of synoviocytes [2,3]. Meanwhile, it is known that autoantibodies to citrullinated proteins are specific for RA and good markers for RA. Peptidyl-Arginine Deiminases 4 (PADI4) is identified as the RA-susceptible gene [3]. However functions of citrulinated proteins are unclear. In this study, we hypothesize that the accumulation of citrullinated proteins in RA synoviocytes could associate for ER stress and explore the crosstalk of ubiquitination and citrullination.

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Neutrophils are the source of IL-17 and RANKL in zymosan induced arthritis

Viktoriya I Milanova*, Nina D Ivanovska, Petya A Dimitrova
Department of Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, 1113, Bulgaria
Arthritis Research & Therapy 2012, **14(Suppl 1):**P46

Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease affecting cartilage and bone. Recently, much attention on the role of neutrophils in the pathology of RA has been paid. However, the capability of RA neutrophils from periphery and bone marrow (BM) to produce cytokines like IL-17 and IFN-γ has not been well understood. Our aim is to analyze neutrophil distribution in BM, blood and synovium and to elucidate IL-17, IL-4 and IFN-γ production and surface expression of RANKL on peripheral and synovial neutrophils during the progression of zymosan-induced arthritis (ZIA).
Materials and methods: In the present study BALB/c and SCID mice were injected intra-articularly with zymosan. Cells from BM, periphery and synovium were collected at day 7 and day 30 of ZIA and the frequencies of $Ly6G^+CD11b^+$ neutrophils and surface expression of RANKL and CD69 on them were evaluated by flow cytometry. In some experiments peripheral neutrophils were isolated at day 7 of ZIA, re-stimulated in vitro with zymosan in the presence or the absence of IL-17, then fixed, permeabilized and used for flow cytometry analyses of IL-17, IL-4 and IFN-γ intracellular levels and of surface RANKL expression. Apoptosis of cultured neutrophils was detected by annexin/propidium iodide kit. The ability of peripheral neutrophils to affect RANKL or IL-17-induced osteoclast differention of bone marrow precursors in vitro was evaluated after TRAP staining of cell co-cultures.
Results: The development of inflammatory process in SCID mice after zymosan injection was related to increased frequencies of $Ly6G^+CD11b^+$ neutrophils in periphery and synovium along with elevated IL-17 production in plasma and serum. We observed that arthritic neutrophils collected at day 7 of disease have higher IL-17, IL-4 and IFN-γ intracellular levels than healthy cells. Exogenous IL-17 increased the cytokine and RANKL expression on healthy and arthritic neutrophils in vitro. While neutrophils were able to inhibit RANKL-induced osteoclast differentiation, they increased the number of TRAP positive mature osteoclasts in the presence of IL-17.
Conclusions: We suggest that $Ly6G^+CD11b^+$ peripheral neutrophils that are positive for IL-17, IL-4, IFN-γ and RANKL can migrate to the synovium where they can affect inflammatory and destructive processes. Our study displays new aspect of the role of neutrophils in the pathology of RA and provides diverse ground for the development of novel therapeutic strategies.

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Role of HLA-antigens class 1 in the development of rheumatoid arthritis in Uzbek women

Nazima N Mirakhmedova[1*], Mavlyuda I Mirzakhanova[2]
[1]Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan; [2]Tashkent Medical Academy, Tashkent, Uzbekistan
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According to the multiple studies women suffer from rheumatoid arthritis (RA) three times more often than men. The women seem to be ill at the age of more active working activity that results in early disability. The great attention is paid to the hereditary factors, particularly, to HLA-system, in the RA development. In this connection the question about early diagnosis and primary prevention of rheumatoid arthritis remain to be important. Consequently, we studied distribution of HLA I class antigens (A, B, C) in 86 Uzbek women with RA. HLA were identified with 2 step standard microlymphocytotoxicity test using antileucocyte HLA-antisera (St-Petersburg, Russia) and rabbit complement. Control group consist of 301 healthy random Uzbeks. In current study 39 antigens were expressed. Higher frequency was found for A25 (15.1% vs. 0.7% in control), A28 (22.1% vs. 4.9%, respectively) with $p < 0.001$. Antigen A19 (3.5% vs. 11.9% in control, $p < 0.01$).

In HLA-A locus (17.4% vs. 4.9% in control); B18 were met in 9.3% vs. 3.7% in control, ($p > 0.05$); B22 (10.5% vs. 1.3% in control, $p > 0.05$); B27 (15.1% vs. 8.9% in control, $p > 0.05$).

Cw4 (12.8% vs. 36.2% in control, $p > 0.05$) met reliably more rare in HLA-A locus.

The highest indicator of risk was established for A25 (RR = 26.6), then for B22 (RR = 8.7), B16 (RR = 4.0), B27 (RR = 2.8), B18 and A10 (RR = 2.7). Results showed that antigens A25 and A28 ($p < 0.001$), have major effect, while the B16, B18, B22, B27 - additive contribution to the predisposition to the RA among Uzbek women.

Analysis of results in different clinical RA forms revealed association of slowly progressing articular form with antigens: A25 ($p < 0.001$, RR = 25.2); A28 ($p < 0.01$, RR = 6.7); whether A10, B16, B27, B22 were not significant ($p > 0.05$). Fast progressing articular-visceral form development was associated with HLA-A28, A25, B16, B27, and significance of association was established only for A28 ($p < 0.001$, RR = 7.6). The important moment in our investigation seems to be the association of RA showed unfavorable development in Uzbek women with antigens HLA-B16 which is a split of antigen B8 and antigen B27, being marker of rheumatoid diseases, that correlates with identical research in different populations.

Thus, the results of our investigation show important contribution of HLA in predisposition to rheumatoid arthritis in Uzbek women.

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SNP algorithms for prediction of efficacy and adverse events of abatacept (ABT)

James E Middleton[1*], Tsukasa Matsubara[1,2], Keiko Funahashi[1,2], Satoru Koyano[1], Takafumi Hagiwara[2], Takako Miura[2], Kosuke Okuda[2], Takeshi Nakamura[2], Mitsuyoshi Iwahashi[3], Tomomi Tsuru[4], Shoichi Uchimura[5], Shigeru Honjo[6]
[1]Research Institute of Joint Diseases, Kobe, Japan; [2]Matsubara Mayflower Hospital, Kato, Japan; [3]Higashi-Hiroshima Memorial Hospital, Higashi-Hiroshima, Japan; [4]PS Clinic, Fukuoka, Japan; [5]Kanzaki Municipal General Hospital, Japan; [6]Honjo Rheumatism Clinic, Japan
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Background: Abatacept (ABT), a CTLA4-Ig fusion protein, which inhibits the binding of CD28 and CD80 agents targeted to T-cells, is a relatively new biological agent for RA treatment in Japan. However, there is no method for prediction of responders, non-responders, or adverse events which can occur during treatment. We established SNP algorithms for prediction of responders (R) or non-responders (NR), and adverse events in ABT-treated patients.

Materials and methods: Forty-six RA patients treated with ABT were included in this study. Efficacy was assessed by DAS28 (CRP) at 48 weeks after the initial treatment. Any adverse events that may have been related to ABT administration and observed at 48 weeks of this long-term administration and during phase II were considered to be side effects. Genome-wide SNP genotyping was performed by Illumina Human610-Quad chip technology. Case-control analyses between 598,821 SNPs and responsiveness or occurrence of adverse events were examined by Fisher's exact test. We selected 10 SNPs associated with ABT-responsiveness, remission, and adverse events ($p < 0.0001$). We scored the relationship between each SNP and responsiveness, the estimated total score of 10 SNPs (estimated scoring in each SNP was as follows: homo allele in the majority in responders: +1 point, hetero allele: 0 points, and homo allele in the majority of non-responders: -1 point), and then examined relationships between responders and non-responders, remission and non-remission, and occurrence of adverse events, plus or minus, and the total score.

Results: Accuracy, specificity, and sensitivity of the algorithm for responsiveness of abatacept ranged from 90-96%. For remission, accuracy, specificity and sensitivity of the algorithm ranged from 91-97%. For adverse events, accuracy, specificity and sensitivity of the algorithm ranged from 95-100%. It is therefore suggested that the SNP algorithms can predict responders and adverse events prior to the initiation of treatment with abatacept.

Conclusions: These highly accurate algorithms using SNP analysis may be useful in the prediction of responsiveness and adverse events before treatment with abatacept, and in this way can contribute to future tailor-made treatment with biologic agents.

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Maintenance of mitochondrial DNA copy number is essential for osteoclast survival

Tsuyoshi Miyazaki[1*], Shuuichi Mori[1], Kazuhiro Shigemoto[1], Nils-Goran Larsson[2], Takeshi Nakamura[3], Shigekaki Kato[3], Tomoki Nakashima[4], Hiroshi Takayanagi[4], Sakae Tanaka[5]
[1]Department of Geriatric Medicine, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo 173-0015, Japan; [2]Division of Metabolic Diseases, Karolinska Institute, Stockholm, Sweden; [3]Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032, Japan; [4]Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo 113-8549, Japan; [5]Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo 113-0033, Japan
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Background: There is accumulating evidence that osteoclasts, the primary cells responsible for bone resorption, are involved in bone and joint destruction in rheumatoid arthritis. Bone resorption is highly regulated by mature osteoclast function as well as osteoclastogenesis. The life span of mature osteoclasts is relatively short both *in vitro* and *in vivo*, and once differentiated, they rapidly die in the absence of supporting cell or growth factors. Mitochondria is known as powerhouse of cell because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell growth, and cell death. Transcription and replication of mitochondrial DNA (mtDNA) are important steps in mitochondrial biogenesis and mitochondrial transcription factor A (Tfam) is essential for mtDNA transcription and replication. However, the functional significance of mitochondria has not been established in osteoclastic bone resorption.

Materials and methods: To address this question, we generated osteoclast-specific Tfam conditional knock-out (cKO) mice by mating $Tfam^{fl/fl}$ mice with cathepsin K-Cre transgenic mice, in which the Cre recombinase gene is knocked into the cathepsin K locus and specifically expressed in mature osteoclasts. The in vivo effects of Tfam deficiency on bone metabolism were examined by histological and histomorphometric analysis. The survival and bone-resorbing activity of Tfam cKO osteoclasts were determined by in vitro survival assay and pit formation assay, respectively.

Results: The expression level of Tfam, mtDNA copy number, and cellular ATP level were markedly reduced in osteoclasts derived from Tfam cKO mice. The body size of Tfam cKO mice was smaller than that of the control mice, although trabecular bone volume remained unchanged by Tfam deficiency. However, histological sections of proximal tibia and lumbar spine of Tfam cKO mice showed significantly decreased osteoclast number. Interestingly, Tfam cKO osteoclasts exhibited increased bone-resorbing activity in spite of their pro-apoptotic tendency.

Conclusions: This study demonstrates that Tfam cKO osteoclasts exhibited increased bone resorption with accelerated apoptosis,

indicating that there may be an inverse correlation between osteoclast survival vs bone resorption. Further investigation of mitochondria in bone-resorbing osteoclasts will give us new insights into the molecular mechanism regulating bone homeostasis.

P50

Over expression of toll-like receptors in peripheral blood and synovial fluid monocytes of enthesitis related arthritis category of juvenile idiopathic arthritis (JIA-ERA) patients contributes to secretion of inflammatory mediators

Arpita Myles*, Amita Aggarwal
Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences Lucknow-226014, India
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Background: TLRs 2, 4 and 9 have been implicated in murine models and human patients of arthritis, but the other TLRs are not well-investigated. Thus, we studied TLR expression and signaling and effect of TLR ligand stimulation in peripheral blood (PB) and synovial fluid (SF) monocytes (MC) of ERA patients.
Methods: Levels of TLR2, TLR4 and TLR9 were measured by flow cytometry in ERA PBMC (n = 26), paired SFMC (n = 13) and healthy PBMC (n = 19) Real time PCR was done for TLRs 1-9 and their adaptors IRAK1, IRAK4, TRIF, TRAF3, TRAF6. PBMC and SFMC were stimulated with ligands for TLR1 (pam3-cys), 2 (peptidoglycan), 3 (polyI:C), 4 (LPS), 5 (flagellin) and 6 (zymosan). Levels of IL-6, IL-8 and MMP3 (ng/ml) were measured in the culture supernatants.
Results: ERA PBMC had higher MFI of TLR2 [295.5(48.1-598) vs 179(68.7-442); $p < 0.05$] and TLR4 [448(178-2581) vs 402(229-569); $p < 0.05$] compared to controls. Intracellular TLR9 expression showed no significant difference between both groups. In paired samples, SFMC had higher MFI of both TLR2 [485(141-1683) vs 353(180-598); $p < 0.05$] and TLR4 [1016 (42.4-3159) vs 513(193-2581); $p < 0.05$] compared to PBMC. Difference in TLR9 expression was not significant [1]. Patient PBMC (compared to healthy control) and SFMC (compared to corresponding PBMC) had higher RNA expression of TLRs1, 2, 3, 4, 5 and 6 and downstream adaptors. Patients PBMC produced significantly higher IL-6 (13.51 vs 6.54) and MMP3 (61 vs 32.9) as compared to controls on stimulation by LPS. With peptidoglycan also IL-6 (30.58 vs 10.84) and MMP-3 (102.54 vs 49.45) was higher than controls. Patient PBMCs produced more IL-6 and IL-8 compared to healthy PBMCs on stimulation with Pam3-cys, poly I:C, flagellin and zymosan. In paired samples, SFMCs showed a trend towards higher IL-6 and IL-8 production compared to PBMCs (Table 1).
Conclusion: Increased TLR expression and signaling on PBMC and SFMC from JIA-ERA patients may exacerbate disease by upregulating IL-6, IL-8 and MMP-3 in response to microbial/ endogenous ligands. TLR pathway is a potential therapeutic target in these patients.

