Meeting report
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Abstract

Major advances in technology now drive how we approach questions in immunology, particularly in analyzing complex data sets commonly encountered in genomics and proteomics studies. Active areas of investigation include development of novel technologies, identification of elusive target antigens for RA and other diseases, dissection of signaling pathways connecting the lymphocyte cell surface with the nucleus, and exploration of new avenues for therapeutic interventions. The European Workshop for Rheumatology Research (EWRR) is a forum for many European and non-European scientists to present research findings of high quality. Arthritis researchers from around the globe should be strongly encouraged to attend future meetings, the next of which is the 22nd EWRR meeting in Leiden, the Netherlands, in 2002.

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Introduction
Vienna provided a delightful forum for the 21st European Workshop for Rheumatology Research (EWRR), March 1–4, 2001, hosted by Professor Josef Smolen. In addition to a reception with the mayor and governor of Vienna, a 'Viennese evening,' and a premeeting dinner and guided tour hosted by world-reknowned figurative painter Adolf Frohner, the program consisted of some novel and insightful presentations by a multitude of arthritis researchers. Participants in the meeting were from Europe, Asia, the Middle East, and the United States, demonstrating that the 'European' EWRR meeting is gaining increasing prominence in the worldwide rheumatology community. The meeting also featured cutting-edge talks from scientists working in industry, highlighting the increasing interaction between academicians and biopharmaceutical scientists. The author apologizes that only a subset of studies is presented and that the sessions on 'Connective tissue and bone remodeling' and 'New aspects on pathogenesis and therapy' are not reported because of incomplete attendance by the author. This year's meeting was the first to schedule concurrent workshops, precluding reporting of all areas of interest.

Novel technologies
The meeting opened with a series of six lectures describing new techniques. Like other areas of medical science, rheumatology is undergoing rapid transition to a field dominated by powerful new technologies. These are likely to replace the traditional methods used to diagnose rheumatic disease, to identify novel therapeutic targets, and to elucidate the pathogenesis of autoimmunity.

EWRR = European Workshop for Rheumatology Research; HC = healthy controls; IL = interleukin; NF = nuclear factor; OA = osteoarthritis; RA = rheumatoid arthritis; TNF = tumor necrosis factor.

The central dogma of 'medical' cell biology is that DNA is transcribed into RNA, which is translated into protein, which is the target of therapeutic agents. It is protein that governs cell structure and function, and a dominant theme of the meeting was that human autoimmune disease is manifested at the level of protein expression. Klaus Wilgenbus (Vienna, Austria) reviewed current methods used to perform transcriptional profiling, including the use of 'spotted' DNA microarray technology and commercially available DNA chips. An application of microarrays was elegantly demonstrated in a later poster presentation by U Ungethüm (Berlin, Germany; P45), in which DNA microarray data from synovial tissue derived from healthy controls (HC) or from patients with osteoarthritis (OA) or rheumatoid arthritis (RA) were compared. These results were then further compared with those obtained using a PCR-based amplification (amplicon) technique. Potentially interesting molecules found to be upregulated (in RA vs HC) included megakaryocyte-stimulating factor, tumor necrosis factor (TNF)-α, cathepsin B, and tissue inhibitor of metalloproteinases (TIMP) 3, as well as (RA vs OA) natural-killer-cell protein NKp58, ribosomal proteins, and collagen. Interestingly, the results found varied with the technique used, highlighting the fact that not all transcriptional profiling methods are created equal.

In the next presentation, the author (PJU, Stanford University, USA) described work performed by William Robinson (in collaboration with Lawrence Steinman), in which autoantigen microarrays containing hundreds of diverse antigens including proteins, peptides, ribonucleoproteins, enzyme complexes, and nucleic acids were spotted and detected by serum autoantibodies on microscope slides. Major autoantigens were detectable in a high-throughput format, including common antigenic targets in systemic lupus erythematosus, scleroderma, RA, myositis, primary biliary cirrhosis, mixed-connective-tissue disease, and Sjögren's syndrome. These arrays should prove useful for performing multiplex serum autoantibody profiling of many human autoimmune diseases and also of autoimmunity in animal models.

Three lectures then described unique approaches to the development of therapeutic agents for treatment of autoimmune disease. 'SELEX' (systematic evolution of ligands by exponential enrichment) is a method used to identify oligonucleotides, ligands or 'aptamers'. Renée Schroeder (Vienna) described SELEX studies, in which libraries of RNA 'aptamers' were screened to identify unique RNA sequences capable of binding a ligand of interest. The approach is similar in concept to identifying a monoclonal antibody. Such ligands include amino acids, cofactors, proteins, antibiotics, and other therapeutics. RNA aptamers are amenable to chemical modifications that might allow them to be used directly as therapeutic agents in clinical medicine, with the potential to revolutionize

drug discovery in much the same way that monoclonal-antibody therapies currently used in clinical rheumatology practice have done. Manfred Auer (Vienna) reported on studies at Novartis using 'AIDA' nanoscreening technology, an on-bead screening assay that allows fluorescent detection of drug-binding to candidate targets, followed by rapid structural decoding. Finally, Ernst Wagner (Vienna) reviewed advances in naked DNA technology and the use of retroviruses, adenoviruses, and adeno-associated viruses to encode immunoregulatory proteins such as IL-1 receptor antagonist, IL-4, IL-10, and a TNF-receptor immunoglobulin Fc fusion protein. Although not discussed by Dr Wagner, DNA vaccine technology has also recently been applied to deliver autoantigens as tolerizing agents and will certainly be expanded in scope during the next decade.

