mAbs 4:4, 426-433; July/August 2012; © 2012 Landes Bioscience

1st French-Israeli international conference on B cells and therapeutic antibodies

October 23–25, 2011, Jerusalem, Israel

Claude-Agnès Reynaud,[1] Sandrine Moutel,[2] Marie-Caroline Dieu-Nosjean,[3] Reuven Laskov[4] and Jean-Luc Teillaud[3,*]

[1]INSERM U. 783; Paris Descartes University; Faculty of Medicine; Necker-Enfants Malades site; Paris, France; [2]Translational Research Department; CNRS UMR144; Institut Curie, Paris, France; [3]Cordeliers Research Center/INSERM U.872; Paris Descartes University and Pierre & Marie Curie University; Paris, France; [4]Department of Developmental Biology and Cancer Research; The Institute for Medical Research Israel-Canada; The Hebrew University-Hadassah Medical School; Jerusalem, Israel

Key words: antibody engineering, antibody therapeutics, B cell

Introduction

The 1st French-Israeli International Conference on B Cells and Therapeutic Antibodies, organized by Reuven Laskov (Israel), Jean-Luc Teillaud (France) and Claude-Agnès Reynaud (France) under the auspices of The Hebrew University of Jerusalem and the University Paris Descartes, was held October 23–25, 2011 in Jerusalem, Israel. The conference featured 36 presentations and a dozen posters. It included two special guest lectures given by Michael Neuberger (Laboratory of Molecular Biology, Medical Research Council, Cambridge, United Kingdom) and by Matthew Scharff (Department of Cell Biology, Albert Einstein College of Medicine, New York, USA). The total number of delegates exceeded 130.

The goal of the meeting was to allow foreign academic scientists, clinicians and scientists from biotechnology companies working on B cells and antibodies to meet and exchange ideas with their Israeli colleagues, and to trigger fruitful collaborations and scientific exchanges. It was organized to ensure complementarity between the academic and industry conferences. The meeting also enabled young Israeli scientists and students to freely attend a world-class conference. It included Israeli, European and Canadian speakers, either academic or from companies. A poster session was organized to allow young European and Israeli scientists to present their work.

One part of the meeting was devoted to fundamental immunology focusing on B cells and antibodies, including sessions on somatic hypermutation (SHM), gene rearrangement, FcγR/IgG interactions, B cell subsets and memory B cells. Another part of the meeting focused on the engineering of therapeutic antibodies and on their clinical use. It included sessions on affinity maturation techniques, optimization of antibody effector functions, and updates on antibodies in the clinic.

Brief summaries of the presentations are included here to give a flavor of the meeting, which was held in a friendly atmosphere, with the helpful support of many of our Israeli colleagues from the Hadassah Medical School of the Hebrew University of Jerusalem. Delegates could enjoy a visit to the Old City of Jerusalem. The success of this first meeting paves the way for planning the 2nd French-Israeli International Conference on B Cells and Therapeutic Antibodies in late 2013 or early 2014 in Israel.

*Correspondence to: Jean-Luc Teillaud; Email:jean-luc.teillaud@crc.jussieu.fr
Submitted: 04/05/12; Accepted: 04/05/12
http://dx.doi.org/10.4161/mabs.20289

Introductory Session

Claude-Agnès Reynaud

"So all that we seem to have acquired is the potential ability to select from an animal any of the antibodies of his repertoire. It is somewhat like selecting individual dishes out of a very elaborate menu: antibodies "à la carte". But surely our "immunological gourmandizing" cannot be satisfied by the menu that the animals are offering to us, astonishingly comprehensive and varied as it may be (...). I am sure that our next step will be to move away from the dining table, where we order and consume our antibodies "à la carte," to the kitchen, where we shall attempt to mess them up!"

(Milstein C. Proc R Soc Lond B 1981; 211:393–412, cited by M. Neuberger).

In the first special guest lecture, **Michael Neuberger** (MRC Laboratory of Molecular Biology) provided an overview of what makes antibodies so successful from both fundamental and applied points of view. A specific emphasis was put on the main actor of affinity maturation, i.e., the activation-induced (cytidine) deaminase (AID) mutator. AID is a portable mutator, the activity of which can be engineered to generate high-affinity mutants, and is also tightly controlled in vivo, its actual catalytic activity representing a compromise between efficient mutagenesis and minimal off-target damage.[1] Examples of recently identified interactors controlling AID stability or subcellular localization were reported, notably REGgamma, mediating a non-ubiquitin proteasomal degradation pathway, and eEF1A, a factor delivering tRNA to the ribosome and sequestering AID in the cytoplasm. Multiple approaches toward high affinity human antibody generation were presented, spanning from human transloci in the mouse for the generation of heavily mutated anti-HIV antibodies, to Burkitt lymphoma hypermutating cell lines, or the use of

the easily targetable avian DT40 cell line to select high affinity scFv variants inserted at the endogenous Ig locus.

An overview of human monoclonal antibodies (mAbs) generated through Epstein-Barr virus (EBV) immortalization was given by a pioneer of the field, **Michael Steinitz** (The Hebrew University-Hadassah Medical School), who discussed passive immunization approaches with fully tolerable, fully human antibodies. He reminded the audience that the idea of producing human mAbs was inspired by the finding that EBV efficiently immortalizes in vitro human B cells. Unlike other in vitro and in vivo methods proposed to generate fully human or humanized antibodies, the antibodies obtained following EBV transformation and subsequent molecular cloning result from the in vivo responses of immunized human donors. Hence, they do not contain T cell epitopes, minimizing the risk of developing HAGA (human anti-globulin antibody) reactions. The approach of mining the natural B cell repertoire for auto-antibody specificities of functional relevance was therefore developed, exemplified through the identification of anti-amyloid β natural antibodies in almost every healthy individual.[2] The possible clinical relevance of the infusion of such antibodies is proposed, based on mouse models of Alzheimer disease.