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P51

Pilocarpine suppresses hyperalgesia induced by intermittent cold stress (ICS) as an experimental fibromyalgia model in mice

Jun Nagai*, Michiko Nishiyori, Hiroshi Ueda
Division of Molecular Pharmacology and Neurosciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8521, Japan
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Fibromyalgia (FM) is a highly populated chronic pain disease, which has unique characteristics including generalized or widespread allodynia and female prevalence of gender difference. Many FM patients are common with Sjögren's syndrome. Pilocarpine, a non-selective muscarinic receptor agonist, is used clinically as a drug that promptes the secretion of salvia for dry eyes and mouth. Otherwise, pilocarpine has been shown to possess antinociceptive effect, which maybe caused by vagal afferents activation. The experimental FM mice exposed to intermittent cold stress (ICS) showed sustained abnormal pain, such as mechanical allodynia and hyperalgesia to nociceptive thermal stimuli for up to 19 days, but those given constant cold stress (CCS) did not. The abnormal pain was bilateral (generalized), female-predominant and specific for A-delta and A-beta, but not C-fiber-stimuli. In ICS mice, intraperitoneal or oral administration of pilocarpine showed potent anti-hyperalgesic effects in doses without excess salivation at post-stress day5 (P5). The anti-hyperagesic effects last for more than 1 h, but disappear at 24 h. Daily administration of pilocarpine showed equivalent anti-hyperalgesic effects without tolerance. These findings suggest that pilocarpine possesses a beneficial effect for the pain treatment of FM patients with dry eyes and mouth symptoms.

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P52

Application of tetraspanin CD81 RNAi for diagnosis and therapy of rheumatoid arthritis (RA)

Tohru Nakanishi[1*], Yuji Arai[2], Hiroki Mori[1], Toshihiro Nakajima[3], Toshikazu Kubo[2]
[1]Shujitsu University School of Pharmacy, Okayama, 703-8516 Japan; [2]Department of Orthopaedics, Kyoto Prefectural University of Medicine, Kyoto, 602-8566 Japan; [3]Institute of Medical Science, Tokyo Medical University, Shinjuku, 160-8402 Japan
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Table 1(abstract P50) Production of IL-6 and IL-8 [median (range) ng/ml] by PBMCs and SFMCs upon TLR ligand stimulation

Cultured with	IL-6			IL-8		
	Normal PBMC (n = 5)	ERA PBMC (n = 7)	ERA SFMC (n = 3)	Normal PBMC (n = 5)	ERA PBMC (n = 7)	ERA SFMC (n = 3)
Medium	4.4 (1.5-5.4)	7.6 (3-16.6) *	18 (9.3-24.2)	10 (4.3-12.6)	12.6 (8.1-35.7)	10 (4.3-12.6)
TNF	13.6 (9.6-14.8)	16 (12-35)	21(18-30)	30.8 (15.8-36.3)	34.4 (30.8-46.1)	31 (15.8-36.3)
Pam3cys	15.1 (13.3-19.6)	44 (26-62) **	53 (28-71)	37.1 (11.4-41.8)	106 (42.6-147.6) **	37.1 (11.4-42)
PolyI:C	13.1 (1.3-25.8)	28 (24-4) *	46 (45-65)	33.6 (31.1-56.3)	126(78-167) *	34(31.1-56.3)
Flagellin	14.1 (6.7-23.5)	34.9 (15-39.2) *	52.6 (40-53.8)#	35.2 (16.1-84.2)	115 (73-162) **	35 (16.1-84.2)
Zymosan	14.7 (8.8-36.3)	34 (28.4-39) *	48 (39.8-56.1)#	56 (12.6-89.6)	106 (103-163) **	56 (12.6-90)

$p < 0.05$** 0.01 ERA PBMC versus control PBMC $p <$#0.05 ERA SFMC versus ERA PBMC.

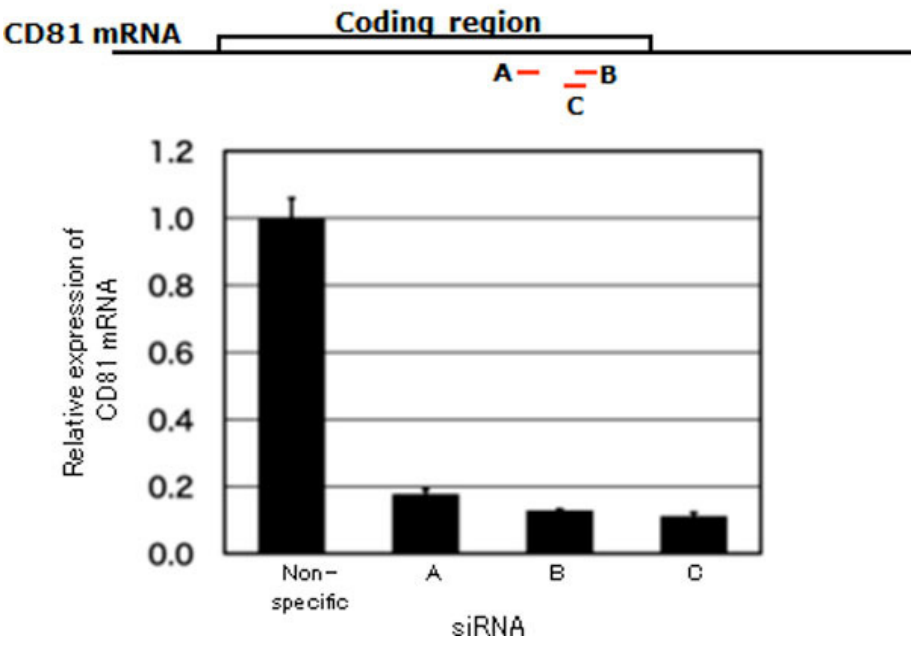

Figure 1(abstract P52) .

CD81 belomgs to a family of cell-surface protein (tetraspanin) which has four transmembrane domains and two outer-membrane loops.Under the DNA chip analysis, we found several genes highly expressed in rheumatoid arthritis (RA) synoviocytes comparing with the expression in OA or normal synoviocytes. Among these genes, tetraspanin CD81 was shown to be involved in the progression of RA through the promotion of Synoviolin expression.Synoviolin is already known as one of the important progressive elements of RA in synoviocytes. We also showed Synoviolin and CD81 highly distributed in RA tissues.

The therapeutic effect of small interfering RNA targeting CD81 (siCD81) was examined by *in vivo* electroporation method. Treatment with siCD81 significantly ameliorated paw swelling of collagen-induced arthritic (CIA) rats. In histological examination, hypertrophy of synovium, bone erosion, and degeneration of articular cartilage were minder in rats treated with siCD81 than in the control group and the non-specific siRNA group. Expression of synoviolin, a rheumatoid regulator, was also suppressed by siCD81 [1]. These results showed that siCD81 would become effective tools for treatment of RA. In addition, siCD81 reduced the amount of CD81 in synovial fluid indicating that quantitative analysis of CD81 opens up the novel and highly sensitive diagnosis for RA.

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P53

The crucial role of osteocyte-derived RANKL in bone homeostasis

Tomoki Nakashima[1,2*], Mikihito Hayashi[1,2], Hiroshi Takayanagi[1,2]
[1]Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima 1-5-45, Tokyo, Japan; [2]Japan Science and Technology Agency (JST), ERATO, Takayanagi Osteonetwork Project, Yushima 1-5-45, Tokyo, Japan

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Receptor activator of NF-κB ligand (RANKL; also known as TNFSF11), a TNF family molecule, and its receptor RANK (TNFRSF11A) are key regulators of osteoclast differentiation and function. Aberrant expression of RANKL explains why autoimmune diseases, cancers, leukemia and periodontal disease result in systemic and local bone loss. In particular, RANKL is the pathogenic factor that cause bone and cartilage destruction in arthritis. Inhibition of RANKL function by the natural decoy receptor osteoprotegerin (OPG; also known as TNFRSF11B) or anti-RANKL antibody prevents bone loss in postmenopausal osteoporosis, cancer metastases and arthritis. RANKL also regulates T cell/dendritic cell communications, dendritic cell survival and lymph node organogenesis. Intriguingly, RANKL and RANK play an essential role in the maturation of mammary glands in pregnancy and lactation. Bone homeostasis depends on the coordination of osteoclastic bone resorption and osteoblastic bone formation. We reported that RANKL induces osteoclast differentiation through activating a transcriptional programme mediated by the master transcription factor nuclear factor of activated T cells (NFAT) c1. Although it is well accepted that the RANKL-NFATc1 pathway is crucially important for osteoclast differentiation, little is known about the major cellular source of RANKL in the skeletal tissue. RANKL has been postulated to be mainly expressed by osteoblasts and bone marrow stromal cells. However, here we show that osteocytes embedded within the bone matrix are the critical source of RANKL in bone remodeling. Osteocytes, the most abundant cell type in bone, are thought to orchestrate bone homeostasis by regulating both osteoclastic bone resorption and osteoblastic bone formation, but *in vivo* evidence and the molecular basis for the regulation has not been sufficiently demonstrated. Using a newly established method for the isolation of high-purity dentin matrix protein 1-positive osteocytes from bone, we have found that osteocytes express a much higher amount of RANKL and have a much greater capacity to support osteoclast formation than osteoblasts and bone marrow stromal cells. The crucial role of RANKL expressed by osteocytes was validated by the severe osteopetrotic phenotype observed in mice lacking RANKL specifically in osteocytes. Thus, we provide *in vivo* evidence for the key role of osteocyte-derived RANKL in bone homeostasis, establishing a molecular basis for osteocyte regulation of bone resorption.

P54

Active repression by Blimp1 play an important role in osteoclast differentiation

Keizo Nishikawa[1*], Tomoki Nakashima[2,3,4], Mikihito Hayashi[2,3,4], Takanobu Fukunaga[2,3,4], Shigeaki Kato[5,6], Tatsuhiko Kodama[7], Satoru Takahashi[8], Kathryn Calame[9], Hiroshi Takayanagi[2,3,4]
[1]Laboratory of Cellular Dynamics Immunology Frontier Research Center, Osaka University, Yamada-oka 3-1, Suita, Osaka 565-0871, Japan; [2]Department of Cell Signaling, Graduate School, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8549, Japan; [3]Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Japan; [4]Japan Science and Technology Agency, ERATO, TakayanagiOsteonetwork Project, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8549, Japan; [5]Institute of Molecular and Cellular Biosciences, Graduate School of Medicine, University of Tokyo, Tokyo 113-0032, Japan; [6]Japan Science and Technology Agency, ERATO, Kato Nuclear Complex, Saitama 332-0012, Japan; [7]Department of Molecular Biology and Medicine, Research Center for Advanced Science and Technology, University of Tokyo, Komaba 4-6-1, Meguro-ku, Tokyo 153-8904, Japan; [8]Institute of Basic Medical Sciences and Laboratory Animal Resource Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8575, Japan; [9]Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
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Regulation of irreversible cell lineage commitment depends on a delicate balance between positive and negative regulators, which comprise a sophisticated network of transcription factors. Receptor activator of nuclear factor-κB ligand (RANKL) stimulates the differentiation of bone-resorbing osteoclasts through the induction of nuclear factor of activated T-cells c1 (NFATc1), the essential transcription factor for osteoclastogenesis. Osteoclast-specific robust induction of NFATc1 is achieved through an autoamplification mechanism, in which NFATc1 is constantly activated by calcium signaling while the negative regulators of NFATc1 are being suppressed. However, it has been unclear how such negative regulators are repressed during osteoclastogenesis. Here we show that B lymphocyte-induced maturation protein-1 (Blimp1; encoded by *Prdm1*), which is induced by RANKL through NFATc1 during osteoclastogenesis, functions as a transcriptional repressor of anti-osteoclastogenic genes such as *Irf8* and *Mafb*. Overexpression of Blimp1 leads to an increase in osteoclast formation and *Prdm1*-deficient osteoclast precursor cells do not undergo osteoclast differentiation efficiently. The importance of Blimp1 in bone homeostasis is underscored by the observation that mice with an osteoclast-specific deficiency in the *Prdm1* gene exhibit a high bone mass phenotype owing to a decreased number of osteoclasts. Thus, NFATc1 choreographs the cell fate determination of the osteoclast lineage by inducing the repression of negative regulators as well as its effect on positive regulators.