Antigens and antibodies in rheumatoid arthritis

Since their identification nearly 40 years ago, antiperinuclear factor and antikeratin antibodies had been all but forgotten before being rediscovered in the 1990s in the laboratories of Walther van Venrooij (Nijmegen, the Netherlands) and Guy Serre (Toulouse, France). The dominant B cell epitope has been definitively identified as one containing citrulline residues, formed by enzymatic deimination of the amino acid arginine. The precise citrulline-containing antigen that may be responsible for driving the synovitis in RA remains elusive. Guy Serre presented data, published soon after the meeting, identifying a candidate citrullinated antigen [1]. His laboratory purified proteins from synovial tissue and identified two molecules, of 66 kDa and 70 kDa, respectively, that reacted with antiperinuclear factor on western blots. Surprisingly, the proteins were identified as the α and β chains of fibrin. In contrast, TJ Smeets and colleagues (Nijmegen, the Netherlands; P4) presented a poster in which they were unable to demonstrate differential binding of anticitrulline antibodies to synovium in patients with RA vs controls. Whether the α and β chains of fibrin represent the antigens driving the autoimmune response in RA is an intriguing possibility that will require further investigation.

A number of posters and discussions described the utility of clinical tests for RA that are based on the above discoveries. I Hromadenikova (Prague, Czech Republic; P1) presented data suggesting that antikeratin antibody may be useful in the diagnosis of juvenile rheumatoid arthritis, while J Vencovsky (Prague; P2) discussed preliminary data regarding the prediction of erosive disease. Three groups described studies using different substrates and different methods in analyzing serum from fairly large populations of patients with arthritis. The results (summarized in Table 1) are of great importance as these diagnostic tests gain increasing acceptance in clinical labs throughout Europe and (hopefully) the United States.

Christophe Benoist updated experiments performed in collaboration with Diane Mathis using their K/BxN mouse model for RA. The details of this interesting model, in which antibodies directed against the protein glucose-6 phosphate isomerase contribute to disease pathogenesis, have been reviewed recently in this journal by Hugh McDevitt [2]. Four unpublished observations were noted by these investigators and presented by Dr Benoist. First, the disease is heterogeneous and frequently asymmetric in presentation, with only certain joints (even within the same limb) becoming involved. Second, antibodies and complement are detectable in the intimal synovial layer. Third, experiments using knockout mice have demonstrated a requirement for the activating receptor FcγRIII and components of the alternative pathway of complement activation. Finally, highly preliminary studies in humans have identified serum antibodies directed against glucose-6 phosphate isomerase in 52% of patients with inflammatory arthritides, including RA and systemic lupus erythematosus, but in only 6% of healthy controls.

Two other poster discussions generated significant interest because of their relation to stress responses. S Hayer and colleagues (Vienna; and Athens, Greece; P11) described studies demonstrating upregulation of the RA autoantigen hnRNP-A2/RA33 in joints of RA patients, and in TNF-α transgenic mice. Surprisingly, the molecule, which is predominantly nuclear in localization, was also present in the cytoplasm of RA synovial cells, particularly the macrophages of the lining layer and the fibroblasts of the sublining layer. U Neuhaus-Steinmetz and colleagues (Berlin, Germany; Ghent, Belgium; Bad Liebenwerda, Germany; P13) identified the stress protein BiP as a novel autoantigen in RA. Antibodies were found in 63% of a cohort of 400 patients with RA by western blot analysis, but in only 3.5% of patients with other rheumatic diseases and in less than 1% of healthy controls. These results are in agreement with the recently reported findings of Corrigal, Panayi, and their colleagues [3].