Session 1—The Life of a B Cell: Diversity, Memory, Aging and Death

Claude-Agnès Reynaud and Sandrine Moutel

Claude-Agnès Reynaud (INSERM; Paris Descartes University) reported recent data from a mouse transgenic model based on an AID-controlled, tamoxifen-inducible Cre reporter system, in which the long-term follow-up of memory B cells can be achieved.[3] This model has allowed the description of IgM and IgG memory subsets with different functions: an effector function for IgG memory, giving rise to antibody secreting cells; and a central memory function for IgM memory, reinitiating the germinal center reaction. The effective role of the IgM subset has been recently questioned, and Dr. Reynaud proposed that its mobilization might require a persistent germinal center response to override the inhibition mediated through FcγRIIB binding by antigen-specific IgG.

Doron Melamed (Technion; Israel Institute of Technology) discussed the issue of B cell immunosenescence, a process occurring through accumulation of long-lived peripheral B cells, with a decreased efficiency in activation and antibody response to vaccination and to infectious agents. Interestingly, B cell depletion appears able to rejuvenate the B cell compartment,[4] indicating that aging for B cells is a peripheral process, not a central one. The use of B cell depleting therapies as an anti-age treatment is an interesting and provocative notion, but not yet a therapeutic indication.

Idit Shachar (Weizmann Institute of Science) described the role of CD74 and its interaction with macrophage migration inhibitory factor (MIF) in the induction of a c-Met operated signaling cascade that conditions B cell survival. Interestingly, this survival pathway operates also in chronic lymphocytic leukemia (CLL) in humans, mediating the upregulation of bcl2 and an activation receptor of the SLAM family (CD84), leading to the production of IL-8 that promotes cell survival.[5] Interfering with various steps downstream of this signaling pathway could thus represent a therapeutic option in CLL.

Returning to the question of affinity maturation in the second guest lecture, **Matthew Scharff** (Albert Einstein College of Medicine) concluded the meeting with his recent work on mismatch repair. Professor Scharff discussed the paradox of the diversion of repair pathways like mismatch repair, which is dedicated in most cells to the repair of replication errors, but mobilized in hypermutation of immunoglobulin genes in B cells for fixing and spreading mutations around the initial sites of AID-mediated deaminations.[6] He reviewed the cascade of mismatch repair complex (MMR) factors and repair signals that are diverted from their canonical error-free role and hijacked by B cells to promote genetic diversification of the Ig locus. Now that the process of mutagenesis is well-described, understanding how mutagenesis takes over repair remains the next challenge. It has obvious biotechnological consequences if engineering of other actors of this unique mutagenic process could also be tailored for affinity maturation in vitro, as discussed at the beginning of the meeting by Professor Neuberger for AID.

Recent advances in the knowledge of human memory B cell compartment are now exploited to derive fully human antibodies. **Esther Breij** (AIMM Therapeutics) described an efficient method for generating antigen-specific B cell lines.[7] Human $CD27^+$ memory B cells are isolated from peripheral blood and modified by introducing BCL6 and Bcl-x_L genes by retrovirus-mediated gene transfer. The culture of these cells with CD40L and IL-21 converts them to highly proliferating, cell surface B cell receptor-positive, Ig-secreting B cells with features of germinal center (GC) B cells, including expression of AID. These cells can then be maintained in culture for several months and used as a source of antibodies for further engineering. The method was used to select and engineer powerful neutralizing antibodies against respiratory syncytial virus (RSV). This method provides a new tool for the rapid generation of high affinity human mAbs.

Another efficient technology to screen, isolate and produce human antibodies was then presented by **Majid Mehtali** (Vivalis). This method, developed in Japan, is based on the use of microwell array chips and allows the screening of thousands of single B cells by ELISA.[8] After two weeks of culture, RNA of selected cells are retrieved and antibody genes cloned and produced as recombinant mAbs. The method also allows isolatation of antigen-specific B cells present at low frequency ($<2 \times 10^{-8}$) in peripheral blood of human donors. This technology has been successfully applied by Vivalis for generatation of high affinity mAbs against an infectious target. Finally, Dr. Mehtali described the use of the EB66® cell line, derived from duck embryonic stem cells, to produce low-fucosylated mAbs with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC).[9] These cells can proliferate in suspension in stirred-tank bioreactors to reach high cell densities in serum-free media, with a yield of mAb production of about 1 g/L.

Session 2—Molecular Events: The Making of an Antibody Molecule

Reuven Laskov, Jean-Luc Teillaud and Sandrine Moutel

Several lectures were devoted to studies on different aspects of the regulation of antibody diversity, such as allelic exclusion, SHM, class switch recombination and some of their implications in normal and pathological states. **Yehudit Bergman** (The Hebrew University; Hadassah Medical School) summarized her studies on the mechanisms of allelic exclusion of the murine kappa light chain gene and showed that this important biological event, which eventually results in the expression of only one B cell receptor in single B cells, is epigenetically regulated at several stages of B cell development. It starts at the early embryonic stages where there is an asynchronous replication of the two kappa light chain alleles in a stochastic manner, where the "early-replicating allele" serves later as a preferential target for epigenetic modifications of its histone-chromatin structure in the pro-B cell stage. Later at the pre-B cell stage, these epigenetic changes dictate the V(J) rearrangement of the modified allele, due to mono-allelic binding of RAG proteins.[10]

Reuven Laskov (The Hebrew University; Hadassah Medical School), described features of SHM in the IgVH alleles in EBV transformed human B cell line, an experimental model system that lacks antigenic selection and enables study of the intrinsic properties of SHM. Similar to the in vivo situation, the mutations were preferentially targeted to the C/G nucleotides in the classical WRCH/DGYW hotspot motifs, and not to A/T nucleotides, suggesting that they were generated during the first stage of AID activity. Lineage tree analysis of the accumulating mutations showed the presence of a significant fraction of independent repetitive mutations and also of two repetitive "mutational-clusters" in the productive IgVH allele. Interestingly, 4 out of the 5 hypermutable sites of these clusters reside in non-hotspot motifs, suggesting that, in addition to the hotspot targeted sites, some transcriptional dependent secondary DNA structures significantly contribute to SHM.[11]