P55

Tks5-dependent formation of circumferential podosomes mediates cell-cell fusion

Tsukasa Oikawa[1*], Masaaki Oyama[2], Hiroko Kozuka-Hata[2], Shunsuke Uehara[3], Nobuyuki Udagawa[3], Hideyuki Saya[4,5], Koichi Matsuo[1]
[1]Laboratory of Cell and Tissue Biology, Institute for Integral Medical Research, School of Medicine, Keio University, Shinanomachi 35, Shinjuku-ku, Tokyo 160-8582, Japan; [2]Medical Proteomics Laboratory, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan; [3]Department of Biochemistry, Matsumoto Dental University, 1780 Gobara, Hiro-oka, Shiojiri, Nagano 399-0781, Japan; [4]Division of Gene Regulation, Institute for Advanced Medical Research, School of Medicine, Keio University, Shinanomachi 35, Shinjuku-ku, Tokyo 160-8582, Japan; [5]CREST, Japan Science and Technology Agency, Tokyo, Japan
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Multinucleation of osteoclasts during osteoclastogenesis requires dynamic rearrangement of the plasma membrane and cytoskeleton, and this process involves numerous previously characterized factors. However, the mechanism underlying osteoclast fusion remains obscure. Live-imaging analysis of osteoclastogenesis revealed that the products of PI3-kinase are enriched at the sites of osteoclast fusion. Among the downstream molecules whose expression was screened, the expression of Tks5, an adaptor protein with the phox homology (PX) domain with multiple Src homology 3 domains, was induced during osteoclastogenesis. Tks5 was localized in the podosomes and fusing membranes of osteoclasts, and reducing its expression impaired both formation of circumferential podosomes and osteoclast fusion without altering osteoclast differentiation. In addition, the expression of a deletion mutant of the PX domain abrogated circumferential podosome formation as well as osteoclast fusion, suggesting that Tks5-dependent circumferential podosomes function as fusion machinery during osteoclastogenesis. As Tks5 is known to promote the formation of podosomes/invadopodia in transformed/cancer cells, we tested if these cells also have the potential to fuse with osteoclasts. Among the cells tested, B16F0 melanoma cells formed circumferential podosomes with Tks5 accumulation in the presence of RANKL, TGFβ and TNFα. Co-culture of B16F0 melanoma cells with osteoclasts in an inflammatory milieu promoted increased formation of melanoma-osteoclast hybrid cells. Our results revealed a previously unknown mechanism of regulation of both circumferential podosome formation and cell-cell fusion by Tks5.

P56

An essential role of IκBζ in the transcriptional program in Th17 development

Kazuo Okamoto[*], Masatsugu Oh-hora, Hiroshi Takayanagi
Department of Cell Signaling, Tokyo Medical and Dental University, Tokyo, Japan. [2]GCOE Program, Tokyo, Japan. [3]JST, ERATO, Takayanagi Osteonetwork Project, Tokyo, Japan
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IL-17-producing helper T (Th17) cells are a distinct T cell subset characterized by its pathological role in autoimmune diseases. Our group previously showed that Th17 cells function as osteoclastogenic helper T cells in bone destruction associated with inflammation, and that inhibition of Th17 development has the potential of a beneficial impact on bone diseases including rheumatoid arthritis (RA) [1]. It is therefore important to comprehend the molecular mechanism underlying Th17 development in order to develop ideal therapeutic strategies against RA.
IL-6 and TGF-β induce Th17 development, in which the orphan nuclear receptors RORγt and RORα play an indispensable role. We found that the expression of a nuclear IκB family member, IκBζ (encoded by the *Nfkbiz* gene), was upregulated by the combination of IL-6 and TGF-β, but independently of RORγt [2]. Not only *Nfkbiz*$^{-/-}$ mice but also *Rag2*$^{-/-}$ mice transferred with *Nfkbiz*$^{-/-}$ CD4^{+} T cells were highly resistant to experimental autoimmune encephalomyelitis, which is a mouse model of multiple sclerosis. *Nfkbiz*$^{-/-}$ mice were also protected from the activation of osteoclastogenesis and bone destruction in a LPS-induced model of inflammatory bone destruction. When activated *in vitro* under Th17-polarizing conditions, IL-17 production in *Nfkbiz*$^{-/-}$ T cells was markedly reduced compared to WT cells. Notably, the expression of RORγt and RORα was comparable between WT and *Nfkbiz*$^{-/-}$ T cells. Thus, it is unlikely that ROR nuclear receptors function downstream of IκBζ or vice versa.
In the absence of IL-6 and TGF-β, neither the ROR nuclear receptors nor IκBζ induced Th17 development efficiently. However, when IκBζ was overexpressed, either RORγt or RORα strongly induced IL-17 production, even in the absence of exogenous polarizing cytokines. In cooperation with RORγt and RORα, IκBζ enhanced *Il17a* expression by directly binding to the regulatory region of the *Il17a* gene. In addition, the expression of *Il17f*, *Il21* and *Il23r* mRNA was decreased in *Nfkbiz*$^{-/-}$ T cells. IκBζ also bound to the promoter or the enhancer region of these genes in Th17 cells. Our study demonstrates the essential role of IκBζ in Th17 development, and points to a molecular basis for a novel therapeutic strategy against autoimmune disease.

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P57

Features of rheumatic fever in adult patients in modern Kyrgyzstan

Nazgul A Omurzakova[1*], Aynagul S Djumagulova[1], Raisa I Rudenko[1], Kusuki Nishioka[2], Toshihiro Nakajima[2]
[1]National Center of Cardiology and Internal Medicine, Bishkek, Kyrgyz Republic; [2]Institute of Medical Science, Tokyo Medical University, Tokyo, Japan

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Objective: Study of peculiarities of rheumatic fever (RF) in adult patients.
Materials and methods: We have studied prospectively for 5 years 200 patients (56 men and 144 women) with acute rheumatic fever (ARF in 27) and recurrent ARF (in 173) at the age of 15-40 years (average age ± 24,5 7 years). Clinical and laboratory (ESR, antistreptolysin-O (ASL-O) and CRP) and instrumental (ECG, ECG monitoring daily, 2-D echocardiography color) studies conducted. The diagnosis of ARF was verified according to the WHO diagnostic criteria in the modification of Jones' criteria, AHA (1999) and WHF (2008).
Results: We found that predisposing factors for the development of ARF was the presence of tonzillopharingitis (47.5%), while carriers of group A streptococcus (GAS) was 38.0% among patients examined. Clinical symptoms of carditis with echocardiographic signs of valvulitis occurred in 196 (98.0%) patients. In 54 (27.5%) of them installed valvulitis mitral valve. Valvulitis aortic valve was detected in 24 (12.2%) patients. In 118 (60.2%) patients observed at the same time valvulitis mitral and aortic valves, while in 22 (39.2%) patients are men and 92 (63.8%) patients are women. In 18 (66.6%) patients with ARF was observed mitral valve prolapse (MVP), in 6 (22.2%) were in men, 12 (44.4%) in women. In 9 (4.5%) patients with ARF proceeded pancarditis (endocarditis, myocarditis and pericarditis). Signs of coronaritis with typical anginal pain with ECG signs of ischemia, arrhythmias, heart block were observed in 12 (6.0%) patients with RF. Verification of diagnosis was carried out using the angiography of coronary arteries. The symptoms of coronaritis in this patients disappeared after anti-inflammatory therapy. Polyarthritis with ARF was observed in 40.7% of patients, 25 (14.4%) of patients with recurrent ARF articular syndrome manifested primarily arthralgia. In addition, 6.5% in patients with RF were observed asymptomatic sacroiliitis stage I-II (Dale), 7 of patients are men and 5 of them are women.
Conclusion: The reducing of clinical manifestations of ARF in adult led to gypo-diagnostics of disease, a consequence of which was the formation of rheumatic heart disease.

P58

Smoking induces expression of ligands of the immune receptor NKG2D

Caroline Ospelt[1*], Giovanni G Camici[2], Fabia Brentano[1], Peter Künzler[1], Christoph Kolling[3], Renate E Gay[1], Beat A Michel[1], Steffen Gay[1]
[1]Center of Experimental Rheumatology and Center of Integrative Human Physiology (ZIHP), University Hospital, Zurich, Switzerland; [2]Institute of Physiology, University of Zurich, Switzerland; [3]Schulthess Clinic, Zurich, Switzerland

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Background: While different studies confirmed an increased risk for smokers to develop rheumatoid arthritis (RA), the mechanisms behind this phenomenon are not known up to now. In all probability, smoking induces expression or post-translational modification of immune activating proteins which then initiate an autoimmune reaction in individuals with a susceptible genetic background. To identify these triggering molecules we screened joints of mice that were exposed to cigarette smoke for differences of gene expression and verified our results in synovial tissues of human smokers.
Methods: C57BL/6 mice were exposed to cigarette smoke (n = 6) or room air (n = 8) in a whole body exposure chamber for 3 weeks. Protein and mRNA was isolated from murine ankle joints and from synovial tissues obtained from smoking (n = 4) and non smoking (n = 5) RA patients undergoing joint replacement surgery. Tissues were further analysed by Affymetrix microarrays, Real-time PCR or immunoblotting.
Results: Since data from microarray experiments had shown increased levels of the immune receptor NKG2D ligand histocompatibility 60 (H60) after cigarette smoke exposure, we measured H60 expression levels by Real-time PCR in ankle joints of smoke exposed and control mice. H60 transcript levels were 3.2 fold higher in joints of smoke-exposed mice compared to control mice (dCT 12.5 +/-0.3 versus 14.2 +/-0.4, p = 0.03). Upregulation of H60 protein after smoke exposure was also seen in immunoblotting experiments. Since H60 is not expressed in humans, we analysed expression of the 7 human NKG2D ligands RAET1E, RAET1G, MICA, MICB, and ULBP1-3 in synovial tissues of RA patients. Transcripts of ULBP1-3 were not detectable in synovial tissues and there was no difference in the expression levels of RAET1G and RAET1E in synovial tissues of smokers compared to non smokers. However, expression levels of MICA and MICB were 2.3 and 2.8 fold higher in synovial tissues of smokers than in non-smokers (dCT 11.3 +/-0.2 versus 10.1 +/-0.3, p = 0.03 and dCT 10.8 +/-0.3 versus 9.3 +/-0.5, p = 0.03).
Conclusion: We found that smoking induces the expression of ligands of the activating immune receptor NKG2D in murine as well as in human joints. Since dysregulated expression of NKG2D ligands has been previously implicated in induction of autoimmune responses, continuous excess of NKG2D ligands in joints of smokers might be a trigger for the development of RA in susceptible individuals.

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Association of microRNA-221/222 and -323-3p with rheumatoid arthritis via predictions using the human TNF transgenic mouse model

Ioannis Pandis[1*], Caroline Ospelt[2], Niki Karagianni[1,3], Maria Denis[3], Martin Reczko[4], Carme Camps[5], Artemis Hatzigeorgiou[4], Jiannis Ragoussis[5,6], Steffen Gay[2], George Kollias[1]
[1]Institute of Immunology, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece; [2]Center of Experimental Rheumatology, University Hospital Zurich and Zurich Center of Integrative Human Physiology, Zurich, Switzerland; [3]Biomedcode Hellas SA, Vari, Greece; [4]Institute of Molecular Oncology, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece; [5]The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; [6]Institute of Molecular Biology & Genetics, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

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Background: MicroRNAs (miRs), a class of small non-coding RNA molecules, act as posttranscriptional regulators and are involved in a plethora of cellular functions. miRs have attracted a great deal of attention as potential therapeutic targets, as the sequence-specific mode in which they act, allows the simultaneous targeting of multiple target genes, often members of the same biological pathway(s) [1]. Previous studies have demonstrated that miRs are dysregulated and functionally involved in rheumatoid arthritis (RA) [2-9]. In this study we sought to identify novel miR associations in synovial fibroblasts (SFs), a key pathogenic cell type in RA [10,11], by performing miR expression profiling on cells isolated from the human TNF transgenic mouse model (TghuTNF, Tg197) [12] and patients biopsies.
Materials and methods: miR expression in SFs from TghuTNF and WT control mice were determined by deep sequencing and the arthritic profile was established by pairwise comparisons. qRT-PCR analysis was utilised for profile validation, miR and gene quantitation in patient SFs. Dysregulated miR target genes and pathways were predicted via bioinformatic algorithms.
Results: Deep sequencing demonstrated that TghuTNF-SFs exhibit a distinct pathogenic profile with 22 significantly upregulated and 30 significantly downregulated miRs (fold change>1.5, p-value<0.05). qRT-PCR validation assays confirmed the dysregulation of miR-223, miR-146a and miR-155 previously associated with human RA pathology, as well as that of miR-221/222 and miR-323-3p. Notably, the latter were also found significantly upregulated in patient RASFs, suggesting their association with human RA pathology. Bioinformatic analysis suggested Wnt/Cadherin signaling as the most significant pathway targets of miR-221/222 and miR-323-3p and CSNK1A1 and BTRC, the negative regulators of β-catenin, amongst predicted gene targets. qRT-PCR assays confirmed the downregulation of these genes in RASFs, validating our hypothesis that the newly identified miRs may function to modulate Wnt/Cadherin signaling.
Conclusions: In this study, by performing comparative analyses between an established mouse model of arthritis and RA patient biopsies, we identified novel dysregulated miRs in RASFs potentially involved in pathways important for the pathogenic phenotype of these cells and highlighting the value of such cross-species comparative approaches [13].
Acknowledgements: This project was funded by the Masterswitch Project (HEALTH-F2-2008-223404), EURO-RA RTN (HPRN-CT-2002-00255) and IMI