Cytokine signaling and autoimmunity

The previous section described advances in understanding the role played by antigen in driving diseases such as RA. However, blockade of 'downstream' effectors – inhibition of inflammatory cytokines – has taken center stage in the treatment of arthritis and inflammatory bowel disease. One session of the meeting described advances in this area. Marc Feldmann (London, UK; L17) provided an overview of cytokine blockade in arguably the most interesting presentation of the conference. Professor Feldmann challenged the role of T cells in RA (i.e. as important or innocent bystanders). In RA, TNF-α production is T-cell dependent, is secreted by macrophages (and perhaps other cells), and is a 'rate-limiting' molecule. Thus there exists a clear reason to block the actions of TNF. T cells can be differentially regulated or activated, e.g. in an

Table 1

Laboratory tests for citrullinated antigens

Author	Substrate	Method	Sensitivity	Specificity	Comments
Bozic (P4)	Cyclic citrullinated peptide (CCP)	ELISA	58%	ND	Tested 97 RA patients who were RF-positive or RF-negative. Small study, lower sensitivity than previously reported.
Union (P6)	In vitro citrullinated filaggrin; 2 citrullinated peptides	Line immunoassay	65% (using 3 markers); 61.8% (using 2 peptides)	97.6% (using 3 markers); 98.4% (using 2 peptides)	Tested 335 RA patients and 254 patients with non-RA rheumatologic conditions retrospectively. Results similar to those reported previously for CCP.
Vincent (P7)	Deiminated recombinant rat filaggrin (ArFA); antikeratin antibodies	ELISA+ indirect immuno-fluorescence of rat esophagus	67%	<99%	Tested 714 patients with rheumatic disease, of whom 241 had RA. Requires sequential testing to maintain utility at 99%. The sensitivity for arFA ELISA alone (at 95% specificity) was 75%.

ELISA = enzyme-linked immunosorbent assay; ND = no data; RF = rheumatoid factor.

antigen-receptor-specific manner (via TCR/CD28 stimulation) and in an antigen-independent fashion (via interleukins such IL-6, and TNF). Unfortunately, blockade of the TNF pathway has the potential to inhibit host defense as well as preventing systemic inflammation. This prediction has been fulfilled by a significant increase in cases of reactivation tuberculosis in patients treated with TNF antagonists, particularly in European patients. Professor Feldmann described the work of B Foxwell, who has explored selective inhibition of cytokine-receptor signaling pathways (e.g. IL-6, IL-2, TNF, and others) that may allow prevention of the synovial inflammatory response without compromising host immunity. These include compounds such as Wortmannin and Ly290402 (PI-3 kinase inhibitors) and overexpression of the natural NF-κB inhibitor, I-κB.

The work of N Busso and colleagues (Lausanne and Geneva, Switzerland; P23) described the surprising finding that mice deficient in leptin and its receptor are significantly less sensitive to collagen-induced arthritis than are wild type animals, as assessed by histology, lymphocyte proliferation assays, and technetium-99 scanning of joints. This result is perhaps less surprising when one considers that leptin and its receptor are homologous to the respective cytokine/receptor pairs IL-6 and IL-11. John O'Shea (Bethesda, MD, USA) presented data in which his group has dissected the JAK/STAT signaling pathway. His studies have identified JAK3 as a potential therapeutic target, as well as P38 mitogen-activated protein kinase (MAPK). A MAPK inhibitor has been developed that blocks the production of interferon γ and possibly prevents the phosphorylation of STAT4.

Apoptosis and autoimmunity

LA Casciola-Rosen and colleagues have published a number of important studies over the past decade implicating apoptosis in connective tissue diseases (reviewed [4]). Presentations by K Nishioka (Kawasaki, Japan; L14) and B Sauter (Erlangen, Germany; L15) of largely published work underscored the importance of developing a better understanding of apoptosis in RA and other diseases. Factors governing lymphocyte and synoviocyte apoptosis, as well as processing and presentation of apoptotic material by antigen-presenting cells including dendritic cells, will continue to be key areas of research. Guido Kroemer (Villejuif, France; L16) presented elegant studies, in press in the journal *Nature,* exploring mechanisms involved in caspase-independent death. Apoptosis-inducing factor, a mitochondrial protein that translocates to the cytoplasm and nucleus in response to certain death stimuli, plays a central role in this process. This factor promotes chromatin peripheralization and condensation. Using an *in vitro* embryogenesis model, Kroemer showed that the factor is required for progression from the morula to the blastula stage, because a subset of the morula cells are unable to undergo apoptosis, which is a requirement for formation of the blastula cavity. AIF is required for large-scale DNA fragmentation (as opposed to internucleosomal DNA cleavage mediated by caspase-activated DNase, and unlike cytochrome *c*, AIF's function is clearly independent of caspases. An important take-home point of these studies is that many of the *in vitro* measures currently used in rheumatology research to 'define' an apoptotic cell rely on indirect detection of activated caspases, e.g. via DNA fragmentation or identification of proteolytic fragments of caspases or their target substrate proteins. The apoptotic cell phenotype now clearly requires redefinition.

Summary

Major advances in technology now drive how we approach questions in immunology, particularly in analyzing complex data sets commonly encountered in genomics and proteomics studies. Active areas of investigation include development of novel technologies, identification of elusive target antigens for RA and other diseases, dissection of signaling pathways connecting the lymphocyte cell surface with the nucleus, and exploration of new avenues for therapeutic interventions. The EWRR represents a forum for many European and nonEuropean scientists to present high-quality research findings. Arthritis researchers from around the globe should be strongly encouraged to attend future meetings, beginning with the 22nd EWRR in Leiden in 2002.

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