Bernardo Reina-San-Martin (Institut de Génétique et de Biologie Moléculaire et Cellulaire) found that specific epigenetic changes are critical for directing the process of class switch recombination (CSR) and generating different antibody isotypes during the immune response. AID is already known to be critical in the initiation of this process by binding to the switch regions (S) and inducing double strand DNA breaks that are a pre-requisite for CSR. He also showed that, both in vitro and in vivo, AID can form a tertiary complex with KAP1 (KRAB domain-associated protein 1), a transcription co-repressor factor and with HP1 (heterochromatin protein 1), a heterochromatin binding protein, and that this complex preferentially associates during transcription with modified histone sites (trimethylated histone H3 at lysine 9, H3K9me3) located in the donor S-regions. It is proposed that this association generates, during transcription of the S-regions, both double strand DNA breaks and somatic point mutations that promote CSR. Of note, the use of KAP1 conditional knockout mice indicated that this transcription factor is required for efficient CSR, but dispensable for SHM.[12]

Michel Cogné (CNRS; Limoges University) described the important role of the cis-regulatory regions located into the constant IgH gene cluster or in its immediate downstream 3' regulatory region (3' RR) encompassing the four hs3a, hs1, 2, hs3b and hs4 transcriptional enhancers, on immunoglobulin B cell receptor expression in mature B cells. Transgenic and knockout murine models of far-apart regions of this locus helped to elucidate the role of their interactions on Ig gene expression, such as shutting-off the expression of the IgH locus in activated mature B cells. Deletion of the RR region from the mouse genome demonstrated that this region only controls IgH locus during the late stages of B cell differentiation.[13] Professor Cogné also described a new recombination mechanism, that takes place in pseudo-switch sequences located within this 3' RR region and mediates the complete deletion of the heavy chain locus. This is a new "suicide mechanism" for B cells that is most probably of physiological relevance because these pseudo-switch elements are conserved between species.

Bertrand Nadel (CNRS; INSERM; Méditerranée University) presented his studies on the early stages of follicular lymphoma (FL) in humans, an incurable long-term disease that originates from the germinal center B cells, and that represents the second most common adult lymphoid neoplasia. Molecular analyses in healthy donors and FL patients indicated that subversion of the molecular processes occurs all along B cell differentiation in vivo, leading to the generation of pre-tumoral clones and to their homing in "niches" in lymphoid organs. It was emphasized that novel methods of studying B cell molecular characteristics allow better insights into the earliest stages of FL and may differentiate between "healthy carriers" and "early patients", which may eventually aid in the development of earlier diagnostic tools.[14]

Dan Eilat (The Hebrew University; Hadassah Hospital) explored whether receptor editing of the light chain or allelic inclusion can explain the failure of self-tolerance and the development of autoimmune systemic lupus erythematosis (SLE) in NZB/NZW mice (a murine model of the human disease). Using sophisticated "knock in" and double-transgenic mice generated on normal or NZB/NZW genetic backgrounds and hybridoma generation, he showed that, in both strains, receptor editing and allelic inclusion occurs to about the same extent and therefore cannot explain the lack of tolerance and the development of autoreactivity and the formation of anti-DNA antibodies in the diseased animals.[15] The striking difference between the two strains was that B cells in the disease-prone NZB/NZW mice used the process of receptor editing to augment, rather than to suppress, the autoimmune response.

When one thinks of therapeutic antibodies, only IgG isotypes are considered in most cases. **Renato Monteiro** (INSERM; Paris Diderot University) discussed the IgA and IgA Fc receptors as tools for immunotherapy. IgA is the second most abundant Ig isotype in serum where it is mostly present as monomer, in contrast to mucosal surfaces where it is present as dimeric secretory IgA. Monomeric IgA have an anti-inflammatory capacity, while being poorly involved in systemic immune responses. Thus, the

question of the underlying mechanisms by which monomeric IgA can down-modulate IgG-mediated phagocytosis, chemotaxis, oxidative burst and cytokine release in absence of antigen has been explored. Professor Monteiro showed that the IgA Fc receptor I (FcαRI or CD89) has inhibitory or activating function depending on whether the receptor is engaged by monomeric or multimeric ligands.[16] This finding has led to the view that immunoreceptor tyrosine-based activation motifs (ITAMs) can also be involved in inhibitory activities. Interestingly, the inhibitory function of ITAM (ITAMi) results from a partial phosphorylation of the ITAM motif of the associated FcγR subunit. Monovalent targeting of FcαRI favors the recruitment of the protein tyrosine phosphatase SHP-1 that impairs signaling induced by activating receptors through the formation of polarized intracellular clusters ("inhibisomes") containing these receptors, signaling effectors and SHP-1. The finding underlines the crucial role of ITAMi signaling in the control of inflammatory responses. Professor Monteiro and his colleagues also identified an anti-CD89 Fab as a new potential therapeutic tool to prevent the progression of renal inflammatory diseases.

Over the last decade, a lot of efforts have been devoted to the increase of the effector functions of therapeutic mAbs exhibiting a human IgG1 Fc domain. **Sven Berger** (Pierre Fabre Research Institute) described the engineering of the hinge region and of Fc domains to increase or decrease the binding of IgG1 to various FcγR, to C1q or to the neonatal Fc receptor (FcRn). Engineering has also targeted the glycosylation pattern of human IgG1, allowing the generation of antibodies with potent ADCC activities due to their increase binding to FcγRIIIa. This binding depends on residues located in the flexible hinge region and the CH2 domain. Dr. Berger showed that, by replacing the human hinge with its less flexible mouse counterpart, one could get an antibody exhibiting an antagonist activity rather than an agonist one. The pharmaceutical industry is now attempting to translate these research tools into serious clinical candidates.[17] The Pierre Fabre Research Institute is currently developing therapeutic mAbs in collaboration with Merck, Sharp and Dohme (dalotuzumab, an anti-IGF-1R mAb now in Phase 2), and with Abbott (h224G11, an antagonist anti-human c-Met mAb that blocks c-Met dimerization). Dr. Berger still recommends remaining cautious about the potential immunogenicity of these new drugs, especially when they are manipulated to increase their half-life.