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P60

Methotrexate alone and methotrexate combined with etanercept in treatment of rheumatoid arthritis

Sylejman Rexhepi[1*], Mjellma Rexhepi[1], Blerta Rexhepi[2], Vjollca Sahatçiu-Meka[3], Vigan Mahmutaj[2]
[1]Rheumatology Department, University of Prishtina, Prishtina, Kosova, 10000; [2]Private Clinic "Rheuma", Prishtina, Kosova, 10000; [3]Physical Medicine Department, University of Prishtina, Kosova, 10000
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Objectives: The aim of this study is to evaluate the efficacy and safety of methotrexate (MTX) alone and combined therapy of Etanercept (ETN) and methotrexate (MTX), in patients with rheumatoid arthritis (RA).
Methods: Patients with RA were treated in combination with ETN (with doses of 25 mg subcutaneously twice weekly), with oral MTX (doses up to 20 mg weekly), and alone MTX (doses up to 20 mg weekly) in period of two years, in Rheumatology Department of Internal Clinic in Prishtina. Clinical response was assessed using American College of Rheumatology (ACR) criteria and the Disease Activity Score (DAS28) in 60 patients with RA. Radiographic changes were measured in the beginning and at the end of the study with Sharp Score.
Results: Of total number of 60 patients (10 of them were males and 50 were females) with mean age of 57.63, 10 or 16.6% of patients were treated with combined therapy (ETN plus MTX) and 50 or 83.3% of patients with monotherapy (MTX). The group of combined therapy (ETN+MTX) after the treatment resulted with improvement of acute phase reactants as erythrocyte sedimentation rate (ESR) for the first hour (41.1 vs. 10.3 mm/hour) and C-reactive protein (CRP) (40.8 vs. 6 mg/liter) comparing to the group treated with MTX alone there were no significant changes (ESR: 45.7 vs. 34.3 mm/hour; CRP: 48 vs. 24 mg/liter). Before treatment the severity of the disease was high, where in group with combined therapy (ETN plus MTX) DAS28 was 5.32, and in the group with monotherapy of MTX DAS28 was 5.90. After 2 years of treatment we had significant changes in the results of DAS28, where in group treated with ETN plus MTX DAS28 was 2.12 ± 0.15, while in the group of patients treated with MTX DAS28 were 3.75 ± 0.39 ($t = 13.03$; $df = 58$; $p < 0.0001$). The group with combined therapy showed less radiographic progression comparing to the group of monotherapy ($p < 0.05$).
Conclusions: According to our results we can conclude that ETN in combination with MTX reduced disease activity, slowed radiographic progression and improved clinical manifestations more effectively than MTX alone within period of 2 years. During the treatment, no serious adverse events were noticed with combination treatment of ETN and MTX.

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P61

SPACIA1/SAAL1: a newly identified gene associated with aberrant proliferation of synovial fibroblasts

Tomoo Sato[1], Ryoji Fujii[1*], Koji Konomi[2], Naoko Yagishita[1], Satoko Aratani[1,3], Natsumi Araya[1], Hiroyuki Aono[2], Kazuo Yudoh[1], Noboru Suzuki[1], Moroe Beppu[1], Yoshihisa Yamano[1], Kusuki Nishioka[3], Toshihiro Nakajima[1,3,4]
[1]St. Marianna University School of Medicine, Kawasaki, Japan; [2]Santen Pharmaceutical Co., Ltd., Osaka, Japan; [3]Institute of Medical Science, Tokyo Medical University, Tokyo, Japan; [4]Misato Marine Hospital, Kochi, Japan
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Background: The bone and cartilage destruction seen inrheumatoid arthritis (RA) is caused by synovial pannus formation, which is characterized by aberrant proliferation of synovial fibroblasts. Inhibition of synovial proliferation has recently been reported to be a promising therapeutic strategy for RA.However, the specific mechanism underlyingdysregulated proliferation of synovial fibroblasts remains unclear.
Objective: We aimed toidentify and characterize genesthat are involved in the aberrant proliferation of synovial fibroblasts.
Methods: Microarray analysiswas performed to identifythe genes that had upregulated expression inmice with collagen-induced arthritis (CIA). The effect of candidate genes on the proliferation of synovial fibroblasts was screened using antisense oligodeoxynucleotides and small interfering RNAs (siRNAs).

Results: We identified a novel gene named *SPACIA1/SAAL1* (synoviocyte proliferation-associated in CIA 1/serum amyloid A-like 1)that was associated with aberrant proliferation of synovial fibroblasts. Immunohistochemical-analysis indicated that SPACIA1/SAAL1 was strongly expressed in the foot joints of mice with CIA and in the thickened synovial lining of the human RA synovium. Transfection of siRNA targeting *SPACIA1/SAAL1*into RA synovial fibroblastscould inhibit tumor necrosis factor (TNF)α-induced proliferation more effectively thanit could inhibit serum-induced proliferation.In addition, the antiproliferative effect of *SPACIA1/SAAL1* siRNA was caused byinhibition of cell cycle progression and not by induction of apoptosis.We established transgenic (Tg) mice that overexpressed SPACIA1/SAAL1. These Tg mice did not spontaneously develop arthritis or cancer. However,inducing CIA causedgreatersynovial proliferation and worse diseasein Tg mice thanin wild-type mice.
Conclusion: SPACIA1/SAAL1 plays an important role in the aberrant proliferation of synovial fibroblasts under inflammatory conditions.

P62

Two cases of multiple-drug-resistant adult-onset Still's disease treated successfully with tocilizumab - the relationship between interleukin 6 and 18

Kojiro Sato*, Akinori Yamamoto, Yoshihiro Yoshida, Toshihide Mimura
Department of Rheumatology and Applied Immunology, Saitama Medical University, Saitama 350-0495, Japan
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Background: Adult-onset Still's disease (AOSD) is an inflammatory disease of unknown cause characterized by a high spiking fever, arthritis and evanescent rash. The mainstay of treatment is glucocorticoids with or without immunosuppressants. Recently, biologics such as anti-tumor necrosis factor (TNF) antibodies have also been tried in certain refractory cases.
Results: We have had two cases of AOSD which were treated successfully with anti-interleukin (IL-) 6-receptor antibody, tocilizumab (TOC). (Case 1) A 36-year-old woman who was diagnosed 8 years previously, and had been treated with various DMARDs plus etanercept (ETA) or adalimumab, presented with a high spiky fever and elevated liver enzymes. After excluding infection, she was treated with TOC. (Case 2) A 26-year-old man with new-onset AOSD, which was shown to be resistant to multiple immunosuppressants including infliximab and ETA, was treated with TOC starting 7 months after the diagnosis. In both cases, serum IL-18 was extremely high, and TOC promptly improved clinical symptoms and liver function. The high level of serum ferritin also became normalized. Interestingly, especially in case 2, the level of IL-18 remained high after the administration of TOC, suggesting that IL-18 is located either upstream of, or at the same level as, IL-6 in the pathogenesis of AOSD.

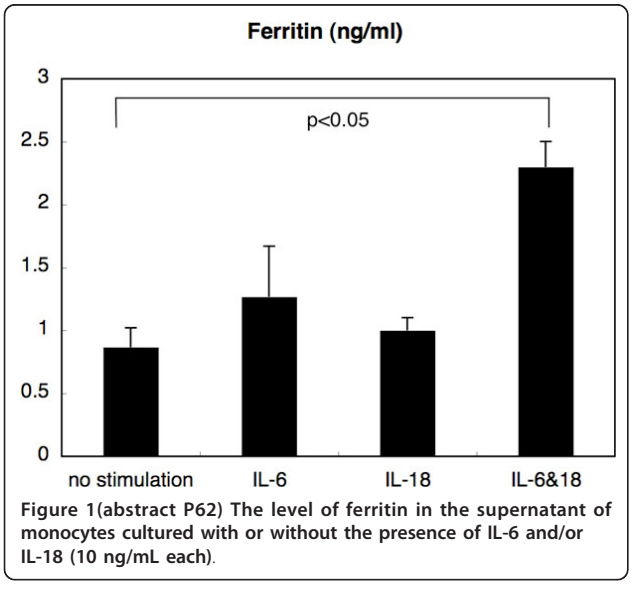

Figure 1(abstract P62) The level of ferritin in the supernatant of monocytes cultured with or without the presence of IL-6 and/or IL-18 (10 ng/mL each).

Next, we cultured human monocytes derived from healthy controls with or without the presence of IL-6 and/or IL-18 *in vitro*. The level of ferritin in the supernatant was significantly increased only when both IL-6 and IL-18 were added, indicating that IL-6 and IL-18 have a synergistic effect on the production of ferritin (Figure 1).
Conclusion: TOC can be a first-line biologic applicable against multiple-drug-resistant AOSD. If an IL-18 blocker is developed, however, it may be even more beneficial in that it may block the cascade of inflammation at a point further upstream.

P63

GI-REASONS: a novel 6-month, prospective, randomized, open-label, blinded end point (PROBE) trial

Byron Cryer[1*], Chunming Li[2], Lee S Simon[3], Gurkirpal Singh[4], Martin J Stillman[5], Manuela Berger[2]
[1]University of Texas Southwestern Medical Center, Dallas, TX, USA; [2]Pfizer Inc. New York, NY, USA; [3]SDG, LLC, Cambridge, MA, USA; [4]Stanford University, Palo Alto, CA, USA; [5]Hennepin County Medical Center, Minneapolis, MN, USA
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Background: The GI Randomized Event and Safety Open-Label NSAID Study (GI-REASONS) was a novel prospective, randomized, open-label, blinded end point (PROBE) study that measured adjudicated clinical outcomes throughout the GI tract. It was designed to assess if celecoxib use in patients with osteoarthritis (OA) at moderate GI risk (≥55 y) is associated with a lower incidence of clinically significant upper and lower GI events compared to nsNSAIDs, with/without proton-pump inhibitors (PPIs), in standard US clinical practice.
Materials and methods: 8067 OA patients were randomized 1:1 for 6-mos with celecoxib or a nonselective (ns)NSAID, stratified by *H pylori* status. The primary end point was a composite of adjudicated clinically significant upper and lower GI events. Aspirin use was not permitted. Treatment doses could be adjusted per US prescribing information. Patients randomized to the nsNSAID arm could switch between nsNSAIDs; however, crossover between treatment arms was not allowed. PPIs and histamine-2 receptor antagonists (H_2RAs) were prescribed at the providers' discretion.
Results: 4035 celecoxib and 4032 nsNSAID patients were randomized and included in the ITT analyses. Baseline demographics were similar. Overall, significantly more nsNSAID users met the primary end point at 6 mos (OR, 1.82; 95% CI 1.31-2.55; p = 0.0003; Table 1). The most commonly used nsNSAIDs were meloxicam (42%), naproxen (21%), diclofenac (20%) and nabumetone (14%). 2596 celecoxib (64.3%) and 2611 (64.8%) nsNSAID users completed the study. 189 patients were lost to follow-up (LTFU; 2.1% celecoxib and 2.6% nsNSAID). Attributing the primary end point to all LTFU patients (worst-case sensitivity analysis), celecoxib remained superior (OR 1.46; 95% CI 1.18-1.82; p = 0.0006). AEs, SAEs and discontinuations were similar in both treatment groups. 23% of celecoxib and 24% of nsNSAID patients used a PPI (p = NS). Moderate to severe abdominal symptoms were experienced by 94 (2.3%) celecoxib and 138 (3.4%) nsNSAID patients (P<.01).
Conclusion: Celecoxib use had a lower risk of clinically significant upper and lower GI events than nsNSAIDs. A major strength of this study is its PROBE design. Simple inclusion and exclusion criteria allowed for a broad patient population of moderate GI risk. Switching among nsNSAIDs and allowing for dose adjustments, along with use of PPIs and H_2RAs as needed, more closely reflects daily clinical practice. GI-REASONS demonstrates the improved GI safety profile of celecoxib throughout the GI tract in patients treated in a "real-world" setting.

