MEETING REPORT

doi: 10.1111/j.1365-3083.2009.02284.x

Frontiers in Clinical Immunology 2008

G. Deniz*, H. B. Oral† & M. Zouali‡,§

*Department of Immunology, Institute of Experimental Medical Research (DETAE), Istanbul University, Istanbul, Turkey; †Department of Microbiology and Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey; ‡Inserm, U606, Lariboisière Hospital, Paris, France; and §University Denis Diderot Paris 7, Paris, France

Received 13 February 2009; Accepted in revised form 18 May 2009

Correspondence to: M. Zouali, Inserm U606, Centre Viggo Petersen, Hôpital Lariboisière, 2, rue Ambroise Paré, 75475 Paris Cedex 10, France. E-mail: moncef.zouali@wanadoo.fr

Abstract

In recent years, investigations in immunology have led to progress in clinical medicine, including understanding transplant rejection, autoimmune diseases, immune deficiencies, inflammation, transplantation, cancer and the development of new vaccines. At a meeting recently held on the Mediterranean shore, advances in several facets of clinical immunology were the focus of discussion. Here, we highlight some of the debates that reflected advances in a variety of human immune disorders.

Introduction

From the early days of immunology, fundamental immunology has been applied to understanding a variety of human disorders. Conversely, clinical immunology has also benefited from progress in fundamental immunology. For example, studies of antibodies by Karl Landsteiner in 1904 in Vienna led to the discovery of the first autoimmune disease in human – paroxysmal cold haemoglobinuria. In the following decades, recognition of clinical immunology as a distinct medical subspecialty with many areas of expertise has provided better opportunities for understanding disease processes. In recent years, investigations in immunology have led to advances in clinical medicine, including understanding allergies, transplant rejection, autoimmune diseases and the development of new vaccines. Currently, understanding the components and functions of the immune system at the molecular and cellular levels is providing clues to processes of pathological immune-mediated disorders and mechanisms of immune responses to micro-organisms. Recent advances in clinical immunology were the focus of discussion at the Second Mediterranean Clinical Immunology Meeting, which was held in Antalya, on the Mediterranean shore of Turkey from 4 to 7 October 2008. The meeting brought together established researchers and young scientists from Europe, America, Asia and Africa.

Vaccine strategies against tuberculosis

According to the World Health Organization, there were, in 2006, an estimated 139 new cases of tuberculosis per 100,000 population, and an estimated 1.5 million deaths from tuberculosis in HIV-1-negative people and 0.2 million among people infected with HIV-1. On the other hand, global epidemiology studies of the disease, have led to the identification of extensively drug-resistant strains of *Mycobacterium tuberculosis* in 50 countries. This heightened threat posed by untreatable and fatal human tuberculosis underscores the need of several approaches to combat the disease. In a keynote lecture, Stefan Kaufmann (Max Planck Institute for Infection Biology, Berlin, Germany) provided insight into the immunity to tuberculosis and discussed novel vaccination approaches. Studies from his laboratory that focused on antigen presentation in the context of products of the major histocompatibility complex class I (MHC class I, responsible for $CD8^+$ T-cell stimulation) showed the critical role played by cross-priming involving apoptosis of infected macrophages, release of vesicles filled with antigenic cargo from *M. tuberculosis* and uptake of these vesicles by dendritic cells [1]. Investigating mycobacterial load in lesions of human tuberculous granuloma and dissemination to different tissue locations, as well as distribution, biological functions and interactions of host immune cells, Kaufmann and co-workers obtained

SCANDINAVIAN JOURNAL OF Immunology AN INTERNATIONAL MONTHLY JOURNAL

evidence that granulomas assume functions of secondary lymphoid organs and hence can prime T cells at the site of *M. tuberculosis* persistence [2]. Like in other infectious diseases, immune phenomena mediate not only protection against tuberculosis, but also disease-pathogenesis, and increasing evidence suggests that exaggerated inflammation contributes to pathogenesis of tuberculosis. The Kaufmann laboratory have identified both host and pathogen factors contributing to exacerbated inflammation. Although the mechanisms underlying reactivation are not fully understood, it is possible that regulatory T cells and T-cell exhaustion are involved.