Session 3A—Antibody Effector Functions: Molecules and Cells in a Complex Environment

Jean-Luc Teillaud

Wolf-Herman Fridman (Georges Pompidou European Hospital—Paris Descartes University) discussed the complexity of tumor microenvironment and the role of immune cells in controlling cancer spread. It is now established that these factors strongly affect the clinical outcome of the patients.[18] Professor Fridman noted that the infiltration of T cells within solid tumors such as colorectal cancers (CRC) and other human malignancies, marked by a Th1/cytotoxic reaction, is the strongest prognostic factor for the overall survival as shown by studies of large cohorts of patients, based on integrated biology approaches. He also reported that CX3CL1, CXCL10 and CXCL9 chemokines are important players in the elaboration of an efficient immune pattern. The presence of tertiary lymphoid structures adjacent to the tumors containing mature dendritic cells (DCs), as observed in lung carcinomas, could polarize naïve T cells toward Th1/cytotoxic subsets. Overall, these immune mechanisms could control the homing of metastatic cancer cells in new areas. All these observations raise the question of how mAb-based therapies affect the host immune system in cancer patients.

The interest of targeting cell surface molecules that supports migration in inflammatory or cancer conditions has been then emphasized by **David Naor** (Hadassah Medical School—The Hebrew University).[19] He showed that the targeting CD44 or RHAMM (receptor hyaluronic acid mediated motility or CD168) by anti-CD44 or anti-RHAMM mAbs is efficient in reducing or eradicating the inflammatory activity in different mouse models [rheumatoid arthritis, multiple sclerosis and type 1 diabetes (T1D)], even when the treatment was initiated after the onset of the disease as is the case with T1D. Interestingly, Professor Naor showed that RHAMM treatment could compensate CD44 in supporting in vitro cell migration and in vivo invasion of inflammatory cells into inflamed joints in a collagen-induced arthritis (CIA) mouse model where CD44 is genetically deleted. The detailed study of the NOD mouse model of T1D also demonstrated that RHAMM and CD44 cooperate in supporting inflammatory cell invasion into the pancreatic islets, although the presence of CD44 is not mandatory. However, the expression of CD44 by insulin secreting cells is needed for killing by invading cells. Professor Naor concluded that RHAMM is a new therapeutic marker in T1D.

The importance of modulating the immune system of cancer patients was then reported by **Rinat Rotem-Yehudar** (CureTech Ltd.).[20] She showed that, in experimental tumor models, treatment with an antibody (CT-011) that targets PD-1 (Programmed Cell), an inhibitory receptor present on effector lymphocytes and whose ligands (PD-L1 and PD-L2) trigger apoptosis, not only induces immediate anti-tumor effect but also facilitates the generation of a tumor-specific memory control. Interim results of a Phase 2 study in patients with diffuse large B cell lymphoma (DLBCL) after autologous stem cell transplantation showed a positive clinical effect as compared with an average of relevant historical controls. A peripheral increase of specific subsets of memory T cells was also observed. Thus, Dr. Rotem-Yehudar concluded that PD-1 blockade by CT-011 could be related to clinical efficacy in aggressive lymphoma patients. Of note, a Phase 2 clinical trial of the combination of rituximab and CT-011 is in progress.

The idea that combinations of mAbs with different molecular targets could enhance the overall response in cancer patients is currently being explored in a number of laboratories and clinical departments. **Hélène Haegel** (Transgène SA) proposed an alternative to this approach. She reported the development of a humanized mAb directed to CD115, TG3003, with multiple intervention points for cancer therapy. CD115, the CSF-1 receptor

is expressed on many epithelial cancers (breast, ovary…), on tumor-associated macrophages that control tumor progression, as well as on osteoclasts that are responsible for metastasis-induced bone destruction. She showed that treatment with anti-CD115 mAb decreases tumor growth, expression of F4/80 macrophage marker in tumors, and inhibits bone destruction in mouse tumor models. Analysis of TG3003 indicated that it exerts selectively ADCC against CD115$^+$ tumor cells and inhibits CD115 function through a non-ligand competitive mode of action. Thus, Dr. Haegel concluded that TG3003 is a promising candidate for the treatment of solid tumors associated with bone metastases.

The engineering and production of new antibody formats that allow the recruitment of cytotoxic effector mechanisms remain a major challenge for antibody-based cancer therapies. **Zelig Eshhar** (The Weizmann Institute of Science) described his efforts to develop antibody-based chimeric receptors for adoptive cell therapy.[21] He showed that "T-bodies," which are T cells engineered to express an antibody-based chimeric receptor (CAR) made of an anti-tumor (HER2/Neu, CEA, CD24) scFv linked to a hinge region, a transmembrane motif and intracellular signaling (CD3zeta or FcRγ chain) moieties, can be used in adoptive cell therapy (ACT) of cancer. Experimental models using immuno-deficient mice showed that medium to high affinity CARs are the best candidates for tumor elimination. However, Professor Eshhar noted that very high affinity CARs can bind target molecules expressed at a low density on normal cells, hence leading to severe side effects. Exploration of T-bodies anti-tumor effect showed that intra-tumor inoculation is an efficient therapeutic method. Current ACT approaches employ the use of autologous lymphocytes. Using allogeneic lymphocytes in ACT is problematic because it causes severe immunological reaction against HLA-mismatched individual. Professor Eshhar and his team have recently set up a new method to overcome this issue. It is based on a mild pre-conditioning of the recipient animal before the injection of allogeneic T cells redirected with a human Her2/neu-specific CAR in a mouse model of metastatic disease. A transient lympho-depletion is achieved by irradiation or cyclophosphamide injection. It delays the rejection of the allogeneic T-bodies that have enough time to destroy the tumor, but not enough to cause significant damage to the host. Professor Eshhar proposed that ex vivo generated HLA-mismatched allogeneic T-bodies could be used as a universal tool for ACT, while their graft-vs.-host reactivity can be controlled by pre-conditioning treatment.