P64

Inhibition of Syndecan-4 by therapeutic antibodies reduces TNFα dependent joint destruction in mice

Athanasios Stratis[1*], Katja Neugebauer[1], Mareike Frohling[1], Peter Paruzel[1], Berno Dankbar[1], Corinna Wemeyer[1], Christoph Cromme[1], Lars Godmann[1], Jessica Bertrand[1], Adelheide Korb[1], Frank Echtermeyer[2], George Kollias[3], Thomas Pap[1]
[1]Institute of Experimental Musculoskeletal Medicine, University Muenster, 48149, Muenster, Germany; [2]Department of Anesthesiology and Intensive Care Medicine, Medical University Hannover, 30625, Hannover, Germany; [3]Institute of Immunology, Biomedical Sciences Research Center, Vari, 16672, Greece
Arthritis Research & Therapy 2012, **14(Suppl 1):**P64

Table 1(abstract P63) Clinically significant upper and lower GI events: primary analysis

		Celecoxib		nsNSAID	
		N	Patients With Event n (%)	N	Patients With Event n (%)
All patients		4035	54 (1.3)	4032	98 (2.4)
***H pylori* status**	**Positive**	1401	25 (1.8)	1386	34 (2.5)
	Negative	2634	29 (1.1)	2646	64 (2.4)
OR (95% CI); P value		1.82 (1.31-2.55); $p = 0.0003$			

Background: Syndecan-4, a member of a syndecan family of transmembrane heparansulfate proteoglycans has been recently associated with cell-matrix-adhesion, cell-migration, differentiation and proliferation, but its specific function in inflammatory pathologies remains unclear. We used the human TNFalpha transgenic mouse (hTNFtg) to analyse the expression and function of syndecan-4 in chronic-destructive-arthritis and answer the question whether inhibition of syndecan-4 by specific antibodies may prevent cartilagedestruction and/or improve the phenotype after onset of the disease in this animal model of human RA.
Methods: Expression of syndecan-4 was investigated by immunohistochemistry in the hind-paws of 8-weeks/12-weeks old hTNFtg mice and wild type controls. In addition, synovial fibroblasts were isolated and analysed for syndecan-4-expression by RT-PCR. For functional analyses, we generated blocking-antibodies against syndecan-4. To investigate their effect on TNFalpha mediated-destructive-arthritis, hTNFtg mice were injected with the antibodies or with IgG-control twice weekly for 4-weeks in a preventive manner (age-4-to-8-weeks) and for disease treatment of joint destruction (age-8-to-12-weeks) into their hind paws. Evaluation of disease severity included clinical parameters (weight, arthritis-score, grip-strength) as well as histomorphometric analysis of toluidin-blue-stained paraffin sections.
Results: As seen in immunohistochemistry, there was a strong expression of syndecan-4 in the synovial membranes of hTNFtg mice, whereas only negligible staining for syndecan-4 was found in synovial tissues of wild type animals. In vitro, synovial fibroblasts isolated from hTNFtg mice showed more than 30-fold higher expression of syndecan-4 than wild type controls. Administration of the anti-syndecan-4 antibodies but not of IgG-control in preventive treated 4-week-old hTNFtg mice clearly ameliorated the clinical signs of arthritis and protected the treated joints from cartilage damage. At histomorphometric analysis, this was evident for all analysed parameters but seen most prominently for area of distained cartilage.
Significantly reduced cartilage damage in the anti-syndecan-4 treated hTNFtg mice was accompanied by a striking reduction in the expression of MMP-3. The treatment with antisyndecan-4 in 8-week-old hTNFtg mice after onset of arthritis clearly ameliorated the jointdestruction, and improved cartilage-damage. The treatment also showed a clear reduction of inflammation in the paws compared to the untreated animals.
Conclusions: Our findings indicate that syndecan-4 is involved prominently in fibroblast-mediated cartilagedamage in hTNFtg mice by regulating the exression of disease-relevant MMPs. More importantly, the data suggest that inhibition of syndecan-4 not only prevens cartilage damage, but also reduces the severity after onset of the disease.

P65

Clinical-experimental assessment of simvastatin efficiency in the treatment of rheumatoid arthritis

Rikhikhon N Tadjikhodjaeva*, Nargiza G Khabibullaeva
Tashkent Medical Academy, Tashkent, Uzbekistan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P65

Subject of the inquiry: 35 patients with rheumatoid arthritis, 50 mature male rats of mixed population.
Aim of the inquiry: Clinical-experimental assessment of simvastatin efficiency and pathogenic justification of its inclusion into the complex treatment for therapy optimization in patients with rheumatoid arthritis.
Methods of investigation: clinical-laboratory, biochemical - determination of total cholesterol, low and high density lipoproteins, triglycerides, calculation of atherogenic coefficient in blood serum of patients with rheumatoid arthritis and in experimental animals.
The results achieved and their novelty: On the systemic and local levels an approach was applied allowing consideration of nitrogen oxide metabolism disorders as an important part of the pathogenesis of rheumatoid arthritis. A number of new data were obtained concerning the relationship of nitrogen oxide metabolism and C-reactive protein formation, clinical course of rheumatoid arthritis. For the first time a complex approach was suggested for the pathogenic justification of simvastatin use in the scheme of conventional treatment to increase the therapy efficiency, to achieve stable early remission in patients with rheumatoid arthritis. It was proved that an important mechanism of increasing the therapeutic efficiency of simvastatin was its action on the system of endothelial function in blood and joint fluid. It was suggested that one should include assessment of blood and joint fluid for nitrogen oxide, nitrate diaphorase and nitrate reductase in the algorithm of investigation and dynamic observation, choice of tactics and therapy efficiency assessment.
Practical value: Obtained new data are necessary for increasing the pharmacotherapy efficacy in patients with rheumatoid arthritis taking into account the metabolic activity of NO-synthetase mechanism in blood and synovial fluid. An algorithm was suggested for screening observation and differentiated management of patients with rheumatoid arthritis taking account of severity of nitrogen oxide metabolism disorders. A differentiated approach was worked out and justified of simvastatin prescription both to increase the efficacy of treatment taking into account the clinical activity of the disease and to correct metabolic disorders in patients with rheumatoid arthritis.

P66

Metabolic syndrome in Indian patients with rheumatoid arthritis and its correlation with disease activity

Aman Sharma[1*], Nilesh Bhilave[1], Kusum Sharma[2], Indu Varma[3]
[1]Internal Medicine, PGIMER, Chandigarh, India,160012; [2]Medical Microbiology, PGIMER, Chandigarh, India,160012; [3]Biochemistry, PGIMER, Chandigarh, India, 160012
Arthritis Research & Therapy 2012, **14(Suppl 1):**P66

Background: Increased prevalence of metabolic syndromein rheumatoid arthritis (RA) has been reported from American and European populations but it has not been studied in Indian patients with RA.
Objectives: The main objective of our study was to assess the prevalence of the metabolic syndrome in Asian-Indian patients with rheumatoid arthritis and also to studyits correlation with disease activity.
Methods: This was a prospective case control study in which 114 patients diagnosed to have rheumatoid arthritis of more than 1 year duration and 114 healthy age (± 5 years) and sex matched controls were included. Height, weight, body mass index, blood pressure and waist circumference of the patients were measured at the enrolment visit. Venous samples were taken after eight hours of overnight fasting for the estimation of serum cholesterol, triglycerides and plasma glucose levels. Metabolic syndrome was diagnosed according to Adult Treatment Panel III criteria [1] and the consensus definition of the metabolic syndrome for adult Asian patients [2]. The disease activity was assessed by DAS 28.
Results: The mean age of patients with RA and control group was 44.8 and 43.2 years (p <0.36) respectively. The mean duration of RA was 6.5 years. Though the mean BMI was similar in both the groups(25.5 and 24.2), there was a statistically highly significant difference in mean waist circumference (92.1 cm and 81.2 cm, p < 0.001) and diastolic blood pressure(80.5 and 75.3

mm Hg, $p < 0.001$) in patients with RA as compared to controls.Metabolic syndrome was present in 36 patients and 17 controls ($p < 0.05$) according to the Adult Treatment Panel III criteria and in 40 patients and 18 controls ($p < 0.01$) according to the consensus definition of the metabolic syndrome for adult Asian patients. There was no significant correlation between the metabolic syndrome and disease activity as measured by DAS-28 using both the criteria.

Conclusions: Indian patients with RA have increased prevalence of metabolic syndrome as compared to their age and sex matched healthy controls, but there is no significant correlation between metabolic syndrome and disease activity.

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P67

Osteoprotegerin induction in response to microbial infection

Yasunari Takada*, Koichi Matsuo
Laboratory of Cell and Tissue Biology, Keio Univ. School of Medicine, Tokyo 160-8582 Japan

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Osteoprotegeirn (OPG) is an endogenous decoy receptor for RANKL, which is a cytokine essential for osteoclast differentiation. Lipopolysaccharide (LPS) is known to induce osteoclast formation when injected onto calvaria in mice. Unexpectedly, we observed that mice injected with LPS up-regulate OPG and down-regulate RANKLlevels in peripheral blood.

In the present study, we examined whether OPG is induced by microbial infection of various kinds, and the sites and significance of OPG production in infected mice. Wild-type mice infected with*Salmonella*, *Staphylococcus*, *Mycobacteria*or influenza virus showed increase in OPG levels in peripheral blood. We also found that the levels of OPG in serum of human patients infected with *M. tuberculosis* and *M. avium* were significantly increased. Moreover, injection of mice with LPS induced OPG production specifically in lymph nodes, especially in high endothelial venule(HEV)cells, but not in other organs. OPG production was suppressed in c-Fos-deficient mice and enhanced in Fra-1 transgenic mice, indicating that OPG production is regulated by AP-1 transcription factors.Loss of OPG in mice did not affect either their survival or *Salmonella* proliferation in spleen and liver after infection with virulent strains of *Salmonella*. Interestingly, however, when wild-type mice were infected with an avirulent*Salmonella* strain, which can induce OPG, osteoclast development was suppressed and bone mineral density was increased. These data reveal for the first time that lymph nodes protect bones from infection-induced bone loss through OPG production.

P68

Expression patterns and function of chromatin protein HMGB2 during mesenchymal stem cell differentiation

Noboru Taniguchi[1,2*], Beatriz Caramés[2], Yasuhiko Kawakami[3], Martin Lotz[2]
[1]Hakuaikai Kaisei Hospital, Obihiro, Hokkaido, Japan; [2]The Scripps Research Institute, La Jolla, CA, USA; [3]University of Minnesota, Minneapolis, MN, USA

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The superficial zone (SZ) of articular cartilage is critical in maintaining tissue function and homeostasis and represents the site of the earliest

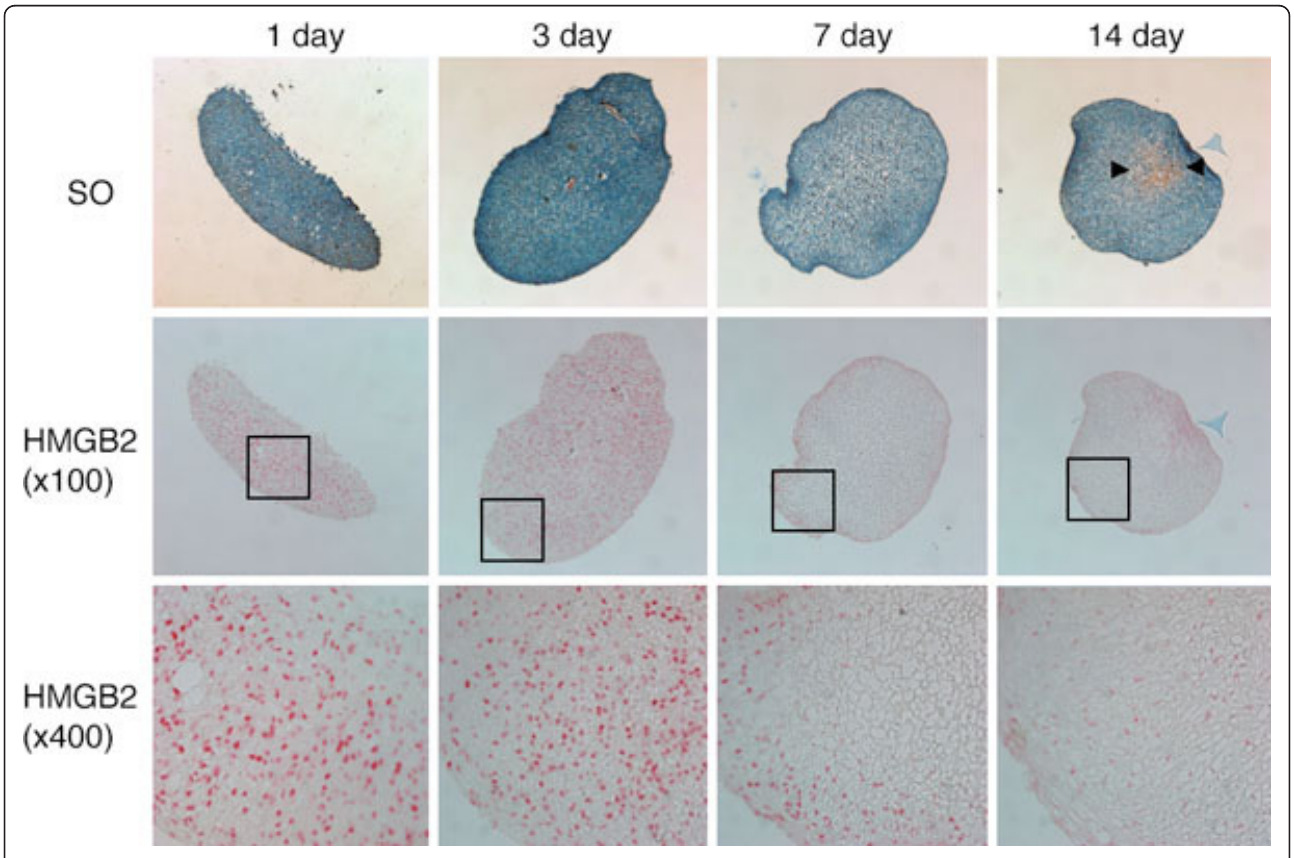

Figure 1(abstract P68) HMGB2 expression during chondrogenesis of human MSC. Immunohistochemistry shows that HMGB2 is expressed at days 1 and 3, but that expression is reduced at days 7, 14 upon induction of chondrogenesis. SO: safranin O staining.

changes in osteoarthritis (OA). The expression of chromatin protein HMGB2 is restricted to the SZ, which contains cells expressing mesenchymal stem cell (MSC) markers [1]. Aging-related loss of HMGB2 and gene deletion are associated with reduced SZ cellularity and early onset OA [2]. This study addressed HMGB2 expression patterns in MSC and its role during differentiation.