Recruitment of effector cytotoxic cells can also be potentiated by selection of structurally well-defined Fc region of IgG therapeutic antibodies, as discussed by **Christophe de Romeuf** (Laboratoire français du Fractionnement et des Biotechnologies). He presented data showing that improvement of IgG Fc interaction with FcγRIIIa expressed on NK cells and macrophages by a careful selection of the Fc glycosylation pattern (low fucosylation) leads to a much stronger ADCC in vitro. Dr. de Romeuf showed as an example that a fully human anti-D antibody (LFB-R593) that strongly binds FcγRIIIa exhibits excellent ADCC in vitro, even when elevated amounts of IVIg (i.e., polyclonal IgG) are present in the assay. Clinical trials showed that LFB-R593 has the same ability to clear RhD$^+$ red blood cells in human volunteers as the reference polyclonal anti-D antibodies currently used for the prevention of fœto-maternal allo-immunization. Similarly, he presented data showing that a low-fucosylated FcγRIII anti-CD20 mAb (LFB-R603) is able to efficiently kill CLL cells that express low density of CD20 in vitro and depletes rapidly peripheral B cells in all CLL patients medicated with methyl-prednisone. The anti-tumor effect of another low-fucosylated mAb, directed against the human Müllerian Inhibiting Substance type II receptor (MISRII) expressed on most ovarian cancer subtypes is currently investigated in mouse models.

Itai Benhar (The George S. Wise Faculty of Life Sciences—Tel Aviv University) then presented the production of a new method to produce IgG and IgG-cargo fusion molecule in bacteria, named "Inclonals" technology.[23] It is based on expert biochemical procedures starting with the different proteins (heavy and light chains of IgG, toxins) produced in bacterial cultures and present in insoluble inclusion bodies. The method has the advantage of leading to a high yield of highly purified full-length antibodies and fusion molecules that exhibit the same affinity and stability properties as their mammalian cell culture-produced counterparts. Professor Benhar stressed that the method also allows the efficient production of toxic proteins as opposed to mammalian cells. Examples of the production and use of fluorescent IgG-based fusion molecules were given. It was concluded that the technology is an attractive option for antibody production.

Session 4—Engineering Antibodies

Sandrine Moutel

Irit Sagi (The Weizmann Institute of Science) presented data on the rational design of antibodies directed to the active sites of metalloenzymes.[24] Among these are the zinc-dependent endopeptidases belonging to the matrix metalloproteinases (MMPs) family, enzymes that are involved in vivo in cell migration and that have been strongly associated with tumor metastasis, inflammation, tissue degeneration and cell death. The catalytic metal centers of zinc-dependent endopeptidases are therefore attractive targets. Molecular inhibitors have already been designed and used in vivo, but these molecules inhibit the entire MMPs family and cause severe side effects. The laboratory of Professor Sagi has recently developed antibodies, termed metallobodies, targeting the MMPs catalytic sites. An original immunization strategy based on molecular mimicry was set up. A synthetic molecule mimicking the conserved structure of the metalloenzyme catalytic zinc-histidine complex residing within the active site of gelatinases (MMP2 and 9) was used. Hence, the antibodies generated appear to be similar to the naturally occurring MMPs inhibitors [endogenous tissue inhibitors of metalloproteinases (TIMPs)].[24] These antibodies bind the catalytic metal ion in the enzyme active conformation and compete with zinc binding, exhibiting an exquisite specificity. The therapeutic potential of these antibodies has been demonstrated in models of inflammatory bowel disease (IBD) using knockout MMP2/9 mouse.

Serge Muyldermans (Vrije Universiteit Brussel) presented an overview on single variable VHH domains (Nanobody®) derived from *Camelidae*. Owing to their small size, VHH can be easily manipulated, formatted as bispecific antibodies or fused to Fc domains or other polypeptides. They offer many advantages for research, industrial and clinical applications over conventional mAbs. They can be produced with a high yield in bacteria, they exhibit excellent stability and good solubility, and their production cost is lower. VHH are usually isolated after immunization of llama or dromedary, cloning of the VHH repertoire and selection by phage display. Professor Muyldermans reported the selection of VHH that modulate the conformation and spectral properties of green fluorescent protein (GFP) and their use as intrabodies to track molecules within living cells.[25] Also, a VHH recognizing a conserved carbohydrate of the variant surface coat glycoprotein that covers the entire surface of *Trypanosoma brucei rhodesiense* responsible for sleeping sickness in East Africa has been developed. Once conjugated with a truncated form of apoL-I (Tr-apoL-I) that is derived from apoL-I-neutralizing serum resistance-associated (SRA) protein expressed by the parasite and lytic for *T. b. rhodesiense*, the molecule could eliminate the parasites from blood. Professor Muyldermans also indicated that, due to their short half-life in blood, VHH can also be used for in vivo imaging and radioimmunotherapy (RIT). An anti-HER2/Neu VHH conjugated either to technetium-99 or to lutetium-177 radionuclides is currently developed for imaging and RIT of HER2-positive breast cancer, respectively.

Roger MacKenzie (National Research Council Canada) also reported the development of therapeutic VHH. AFAI is a VHH targeting carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) that was isolated from a non-immune camelid. He noted that AFAI has been formatted as a pentamer. The molecule strongly stains several tumor types and, unlike conventional antibodies to CEACAM6, discriminates between tumor and normal tissues. Dr. MacKenzie hypothesized that this VHH binds a hidden epitope otherwise inaccessible to conventional antibodies due to its small size and a long complementarity determining region 3 (CDR3). The company HelixBiopharma has fused the pentameric AFAI with a plant enzyme, urease (DOS47), which converts urea to ammonia, a toxic metabolic product. The resulting drug candidate, L-DOS47, is designed to act in a targeted manner by selectively recognizing non-small cell lung cancer cells to produce a potent anti-tumor effect based on the local production of ammonia at the tumor sites.

The presentation of **Yoram Reiter** (Technion—Israel Institute of Technology) detailed two strategies for cancer immunotherapies that involve combining high affinity antibody fragments with the recruitment of cytotoxic $CD8^+$ T cells. The first consists of fusing a scFv specific for tumor cell surface antigens to a human single-chain HLA-A2 molecule covalently linked to a tumor or viral-derived peptide to recruit specific cytotoxic T cells that are able to kill the scFv-decorated target cells.[26] The second strategy is developed in collaboration with Applied Immune Technologies (AIT). AIT has created a platform technology for the development of human recombinant T-Cell Receptor-Like (TCRL™) antibodies capable of binding to intracellular-derived peptides presented by MHC molecules. Novel therapeutic scFv mimicking the T cell receptor, binding to various peptide-MHC complexes, have been generated for therapeutic and diagnostic applications in a variety of cancer, viral and autoimmune diseases. The identification of candidate peptides derived from the proteasome of patients has been accelerated by a new tool that combines bioinformatic analysis and mass spectroscopy (EpiTarget™ Discovery). This made it possible to identify more than 2,000 new peptides presented on MHC molecules from patient cells, from which 33 have been selected as peptide candidates for novel MHC/peptide based targets.

Session 5—Learning from Treatment with Therapeutic Antibodies

Marie-Caroline Dieu-Nosjean

Josée Golay (Laboratory of Cellular therapy «*G. Lanzani*», Division of Hematology, Ospedali Riuniti) reported on the role of macrophages on the therapeutic activity of the anti-CD20 antibody rituximab. In vivo, macrophages can have a dual role with a negative impact on tumor growth and a positive impact on rituximab-mediated therapeutic activity. Dr. Golay showed that M2 type tumor-infiltrating macrophages, although producing IL-10 and VEGF that negatively impact anti-tumor response and angiogenesis and lead to tumor growth, exhibit a strong phagocytic activity against rituximab-opsonized CLL targets.[27] In vitro experiments showed that addition of IL-10 increased phagocytosis by both M-CSF- and GM-CSF-differentiated macrophages. These data demonstrate that future therapeutic antibody-based strategies combined with cellular therapies should also aim at improving antibody-dependent phagocytosis (ADP), as well as complement-dependent cytotoxicity (CDC) and ADCC.

Recent reports have suggested that antibody treatment in oncology not only involves cells from the innate immunity but also cells from the adaptive immunity. **Jean-Luc Teillaud** (INSERM-Paris Descartes University) first reminded the conference participants that many advances have been achieved over the last decade to improve the effector function of therapeutic antibodies via better recruitment of $Fc\gamma R^+$ cells of the innate immunity. In particular, he showed that ADCC against CLL cells can be strongly increased in vitro when using a low-fucosylated antibody directed to CD20. Dr. Teillaud then showed that antibody treatment of $CD20^+$ tumor-bearing mice induces the recruitment of $CD4^+$ cells that are required to induce long-term protection.[28] The presence of $CD4^+$ cells is also required when mAb-treated surviving mice are challenged with tumor cells. Interestingly, the presence of $CD8^+$ cells was not required at the initiation of the treatment, but was necessary after tumor challenge. Thus, these findings indicate that mAb-based treatment of tumors induces an adaptive cellular immunity. It paves the way to the development of new therapeutic strategies aimed at strengthening this adaptive response, as exemplified by Dr. Teillaud who showed that IL-2 improves the overall survival rate when given after tumor challenge. The induction of a long-lasting anti-tumor response following anti-CD20 treatment has been also explored by **Nurit**

Hollander (Sackler School of Medicine—Tel Aviv University).[29] She discussed the use of anti-CD20 mAbs in combination with dendritic cells (DCs) in order to supplement the anti-CD20-induced depletion of normal B cells, another subset of antigen-presenting cells. In a therapeutic model of disseminated tumor, Professor Hollander reported a synergistic effect of tumor cell-loaded DC injected subcutaneously with chemotherapy and anti-CD20 antibody. Alternatively, intra-tumoral injection of naïve DCs in addition to mAb treatment and chemotherapy (cyclophosphamide) was also shown to be effective. All these observations suggest that the clinical efficacy of anti-CD20 mAb treatment can be increased by inducing T cell-mediated antitumor immune responses with long-term memory.

Session 6—Monoclonal Antibodies in the Treatment of Auto-Immune and Viral Diseases

Marie-Caroline Dieu-Nosjean and Sandrine Moutel

Luc Mouthon (Cochin Hospital, Paris Descartes University) discussed the clinical use of intravenous immunoglobulin (IVIg) preparations and mAbs as therapeutic antibodies. Professor Mouthon presented an overview of the efficacy, as well as the limitation, of antibody-based treatment through many examples of inflammatory and auto-immune diseases.[30] He pointed out that the consumption of IVIg increases each year due to their use in a large number of these diseases. The interest of different mAbs and fusion proteins in the treatment of inflammatory and auto-immune diseases such as anti-TNF mAbs, the TNFαRII-Fc IgG1 fusion protein etanercept and abatacept, an IL-1RA recombinant molecule in rheumatoid arthritis, as well as rituximab in systemic vasculitis, was also discussed.

Yaakov Naparstek (Hadassah, Hebrew University Medical Center) described an IL-10-mediated mechanism involved in the resistance to adjuvant arthritis that has been exploited to develop a new therapeutic mAb. He noted that the serum of rheumatoid arthritis patients contains anti-heat shock protein (HSP) antibodies directed against peptide 6, a 16 amino-acid surface epitope. These antibodies cross-react with an epitope of adenyl cyclase associated protein (CAP1) present on the surface of monocytes. Professor Naparstek showed that the binding of a humanized anti-peptide 6 mAb to monocytes results in the secretion of the immunosuppressive cytokine IL-10 and the suppression of the disease in different animal models such as collagen-induced arthritis and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. He concluded his presentation by indicating that this humanized antibody may be used as a therapeutic tool in human auto-immune and inflammatory diseases.