HMGB2 was detected at higher levels in human MSC as compared to human articular chondrocytes and its expression declined during chondrogenic differentiation of MSC (Figure 1). Lentiviral HMGB2 transduction of MSC suppressed chondrogenesis as reflected by an inhibition of Col2a1 and Col10a1 expression. Conversely, in bone marrow MSC from Hmgb2-/- mice, Col10a1 was more strongly expressed than in wildtype MSC. This is consistent with in vivo results from mouse growth plates showing that Hmgb2 is expressed in proliferating and prehypertrophic zones but not in hypertrophic cartilage where Col10a1 is strongly expressed. Osteogenesis was also accelerated in Hmgb2-/- MSC. The expression of Runx2, which plays a major role in late stage chondrocyte differentiation, was enhanced in Hmgb2-/- MSC and HMGB2 negatively regulated the stimulatory effect of Wnt/β-catenin signaling on the Runx2 proximal promoter.

These results demonstrate that HMGB2 expression is inversely correlated with the differentiation status of MSC and that HMGB2 suppresses chondrogenic differentiation. The aging-related loss of HMGB2 in articular cartilage may represent a mechanism responsible for the decline in adult cartilage stem cell populations.

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P69

Age features of metabolic syndrome and cardiovascular disorders in gout

Surayo Shukurova*, Khisrav Toirov, Nabijon Hamidov, Dilfuza Jonnazarova
AvicennaTajik State Medical University, Tajikistan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P69

Materials and methods: Are surveyed 76 gout patients, middle age equaled 56.6 ± 7.5 year. Have been distributed on 3 groups: more younger 50, from 50 to 60 and more senior 60 years. Metabolic syndrome was diagnosed by criteria Adult Treatment Panel III (National Institute of Health, USA) [1]. Serum level of Uric Acid defined by colorimetric enzyme method, glucose - by glucose oxidize method, cholesterol, triglycerides and high density lipoproteides-cholesterol - by colorimetric method [2]. Low and very low density lipoproteides-cholesterol defined by "WT Friedewald Equation" (1972) [3].

Table 1(abstract P69) Frequency of revealing of signs of metabolic syndrome at gout patients (n = 76)

Sign	Frequency
CW > 102 cm	48 (63.2%)
SBP > 140 mm Hg and/or DBP > 90 mm Hg	50 (65.8%)
TG ≥ 120 mg/dl	22 (29%)
Glucose ≥ 110 mg/dl	32 (42.1%)
HDL-cholesterol < 50 mg/dl	58 (76.3%)

CW - circle waist; TG - triglycerides; SBP - systolic blood pressure; DBP - diastolic blood pressure; HDL - high density lipoproteides.

Results: Metabolic syndrome has been diagnosed at 46 (60.5%) patients. Middle age patients with presence of metabolic syndrome has made 55.7 ± 4.7, without - 57.9 ± 8.3 year.

Conclusions: At the same time we have not revealed age distinctions in occurrence of metabolic syndrome at patients with primary gout, however frequency of IHD of gout patients naturally increased with the years - from 38% to 68%. Patients of the senior age groups the increase in frequency of hypertension and IHD while patients of younger age have obesity, hypertriglyceridemia and hyperglycemia is more often noted.

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P70

Unfolded protein response mediator, the IRE1α-XBP1 pathway is involved in osteoblast differentiation

Takahide Tohmonda[1,2*], Kazuhiro Chiba[2], Yoshiaki Toyama[2], Keisuke Horiuchi[1,2]
[1]Department of Anti-aging Orthopedic Research, School of Medicine, Keio University, Tokyo, Japan; [2]Department of Orthopedic Surgery, School of Medicine, Keio University, Tokyo, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P70

Background: To maintain the bone strength and functions, the balance between bone resorption and bone formation has to be tightly regulated. However, under certain pathological conditions, including osteoporosis and rheumatoid arthritis, the equilibrium gets disrupted, resulting in a severe bone loss. Recent studies have shown that signaling molecules involved in the unfolded protein response (UPR) are potentially involved in the coupling of bone resorption and bone formation [1-3]. In the present study, we investigated the roles of UPR mediator, the IRE1α-XBP1 pathway in osteoblast differentiation.

Materials and methods: To induce osteoblast differentiation *in vitro*, we used recombinant human BMP-2 and mouse embryonic fibroblasts (MEFs) obtained from wild-type and *Ire1*$^{-/-}$ embryos. Small interfering RNA-mediated gene silencing was used to suppress the expression of the target molecules of IRE1 (XBP1 and TRAF2) in wild-type MEFs. Osteoblast differentiation was evaluated by analyzing the expression levels of the transcripts for osteoblast differentiation markers (*Runx2*, *Osterix*, *Osteoclacin* and type I collagen) and alkaline-phosphatase activity.

Results: We found that UPR is induced during osteoblast differentiation in *in vitro* and *ex vivo* experiments. Most importantly, *Ire*$^{-/-}$ MEFs and *Xbp1*-

Table 2(abstract P69) Frequency of revealing of signs metabolic syndrome at gout patients depending on age, n (%)

Sign	Age groups		
	<50 y (*n = 26*)	**50-60 y (*n = 26*)**	**>60 y (*n = 24*)**
CW > 102 cm	22 (84.6%)	20 (76.9%)	6 (25%)
SBP > 140 mm Hg and/or DBP > 90 mm Hg	20 (76.9%)	14 (53.8%)	20 (83.3%)
TG ≥ 120 mg/dl	8 (30.8%)	10 (38.45%)	4 (16.7%)
Glucose ≥ 110 mg/dl	14 (53.85%)	14 (53.85%)	4 (16.7%)
HDL-cholesterol < 50 mg/dl	14 (53.85%)	24 (92.3%)	20 (83.3%)

silenced MEFs were defective in BMP2-induced osteoblast differentiation, indicating that the IRE1α-XBP1 pathway is essential for the maturation of osteoblasts. Furthermore, we found that UPR induces transcription of *Osterix* (a transcription factor indispensable for bone formation) via the IRE1α-XBP1 pathway, and that XBP1 directly binds to the promoter region of the *Osterix* gene and functions as a transcription factor. Taken together, the present study indicates that the UPR induced during osteoblast differentiation stimulates *Osterix* transcription through the IRE1α-XBP1 pathway.

Conclusions: The present study shows that the IRE1α-XBP1 pathway is a critical component of osteoblast differentiation. Since the IRE1α-XBP1 is also involved in the production of a potent regulator for osteoclast differentiation, interferon beta [1,2], the IRE1α-XBP1 pathway may be an attractive molecular target in modulating the equilibrium between bone formation and bone resorption under pathological conditions.

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P71

Resistance to morphine analgesia and its underlying mechanisms in an experimental mouse model of fibromyalgia

Hitoshi Uchida*, Michiko Nishiyori, Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8521, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P71

Fibromyalgia (FM) is a common condition with generalized or widespread allodynia that affects at least 2% of the US, European and Japanese populations. Although the etiology of this disease remains poorly understood, physical and psychological stressors have been assumed to play a role in the development of FM. Previously, we have established an experimental mouse model of FM pain, using intermittent cold stress (ICS) exposure. This model was found to produce mechanical allodynia and thermal hyperalgesia in a female-predominant manner, as often observed in FM patients. In contrast, exposure to constant cold stress produced a transient allodynia. Importantly, we found that anticonvulsant agent gabapentin, especially when injected intracerebroventricularly, exerts powerful anti-allodynic and anti-hyperalgesic effects in the ICS-exposed mice. In this study, we found that ICS model mice show morphine resistance, as often observed in FM patients. To be concrete, systemic or intracerebroventricular, but not intrathecal or intraplantar, injection of morphine caused no significant analgesia in the ICS-exposed mice. In addition, we found that intracerebroventricularly administrated morphine increases the 5-hydroxytryptamine turnover ratio in the dorsal half of the spinal cord of control mice, but not in the ICS-exposed mice. These findings indicate that ICS model well reflects pathological and pharmacotherapeutic features of FM pain, and the loss of descending serotonergic activation seems to be a crucial mechanism underlying the absence of morphine-induced analgesia in the ICS model.

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P72

Brain perfusion in fibromyalgia patients and its differences between responders and poor responders to gabapentin

Chie Usui[1*], Kotaro Hatta[1], Nagafumi Doi[2], Atsushi Nakanishi[3], Hiroyuki Nakamura[4], Kusuki Nishioka[5]
[1]Department of Psychiatry, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan; [2]Ibaraki Prefectural Tomobe Hospital, 654 asahi-cho, kasama-city, Ibaraki 309-1717, Japan; [3]Department of Radiology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan; [4]Department of Environmental and Preventive Medicine, Graduate School of Medical Science, Kanazawa University, kakuma-cho, Kanazawa-city, Kanazawa 920-1192, Japan; [5]Institute of Innovative Medical Science and Education, Tokyo Medical University, 6-1-1 Shinjyuku, Shinjyuku-ku, Tokyo 160-8402, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P72

Purpose: The aim of the present study was to determine the brain areas associated with fibromyalgia, and whether pretreatment regional cerebral blood flow (rCBF) can predict response to gabapentin treatment.

Methods: A total of 29 women with fibromyalgia and 10 healthy women without pain matched for age were finally enrolled in the study. Technetium-99 m ethyl cysteinate dimer single photon emission computed tomography ($^{99\ m}$Tc-ECD SPECT) was performed in the fibromyalgia patients and controls. A voxel-by-voxel group analysis was performed using SPM2. After treatment with gabapentin, 16 patients were considered "responders", with decrease in pain of greater than 50% as evaluated by visual analogue scale (VAS). The remaining 13 patients were considered "poor responders".

Results: Compared to control subjects, we observed rCBF abnormalities in fibromyalgia including hypoperfusion in the left culmen and hyperperfusion in the right precentral gyrus, right posterior cingulate, right superior occipital gyrus, right cuneus, left inferior parietal lobule, right middle temporal gyrus, left postcentral gyrus, and left superior parietal lobule (Table 1, Figure 1). Compared to responders, poor responders exhibited hyperperfusion in the right middle temporal gyrus, left middle frontal gyrus, left superior frontal gyrus, right postcentral gyrus, right precuneus, right cingulate, left middle occipital gyrus, and left declive

Table 1(abstract P72) Regions of significant hyperperfusion and hypoperfusion in the FM group

	κ	Z score	x(mm)	y(mm)	z(mm)	Localisation
Hyperperfusion	134	4.55	66	-10	30	R **Precentral Gyrus**
	262	4.16	2	-62	14	R **Posterior Cingulate**
	824	3.98	36	-82	32	R **Superior Occipital Gyrus**
	429	3.95	18	-96	-6	R **Cuneus**
	220	3.57	50	-38	52	L **Inferior Parietal Lobule**
	55	3.54	52	-46	6	R **Middle Temporal Gyrus**
	113	3.52	-30	-42	68	L **Postcentral Gyrus**
		3.74	-14	-74	56	L **Superior Parietal Lobule**
	709	4.66	-2	56	-22	L **Superior Frontal Gyrus**
Hypoperfusion	1111	4.38	-12	-32	-18	L **Culmen**

Results are listed by clusters.κvalue, Z score, Talairach coordinates of peak voxel, and anatomic localization are provided for each cluster.