Yves Poulain (Hôtel Dieu Hospital of Quebec—Laval University) presented the physiopathology of the psoriasis and the limitation of the existing therapies. Professor Poulain reported new data obtained in clinical Phase 2 and 3 studies in patients treated with antibodies that target cytokines and receptors or block the DC-T cell interaction. He noted that a significant improvement of the quality of life of patients have been obtained with the advent of biotherapies based on the use of anti-TNF molecules. Professor Poulain also stressed that the use of antibodies directed against the IL-12/IL-23 common p40 subunit such as the human mAb ustekinumab, had led to major improvement in patients with moderate or severe plaque psoriasis.[31] This approach has been elaborated because IL-23 is essential for the differentiation of Th17 lymphocytes, a T cell subset involved in chronic inflammatory and autoimmune diseases. However, as already observed with anti-TNF treatments, the use of neutralizing anti-IL-12/IL-23 antibody has been associated with serious adverse events, including an increased risk of infection. Long-term follow-up of treated patients will give a more accurate insight in the benefit-risk ratio of this treatment, although no evidence of cumulative toxicity has been observed to date with up to four years of exposure.

Roberto Mancini (Università Vita Salute San Raffaele) presented studies on mAbs isolated from human antibody repertoires directed to influenza[32] and hepatitis C (HCV) viruses. Two antibodies were obtained from a patient exposed to H1N1 influenza A strains with a negative clinical history for influenza infection over the last years, but with a detectable serum neutralizing activity against a 1934 influenza A strain. These two mAbs exhibited a strong neutralization activity against both swine-origin influenza virus (S-OIV) and a broad range of human and swine H1N1 isolates. Thus, these two mAbs could constitute the basis, alone or in a combination with other mAbs, for a new class of drugs to be used to design a vaccine and in the prophylaxis of this disease. Similarly, Dr. Mancini and his colleagues developed from a chronically infected patient an "intrabody" mAb that inhibits the activity and replication of HCV. This mAb could be an attractive tool when a gene delivery method of antibody to liver cells will be effective.

Jamie Scott discussed the properties of antibodies produced during acute vs. chronic viral infections.[33] She showed that neutralizing anti-HIV antibodies are raised only after protracted rounds of viral escape from the antibody response. Interestingly, antibodies derived from chronically infected patients demonstrate peculiar structural features in terms of CDR-H3 length, somatic mutations, likely due to persistent antigen selection during chronic infection and favor distal germline V(H)-genes usage. Thus, Professor Scott raised the issue of whether the generation of antibodies sharing the characteristics of those elicited during chronic infections should be a major goal when developing a protective vaccine, in particular in the case of HIV infection.