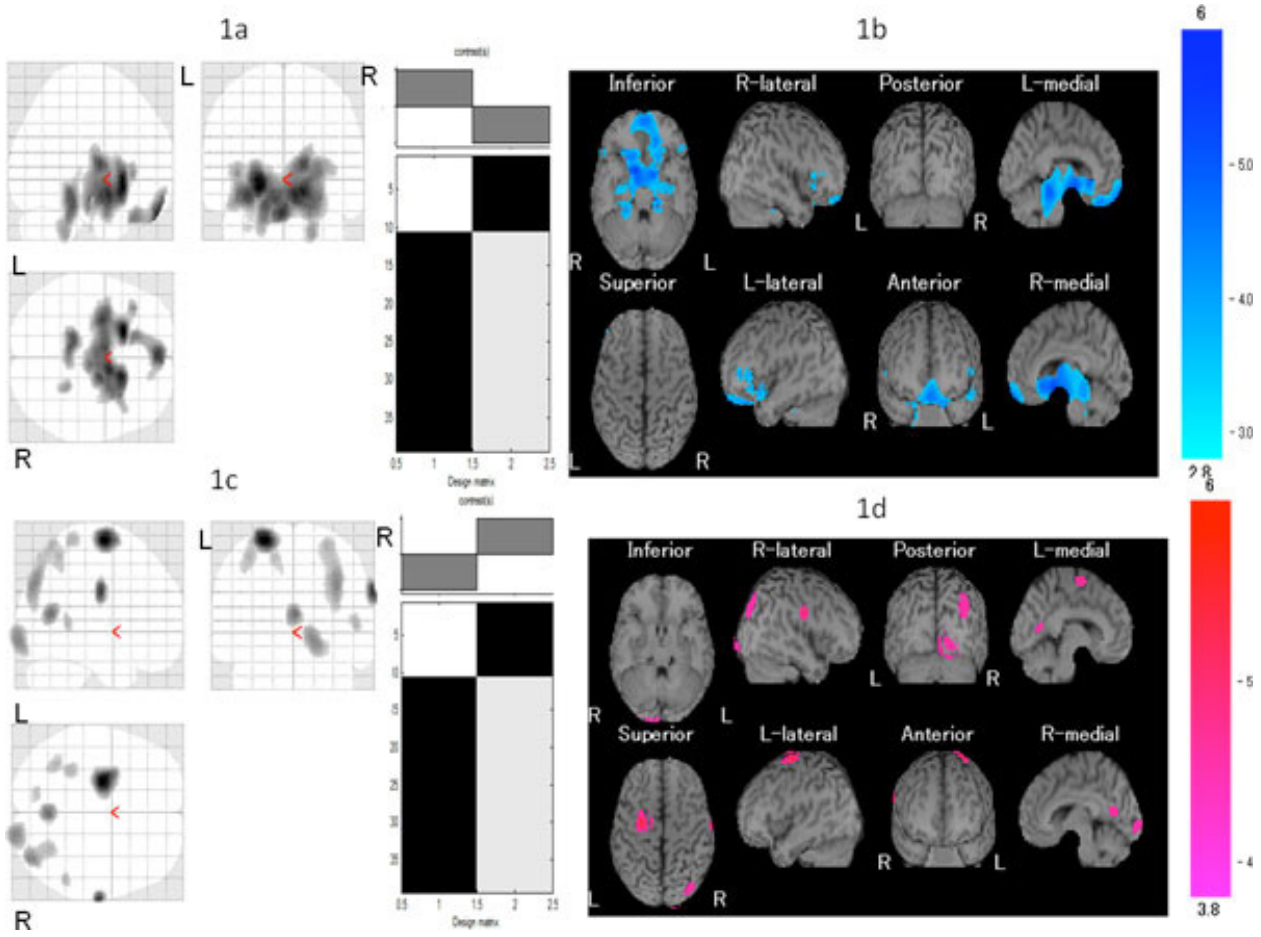

Figure 1(abstract P72) Comparison of rCBF between patients with FM and age-matched healthy controls. Maximum intensity projections of SPM2 results from comparison of rCBF between patients with FM and age-matched healthy controls. **a, b** The FM patient group exhibited significant hypoperfusion in the left culmen. **c, d** The FM patient group exhibited significant hyperperfusion in the right precentral gyrus, right posterior cingulate, right superior occipital gyrus, right cuneus, left inferior parietal lobule, right middle temporal gyrus, left postcentral gyrus, and left superior parietal lobule. Height threshold is < 0.001, corrected for multiple comparison.

Table 2(abstract P72) Regions of significant hyperperfusion in the poor responder group compared to the responder group

	κ	Z score	x(mm)	y(mm)	z(mm)	Localisation
Hyperperfusion	1260	4.08	42	-62	16	R **Middle Temporal Gyrus**
	95	3.88	-46	6	50	L **Middle Frontal Gyrus**
	95	3.88	-20	38	52	L **Superior Frontal Gyrus**
	69	3.67	56	-12	56	R **Postcentral Gyrus**
	578	3.67	14	-76	28	R **Preuneus**
	59	3.58	4	20	36	R **Cingulate**
	70	3.54	-20	-80	4	L **Middle Occipital Lobule**
	77	3.51	-20	-80	-26	L **Declive**

Results are listed by clusters. κvalue, Z score, Talairach coordinates of peak voxel, and anatomic localization are provided for each cluster.

(Table 2). The right middle temporal gyrus, left superior frontal gyrus, right precuneus, left middle occipital gyrus, and left declive exhibited high positive likelihood ratios.

Conclusion: The present study revealed brain regions with significant hyperperfusion associated with the default-mode network, in addition to abnormalities in the sensory dimension of pain processing and affective-attentional areas in fibromyalgia patients. Furthermore, hyperperfusion in these areas was strongly predictive of poor response to gabapentin.

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P73

Pathogenic protease expression in murine OA is critically dependent upon mechanical joint loading

Annika Burleigh*, Anastasios Chanalaris, Jeremy Saklatvala, Tonia Vincent
Department of cell signalling, Kennedy Institute of Rheumatology, Oxford University, London W6 8LH, UK
Arthritis Research & Therapy 2012, **14(Suppl 1):**P73

Background: Once considered a passive disease of 'wear and tear' of the joint, osteoarthritis (OA) is now known to be driven by the expression and activation of specific proteases that degrade the extracellular matrix of articular cartilage. Such proteases include aggrecanases, principally adistintegrin and metalloproteinase (Adamts) 4 and 5, and collagenases which are members of the matrix metalloproteinase (Mmp) family. In mice, Adamts5 and Mmp13 are considered to be the critical proteases in disease, as mice in which these proteases have been knocked out are protected from developing OA [1,2]. What drives these proteases in vivo is unknown, but one possibility is that mechanical factors alone are sufficient to lead to their expression and activation.
To test this hypothesis we investigated the effects of joint immobilisation on protease expression and the course of disease in mice with surgically induced OA.
Materials and methods: Destabilisation of the medial meniscus or sham surgery was performed in 10 week old male mice. Joints were immobilised either by prolonged anaesthesia (for a max of 4 h to examine gene expression changes) or by sciatic neurectomy (for 4-6 h or for 12 weeks). mRNA was extracted from whole joints at 4-6 h following induction of OA. A microarray was performed and 47 genes validated by RT-PCR. Joints were examined histologically after 12 weeks forcartilage damage.
Results: Many genes were regulated within 6 hours of OA surgery (compared to sham surgery) including Adamts5, Mmp3, IL1b, Ccl2, activin and TNF-stimulated gene 6 (Tsg6). Mmp13 was not regulated at this early time point. Of the 47 genes studied, all gene responses were strongly suppressed if the joint was immobilised (by prolonged anaesthesia). Joint immobilisation by sciatic neurectomy also suppressed a number of genes (approx. 50%) including Adamts5, and protected the joints from cartilage degradation at 12 weeks.
Conclusion: Pathogenic protease expression occurs rapidly upon induction of OA in mice (within 6 h) and is highly mechanosensitive. Suppression of Adamts5 also occurs following sciatic neurectomy in which the joint is immobilised but the mice are able to bear weight (they walk with a 'splinted' knee). This suggests that dynamic flexion of the destabilised knee joint is important for induction of proteases and subsequent disease.The pathway by which joint cells sense and respond to these mechanical signals could represent a novel target for disease intervention.

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P74

Helicobacter pylori infection in rheumatic diseases

Hongyan Wen*, Jing Luo, Junxia Li, Xiaofeng Li
Department of Rheumatology, Shanxi Medical University, The Second Hospital of Shanxi Medical University, 56 South Xinjian Road, Taiyuan, Shanxi Province, China
Arthritis Research & Therapy 2012, **14(Suppl 1):**P74

Background: Due to a number of factors, Helicobacter pylori (Hp) infection is increasingly recognized as highly prevalent in many populations and of increasing health concern. Hp infection has been associated with digestive diseases and rheumatic diseases[1]. It remains unclear whether all or part patients of rheumatic diseases should be routinely screened for Hp infection. We have examined predictors of Hp infection in rheumatic diseases so as to define who might benefit most from screening.
Methods: 292 patients with rheumatic diseases were recruited through outpatient rheumatology clinics between 2005-2008. The study was approved by the Second Hospital of Shanxi Medical University Ethics Committees, and all participating patients signed an informed consent form. The description of this study is 3-fold: to evaluate the relationship between Hp and rheumatic diseases, to assess the relationship between Hp and rheumatoid arthritis (RA), to explore the relationship between Hp and ankylosing spondylitis (AS).
Results: Patients of rheumatic diseases were significantly more likely to be Hp infection than health control (89 vs 42%, $P < 0.01$). The study revealed that 88% of RA patients and 90% AS patients suffer from Hp infection. RA patients carried a diagnosis of Hp, a higher prevalence of the value of CRP was associated with the DAS28(Disease Activity Scor-28) ($r = 0.287, P = 0.034$). AS patients carried a diagnosis of Hp, a higher prevalence of the value of MMP-3(matrix metalloproteinase-3, MMP-3) was associated with the BASDI(Bath AS Disease Activity Index) ($r = 0.435, P = 0.009$).
Conclusions: Patients of RA and AS are associated with a high prevalence of Hp infection rate. Hp infection may be play an important role in RA and AS.
Next steps: Further investigation with other rheumatic diseases are planned.

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P75

Importance of E3 ubiquitin ligase Synoviolin in fibrogenesis

Naoko Yagishita[1], Daisuke Hasegawa[1], Satoko Aratani[1,2], Yoshihisa Yamano[1], Toshihiro Nakajima[1,2,3*]
[1]Institute of Medical Science, St.Marianna University School of Medicine, Kawasaki, Kanagawa 216-8512 Japan; [2]Institute of Medical Science, Tokyo Medical University, Shinjuku-ku, Tokyo 160-8402 Japan; [3]Bayside Misato Medical Center, Kochi 781-0112 Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P75

The symptoms of rheumatoid arthritis (RA) are based on the many processes; chronic inflammation, overgrowth of synovial cells, bone and joint destruction and fibrosis. To clarify the mechanism of outgrowth of synovial cells, we carried out immunoscreening using anti-rheumatoid synovial cell antibody, and cloned 'Synoviolin'. Synoviolin, a mammalian homolog of Hrd1p/Der3p, is endoplasmic reticulum (ER)-resident E3 ubiquitin ligases with a RING motif, and is involved in ER-associated degradation (ERAD). Synoviolin is highly expressed in synoviocytes of patients with RA. Overexpression of synoviolin in transgenic mice leads to advanced arthropathy caused by reduced apoptosis of synoviocytes. We postulate that the hyperactivation of the ERAD pathway by overexpression of synoviolin results in prevention of ER-stress-induced apoptosis leading to synovial hyperplasia. Indeed, synoviolin$^{+/-}$ knockout mice showed resistance to the development of collagen-induced arthritis owing to enhanced apoptosis of synovial cells. In addition, Synoviolin ubiquitinates and sequesters the tumor suppressor p53 in the cytoplasm, thereby negatively regulating its biological functions in transcription, cell-cycle regulation and apoptosis by targeting it for proteasomal degradation. Therefore Synoviolin regulates, not only apoptosis in response to ER stress, but also a p53-dependent apoptotic pathway. These studies indicate that Synoviolin is one of the causative factors of arthropathy. Further analysis using gene targeting approaches showed that in addition to its role in RA, Synoviolin is essential for embryogenesis. Synoviolin deficient (*syno*$^{-/-}$) mice exhibited severe anemia caused by enhancement of apoptosis in fetal liver, and the results suggested that the liver is sensitive organ for Synoviolin.
Thus, this study aimed to explore the involvement of the Synoviolin in fibrosis process of RA using mice model of liver fibrosis. In CCl_4-induced hepatic injury model, *syno*$^{+/-}$ mice are resistant to onset of liver fibrosis. The number of activated HSCs was decreased in *syno*$^{+/-}$ mice, and some of these cells showed apoptosis. Furthermore, collagen expression in HSCs was upregulated by synoviolin overexpression, while synoviolin knockdown led to reduced collagen expression. Moreover, in *syno*$^{-/-}$ MEFs, the amounts of intracellular and secreted mature collagen were significantly decreased, and procollagen was abnormally accumulated in the endoplasmic reticulum. In

conclusion, Synoviolin is involved in not only overgrowth process of synovial cells but also fibrosis process.

P76

Human retrovirus promotes the plasticity of regulatory T cells into T helper type 1-like cells through the T-bet transcriptional activation in neuroinflammatory disease

Yoshihisa Yamano
Department of Rare Diseases Research, Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P76

Recently, it has become increasingly clear that some committed effecter and regulatory T (Treg) cells are not stable, and the plasticity of these T-cells may be related to the pathogenesis of autoimmunity and inflammatory diseases [1]. However, the precise mechanisms that allow for T cell plasticity have not yet been clearly understood. Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus that is associated with multiorgan inflammatorydisorders such as HTLV-1- associated myelopathy (HAM/TSP), HTLV-1- associated arthropathy (HAAP), uveitis, Sjögren syndrome, and polymyositis [2-5]. HTLV-1-infected T cells may contribute to development of these disorders, since the number of HTLV-1-infected T cells circulating in the peripheral blood is higher in patients [6]. HTLV-1 mainly infects $CD4^+$ T helper (Th) cells that play central roles in adaptive immune responses. Based on their functions, patterns of cytokine secretion, and expression of specific transcription factors and chemokine receptors, Th cells differentiated from naïve $CD4^+$ T-cells are classified into 4 major lineages: Th1, Th2, Th17, and T regulatory (Treg) cells. We recently demonstrated that $CD4^+CD25^+CCR4^+$ T cells, which mainly include suppressive T-cell subsets such as Treg and Th2 under healthy conditions, are the predominant viral reservoir of HTLV-1 in both adult T-cell leukemia/lymphoma (ATL) and HAM/TSP [7]. Interestingly, T-cells of this subset become Th1-like cells with overproduction of IFN-γ in HAM/TSP, suggesting that HTLV-1 may intracellularly induce Tcell plasticity from Treg to IFN-γ^+ T cells [7].In this study, using human T-cell line and HTLV-1 infected $CD4^+CD25^+CCR4^+$ T-cells of HAM/TSP patients, the virus-encoded transactivating HTLV-1 Tax protein was demonstrated to induce the IFN-γ production through the expression of T-box 21(Tbx21)/T-bet, a transcription factor that is known to direct the differentiation of naive $CD4^+$ cells into IFN-γ-expressing Th1 cell. HTLV-1 Tax was also demonstrated to enhance promoter activity of Tbx21/T-bet cooperatively with transcription factor Specificity Protein 1 (Sp1). Furthermore, transfer of HTLV-1 tax gene in $CD4^+CD25^+CCR4^+$ T-cells using a lentiviral vector resulted in the loss of regulatory function of these T cells. This is the first report to our knowledge demonstrating the role of a specific viral product (HTLV-1 Tax) on the expression of genes associated with T-cell differentiation resulting in plasticity of Treg cells into Th1-like cells. These results suggest that HTLV-1 infection-induced immune dysregulation may play an important role in the development and pathogenesis of HTLV-associated immunological diseasesthrough its interference in the equilibrium maintained among host immune responses.

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P77

AJAK inhibitor, tofacitinibreduces IL-6 and matrix metalloproteinase-3 productionin rheumatoid arthritis with suppressed cartilage destruction

Kunihiro Yamaoka[1*], Satoshi Kubo[1], Koshiro Sonomoto[1], Keisuke Maeshima[2], Yoshiya Tanaka[1]
[1]The First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan, 807-8555; [2]Department of Internal Medicine I, Faculty of Medicine, Oita University, Japan
Arthritis Research & Therapy 2012, **14(Suppl 1):**P77

Background: Tofacitinib, targeting Janus kiase (JAK) has gained attention as anorally available new disease modifying anti-rheumatic drug with high clinical efficacy against rheumatoid arthritis (RA). While the clinical trial has progressed and the wide usage of tofacitinib is conceivable in the near future, the precise mechanism of action in RA patients remains to be solved.
Materials and methods: Fifteen RA patients enrolled in tofacitinib clinical trial were randomized to 1, 3, 5 or 10 mg BID for 12 weeks. Serumwas collected at 0 and 12 weeks for further cytokine measurement by ELISA.To analyze the effect at the local inflammatory site, synovium and cartilage from a RA patient undergoing joint replacement was implanted to severe combined immunodeficiency (SCID) mice (SCID-huRAg mouse) andtofacitinib was administered via osmotic mini-pump and serological and histological investigation was performed.
Results: Background of patients in clinical trial: mean age; 56.4 years, mean disease duration; 95.1 months, methotrexate (MTX) and tofacitinib were administered in all patients, median doses were 9.4 mg/week and 4.1 mg BID, glucocorticoids were administered in 6 patients, median dose was 5.4 mg/day. Baseline characteristics of the disease activity; SDAI 30.0, DAS28 (ESR) 6.3, HAQ 1.1, CRP 21.0 mg/l, ESR 57.1 mm/h, MMP-3 259.3 ng/ml, RF 216.2 U/ml. After 12 weeks treatment, disease activity decreased with statistical difference ($p < 0.05$) as follows; SDAI13.8, DAS28(ESR) 4.0, HAQ 0.8, CRP 8.1 mg/l, ESR 30.9 mm/h, MMP-3 149.9 ng/ml, RF 150.8 U/ml. Among the multiple cytokines measured, IL-6 and IL-8 tended to decrease, from 52.2 pg/ml to 28.2 pg/ml ($p < 0.05$) and from 41.7 pg/ml to 29.5 pg/ml (not significant), respectively. There was a statistically significant correlation between reduction of IL-6 and reduction of MMP-3.
In SCID-huRAg mouse, apparent invasion of RA-derived synoviuminto cartilage was observed, whileadministration of tofacitinibmarkedly suppressed invasion. In order to investigate the relevance with our findings from the patients in the clinical trial, cytokines in SCID-huRAg mouse serum was measured after administration of tofacitinib for 7 days. Interestingly, tofacitinib significantly decreased production of human IL-6 and IL-8 as well as human MMP-3 from 29.79 pg/ml to 2.89 pg/ml, 17.89 pg/ml to 4.22 pg/ml and 65.96 pg/ml to 33.13 pg/ml respectively.
Conclusions: Tofacitinib improved disease activity and suppressed cartilage destruction with decreased serum IL-6 and IL-8 in both, RA patients and SCID-huRAg mouse in connection with reduced MMP-3. These results indicate that tofacitinib reduces inflammation by suppressing IL-6 production and consequently inhibiting cartilage destruction in the initial several months of administration.

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Regulation of macrophage-mediated chronic inflammation by JAK inhibitors

Anna Yarilina[1*], Kai Xu[1], Chunhin Chan[1], Lionel B Ivashkiv[1,2]
[1]Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, USA; [2]Weill Medical College of Cornell University, New York, NY, 10021 USA
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Small molecule inhibitors of the Janus kinases (JAK) have been developed as anti-inflammatory and immunosuppressive agents and are currently subjects of clinical trials. Tofacitinib/CP-690,550 (more potent in inhibiting JAK3 and JAK1) and Ruxolitinib/INCB-018424 (selective inhibitor of JAK1/2) have demonstrated clinical efficacy in rheumatoid arthritis (RA), however, the exact mechanisms that mediate the inhibitory effects of these compounds are not known.
In this study, we examined the effects of CP-690,550 (CP) and INCB-018424 (INCB) on inflammatory responses in human macrophages (hMΦs). In our

study, we used long term exposure to TNF as a model of chronic inflammation to investigate mechanisms regulating hMΦ activation and functions, and have shown that TNF can activate an IFN-JAK-STAT-dependent autocrine loop that regulates expression of pro-inflammatory chemokines and interferon stimulated genes (ISGs), followed by an increase of NFATc1, that regulates osteoclastogenesis.
As expected, both inhibitors abrogated TNF-induced STAT1 activation and expression of genes encoding inflammatory chemokines (*CXCL9, 10, 11* and *CCL5*) and ISGs (*IFIT1* and *2*, *IRF7*). Interestingly, both compounds attenuated a late wave of *IL-1* induction and nuclear expression of NF-κB subunits. Furthermore, ex vivo treatment with inhibitors decreased *IL-1* and *IL-6* expression in synovial MΦs isolated from the patients with arthritis. Next, we analyzed the effects of JAK inhibitors on TNF-induced osteoclastogenesis and discovered that both compounds augmented nuclear levels of NFATc1 and cJun, followed by increased formation of TRAP positive multinuclear cells. Lastly, we examined an in vivo effect of CP on innate immune response in arthritis using K/BxN serum transfer arthritis model and found that CP treatment significantly inhibited inflammation and joint swelling. Taken together, our data suggest that JAK inhibitors can affect inflammatory responses in hMΦs and thus, can target both acquired and innate immunity in RA and other chronic inflammatory diseases.

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Th17 is involved in the pathogenesis of Bechet's disease via CCL20-CCR6 axis

Hidekata Yasuoka[1*], Zhu Chen[1,2], Tsutomu Takeuchi[1], Masataka Kuwana[1]
[1]Department of Internal Medicine, Keio University School of Medicine, Tokyo, 160-8582, Japan; [2]Department of Rheumatology and Immunology, Anhui Provincial Hospital, Hefei, 230001, China
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Background: Behcet's disease (BD) is an autoinflammatory disease with a unique distribution characterized by uveitis, and mucosal and skin lesions, which are characterized by the prominent infiltration of immune cells such as lymphocytes and neutrophils. A novel helper T-cell subset Th17, IL-17-producing helper T cells, has been appreciated [1]. IL-17 is involved in the induction of a series of chemokines, growth factors, proteases, and cytokines, and production of IL-17 results in induction of neutrophil migration and chronic inflammation [2]. Based on these findings, we hypothesized that Th17 is involved in the pathogenesis of BD.
Materials and methods: To examine a role of Th17 response in the pathogenic process of BD, peripheral blood samples from 20 patients with BD and 14 controls were used to evaluate phenotypic and functional properties relevant to the Th17 response. Plasma IL-17 and CCL20 levels were examined using ELISA. Expression levels of RORC mRNA in $CD4^+$ T cells were examined by RT-PCR and $CD4^+$ cells expressing IL-17, CCR6 was examined by flow cytometry. Evaluation of chemotaxis of $CD4^+$ T cells toward CCL20 was examined by migration assay using TransWell® double chamber system.
Results: Plasma IL-17 was higher in active BD compared with healthy controls ($P < 0.05$). Expression levels of RORC mRNA in peripheral blood mononuclear cells by RT-PCR and proportion of $CD4^+$ cells expressing intracellular IL-17 were increased in patients with BD than in controls ($P < 0.05$ in both comparisons). Expression of chemokine receptor CCR6 was detected in nearly all IL-17-expressing cells. The proportion of $CD4^+CCR6^+$ was higher in BD patients in remission compared those with active disease ($P < 0.05$), suggesting that these cells are migrated to the lesions at active disease phase. In addition, $CD4^+$ T cells from BD patients had enhanced migration capacity induced by CCL20, than did those from controls. Finally, CCL20 level was higher in BD patients than in controls ($P < 0.05$).
Conclusions: These results together suggest that Th17 are involved in the pathogenesis of BD by migrating into the lesions of BD through the CCL20-CCR6 axis.
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The association of autoantibodies expression, Th1/Th2 cytokines balance and IFNG polymorphism with histological phenotype of lupusnephritis

Kusworini Handono[1*], Atma Gunawan[2], Singgih Wahono[2], Rulli Rosandi[2], Wivina[1], Natalina Mallo[1], Handono Kalim[2]
[1]Clinical Pathology Department, Medical Faculty Brawijaya University/Dr Saiful Anwar Hospital Malang, Indonesia; [2]Internal Medicine Department, Medical Faculty Brawijaya University/Dr Saiful Anwar Hospital Malang, Indonesia
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Background: Racial differences were observed in clinical, serologic and histologic presentation of lupus nephritis (LN)[1]. It has been suggested that Th1/Th2 cytokines balance and IFNG polymorphism play important role in the development of different pathologic pattern of lupus nephritis (LN)[2-4]. The objective of our study is to determine the association between autoantibodies expression, Th1/Th2 cytokines balance and IFNG polymorphisms with pathologic class of LN in Javanese patients.
Patients and methods: We studied 60 female patients with LN (ARA criteria,1992), and 20 healthy individual as control. Histopathologic classification was based on WHO criteria (1995). Anti ds-DNA, anti RO, anti nRNP and anti Sm autoantibodies were assayed by ELISA.IFNg-IL-4 balance were used to assess Th1/Th2 cytokines balance, IFNγ and IL4 serum levels assayed by ELISA. Microsatelitepolymorphisms within the first intron of the IFNG gene on chromosome 12q24.1 was performed by DNA sequencing. The association of histopathologic phenotype of LN with Th1/Th2 balance (IFNγ/IL4),and autoantibodies expression were analysed by Chi-square and Student T test with $p < 0.05$ is significant. The IFNG allele difference between LN classes were analysed by Chi-square. The risk of LN in patients with certain IFNG allele was calculated using Odds Ratio.
Results: Our study showed that the frequency of anti Ro, and anti nRNP antibodies in patients with LN WHO class III, IV and V LN weresignificantly higher compared with patients with class I and II LN. There is no autoantibodies expression differences between class III, IV and clas V LN. The IFNγ/IL4 ratio in patients with classIII and IV LN was significantly higher than patients with class I,II and class V LN (2.30 ± 0.89 vs 0.94 ± 0.24 and 0.69 ± 0.30, $p = 0.000$), but the serum level of IL4 in patient with WHO class III and IV was significantly lower than class V (77.72 ± 40.28 pg/ml vs 145.68 ± 71.21 pg/ml. $p = 0.014$). The result showed that the activity of Th1 immune response tent to be higher in patient with WHO class III and IV LN. The frequency of IFNG 112 allele were higher in patients with SLE compared with healthy controls (55% vs 25%, $p = 0.0471$) and the risk to have LN class V in patients with IFNG 112 was 6 times higher compared with patients without these allele (OR 6.1, CI 95% 3.1- 12.4, $p = 0.0427$).
Conclusion: The results showed different underlying mechanism of inflammation in different pathologic class of LN.
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