References

1. Wang M, Yang Z, Rada C, Neuberger MS. AID upmutants isolated using a high-throughput screen highlight the immunity/cancer balance limiting DNA deaminase activity. Nat Struct Mol Biol 2009; 16:769-76; PMID:19543289; http://dx.doi.org/10.1038/nsmb.1623.
2. Steinitz M. Three decades of human monoclonal antibodies: past, present and future developments. Hum Antibodies 2009; 18:1-10; PMID:19478393.
3. Dogan I, Bertocci B, Vilmont V, Delbos F, Mégret J, Storck S, et al. Multiple layers of B cell memory with different effector functions. Nat Immunol 2009; 10:1292-9; PMID:19855380; http://dx.doi.org/10.1038/ni.1814.
4. Keren Z, Naor S, Nussbaum S, Golan K, Itkin T, Sasaki Y, et al. B-cell depletion reactivates B lymphopoiesis in the BM and rejuvenates the B lineage in aging. Blood 2011; 117:3104-12; PMID:21228330; http://dx.doi.org/10.1182/blood-2010-09-307983.
5. Shachar I, Haran M. The secret second life of an innocent chaperone: the story of CD74 and B cell/chronic lymphocytic leukemia cell survival. Leuk Lymphoma 2011; 52:1446-54; PMID:21417823; http://dx.doi.org/10.3109/10428194.2011.565437.
6. Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, et al. The biochemistry of somatic hypermutation. Annu Rev Immunol 2008; 26:481-511; PMID:18304001; http://dx.doi.org/10.1146/annurev.immunol.26.021607.090236.
7. Kwakkenbos MJ, Diehl SA, Yasuda E, Bakker AQ, van Geelen CMM, Lukens MV, et al. Generation of stable monoclonal antibody-producing BCR$^+$ human memory B cells by genetic programming. Nat Med 2010; 16:123-8; PMID:20023635; http://dx.doi.org/10.1038/nm.2071.
8. Jin A, Ozawa T, Tajiri K, Obata T, Kondo S, Kinoshita K, et al. A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. Nat Med 2009; 15:1088-92; PMID:19684583; http://dx.doi.org/10.1038/nm.1966.
9. Olivier S, Jacoby M, Brillon C, Bouletreau S, Mollet T, Nerriere O, et al. EB66 cell line, a duck embryonic stem cell-derived substrate for the industrial production of therapeutic monoclonal antibodies with enhanced ADCC activity. MAbs 2010; 2:405-15; PMID:20562528.
10. Mostoslavsky R, Singh N, Tenzen T, Goldmit M, Gabay C, Elizur S, et al. Asynchronous replication and allelic exclusion in the immune system. Nature 2001; 414:221-5; PMID:11700561; http://dx.doi.org/10.1038/35102606.
11. Laskov R, Yahud V, Hamo R, Steinitz M. Preferential targeting of somatic hypermutation to hotspot motifs and hypermutable sites and generation of mutational clusters in the IgVH alleles of a rheumatoid factor producing lymphoblastoid cell line. Mol Immunol 2011; 48:733-45; PMID:21194753; http://dx.doi.org/10.1016/j.molimm.2010.10.009.
12. Jeevan-Raj BP, Robert I, Heyer V, Page A, Wang JH, Cammas F, et al. Epigenetic tethering of AID to the donor switch region during immunoglobulin class switch recombination. J Exp Med 2011; 208:1649-60; PMID:21746811; http://dx.doi.org/10.1084/jem.20110118.
13. Rouaud P, Vincent-Fabert C, Fiancette R, Cogné M, Pinaud E, Denizot Y. Enhancers located in heavy chain regulatory region (hs3a, hs1,2, hs3b and hs4) are dispensable for diversity of VDJ recombination. J Biol Chem 2012; 287:8356-60; PMID:22270371; http://dx.doi.org/10.1074/jbc.M112.341024.
14. Roulland S, Faroudi M, Mamessier E, Sungalee S, Salles G, Nadel B. Early steps of follicular lymphoma pathogenesis. Adv Immunol 2011; 111:1-46; PMID:21970951; http://dx.doi.org/10.1016/B978-0-12-385991-4.00001-5.
15. Makdasi E, Fischel R, Kat I, Eilat D. Autoreactive anti-DNA transgenic B cells in lupus-prone New Zealand black/New Zealand white mice show near perfect L chain allelic exclusion. J Immunol 2009; 182:6143-8; PMID:19414767; http://dx.doi.org/10.4049/jimmunol.0803610.
16. Pfirsch-Maisonnas S, Aloulou M, Xu T, Claver J, Kanamaru Y, Tiwari M, et al. Inhibitory ITAM signaling traps activating receptors with the phosphatase SHP-1 to form polarized "inhibisome" clusters. Sci Signal 2011; 4:24; PMID:21505186; http://dx.doi.org/10.1126/scisignal.2001309.
17. Beck A, Wurch T, Bailly C, Corvaïa N. Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol 2010; 10:345-52; PMID:20414207; http://dx.doi.org/10.1038/nri2747.
18. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12:298-306; PMID:22419253; http://dx.doi.org/10.1038/nrc3245.
19. Turley EA, Naor D. RHAMM and CD44 peptides-analytic tools and potential drugs. Front Biosci 2012; 17:1775-94; PMID:22201835; http://dx.doi.org/10.2741/4018.
20. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res 2008; 14:3044-51; PMID:18483370; http://dx.doi.org/10.1158/1078-0432.CCR-07-4079.
21. Marcus A, Eshhar Z. Tumor-specific allogeneic cells for cancer therapy. Expert Opin Biol Ther 2011; 11:1551-4; PMID:21995504; http://dx.doi.org/10.1517/14712598.2011.628934.
22. de Romeuf C, Dutertre CA, Le Garff-Tavernier M, Fournier N, Gaucher C, Glacet A, et al. Chronic lymphocytic leukaemia cells are efficiently killed by an anti-CD20 monoclonal antibody selected for improved engagement of FcgammaRIIIA/CD16. Br J Haematol 2008; 140:635-43; PMID:18302712; http://dx.doi.org/10.1111/j.1365-2141.2007.06974.x.
23. Vaks L, Benhar I. In vivo characteristics of targeted drug-carrying filamentous bacteriophage nanomedicines. J Nanobiotechnology 2011; 9:58; PMID:22185583; http://dx.doi.org/10.1186/1477-3155-9-58.
24. Sela-Passwell N, Kikkeri R, Dym O, Rozenberg H, Margalit R, Arad-Yellin R, et al. Antibodies targeting the catalytic zinc complex of activated matrix metalloproteinases show therapeutic potential. Nat Med 2012; 18:143-7; PMID:22198278; http://dx.doi.org/10.1038/nm.2582.
25. Kirchhofer A, Helma J, Schmidthals K, Frauer C, Cui S, Karcher A, et al. Modulation of protein properties in living cells using nanobodies. Nat Struct Mol Biol 2010; 17:133-8; PMID:20010839; http://dx.doi.org/10.1038/nsmb.1727.
26. Dahan R, Reiter Y. T-cell-receptor-like antibodies—generation, function and applications. Expert Rev Mol Med 2012; 14:6; PMID:22361332; http://dx.doi.org/10.1017/erm.2012.2.
27. Leidi M, Gotti E, Bologna L, Miranda E, Rimoldi M, Sica A, et al. M2 macrophages phagocytose rituximab-opsonized leukemic targets more efficiently than m1 cells in vitro. J Immunol 2009; 182:4415-22; PMID:19299742; http://dx.doi.org/10.4049/jimmunol.0713732.
28. Abès R, Gélizé E, Fridman WH, Teillaud JL. Long-lasting antitumor protection by anti-CD20 antibody through cellular immune response. Blood 2010; 116:926-34; PMID:20439625; http://dx.doi.org/10.1182/blood-2009-10-248609.
29. Gadri Z, Kukulansky T, Bar-Or E, Haimovich J, Hollander N. Synergistic effect of dendritic cell vaccination and anti-CD20 antibody treatment in the therapy of murine lymphoma. J Immunother 2009; 32:333-40; PMID:19342972; http://dx.doi.org/10.1097/CJI.0b013e31819b7c17.
30. Pham T, Bachelez H, Berthelot JM, Blacher J, Bouhnik Y, Claudepierre P, et al. TNFalpha antagonist therapy and safety monitoring. Joint Bone Spine 2011; 78:15-185; PMID:21703545; http://dx.doi.org/10.1016/S1297-319X(11)70001-X.
31. Papp K, Menter A, Poulin Y, Gu Y, Sasso EH. Long-term outcomes of interruption and retreatment vs. continuous therapy with adalimumab for psoriasis: subanalysis of REVEAL and the open-label extension study. [Epub ahead of print]. J Eur Acad Dermatol Venereol 2012; PMID:22429586; http://dx.doi.org/10.1111/j.1468-3083.2012.04515.x.
32. Burioni R, Canducci F, Mancini N, Clementi N, Sassi M, De Marco D, et al. Monoclonal antibodies isolated from human B cells neutralize a broad range of H1 subtype influenza A viruses including swine-origin Influenza virus (S-OIV). Virology 2010; 399:144-52; PMID:20096915; http://dx.doi.org/10.1016/j.virol.2009.12.014.
33. Breden F, Lepik C, Longo NS, Montero M, Lipsky PE, Scott JK. Comparison of antibody repertoires produced by HIV-1 infection, other chronic and acute infections, and systemic autoimmune disease. AIDS 2011; 25:1247-57; PMID:21508803.