As for other infectious diseases, vaccine design is a central issue in tuberculosis and vaccination strategies focus either on subunit vaccines or on viable attenuated vaccines. The first strategy is based on the assumption that one or few antigens suffice for an efficient immune response. Hence, the identification of protective antigens represents an essential prerequisite for the success of this type of vaccines. On the other hand, variable-attenuated vaccines are based on the assumption that multiple antigens are required for efficacious protection against *M. tuberculosis*. Two approaches are followed by the Berlin group, namely knockout mutants of *M. tuberculosis* and improved recombinant (r) BCG vaccines [3]. To improve this latter vaccine, the Kaufmann laboratory constructed a r-BCG strain expressing listeriolysin, but devoid of urease, and showed that, in experimental animals, this vaccine candidate induces a better protection than the wild-type parental BCG and is safer when tested in immunocompromised mice (including RAG-deficient and nude animals). Recently, the candidate vaccine has entered a phase I clinical trial. Kaufmann speculated that a prime–boost scheme comprising priming with improved r-BCG and boosting with the most efficacious subunit vaccine candidate would provide optimal protection. Identification of a bio-signature that allows distinction between infection/protection and infection/disease in tuberculosis could accelerate efficacy testing of vaccines in clinical trials.

Escape mechanisms implemented by infectious agents

The capacity to subvert functions of the immune system is a striking hallmark of infectious agents. This is further supported by recent studies of anthrax, a disease caused by gram-positive, aerobic, spore-forming *Bacillus anthracis* that affects primarily herbivores. Humans are accidental hosts through food of animal origin and animal products and the disease is prevalent in most parts of the world and cases reported in almost all countries. Lhousseine Touqui (Institut Pasteur, Paris, France) showed that *B. anthracis* represses the expression of factors such as the anthracidal enzyme type-IIA secreted phospholipase A2 (sPLA2-IIA) produced by alveolar macrophages and interleukin-8 (IL-8) secreted by endothelial cells. Inhibition of sPLA2-IIA production occurs through a process involving elevation of cAMP concentrations in alveolar macrophages, whereas repression of IL-8 expression is because of the alteration of MAPK activation in endothelial cells [4, 5]. These studies reveal new molecular mechanisms implemented by *B. anthracis* to escape innate host defence.

Acquired Immunodeficiency Syndrome (AIDS), another infectious disease with global impact, was also the focus of discussion. In this disease, 'polyfunctional' $CD8^+$ T cells producing multiple antiviral factors reportedly emerge during prolonged suppression of HIV-1 replication by antiretroviral therapy [6]. Investigating protection correlates, Reinhold E. Schmidt (Hannover Medical School, Germany) discussed the polyfunctional potential of $CD8^+$ T cells in the control of HIV-1 infection. In studies of natural killer (NK) cells, the Hannover group had previously demonstrated that low NK cell numbers are associated with rapid HIV-1 progression. That NK cells may have a critical role in the natural history of HIV-1 infection comes from investigation of allotypes of the NK cell receptor KIR3DL1 that vary in both NK cell expression patterns and inhibitory capacity upon binding to their HLA-B Bw4 ligands present on target cells. Thus, it has been demonstrated that HLA-B57, together with expression of the killer cell inhibitory molecule $KIR3DL1^{high}$, is a good prognostic factor for HIV-1 patients [7]. The Hannover group also could demonstrate that NK cells from HIV-1-seropositive patients display different expression patterns of CD57, a marker of replicative senescence. NK cells are also considered as important regulators of antiviral immune responses because they secrete a multitude of soluble mediators and because they can directly interact with other immune cells, *e.g.* plasmacytoid dendritic cells that can produce high amounts of interferon-α. During HIV-1 infection, the virus has also developed tactics to suppress these host cellular responses. Among the many viral offensive strategies, viral protein R (Vpr) plays a particularly active role. Vpr is involved in nuclear transport of the viral pre-integration complex, activation of viral transcription, induction of cell cycle G2/M arrest and apoptosis of the host cells. Infection has been implicated in impairing various aspects of NK cell function, and the results illustrate the ability of Vpr to impair NK cell-mediated innate immune functions indirectly by deregulating multiple cytokines in the infected target cells, thus increasing disease severity and affecting the final outcome in HIV-1 infection [8]. In the gut, the depletion of lymphoid tissue is dependent on various mechanisms, such as CD95-mediated apoptosis and high expression of CCR5 on lymphatic $CD4^+$ cells in the gut. In addition, integrin $\alpha4\beta7$ has more recently been described as a new HIV-1 co-factor in the lamina propria of the gut [9].

A number of bacteria produce toxins exhibiting a heterogeneous array of potent activities demonstrable in primary cells, such as inhibiting protein synthesis, actin polymerization, signal transduction pathways, intracellular trafficking of vesicles and immune and/or inflammatory responses detectable in animal models. As discussed by Massimo Alfano (San Raffaele Scientific Institute, Milan, Italy), the specificity and extreme potency of bacterial toxins, and the possibility to separate catalytic domains from receptor binding domains have enabled engineering of 'modified' toxins that could be tested against tumour or HIV-1-infected cells. These include immunotoxins active against *ex vivo*-HIV-1 infected cells, toxins exhibiting anti-HIV-1 activities, immunotoxin derived from *Pseudomonas aeroginosa* exotoxin A [10] and *Bordetella pertussis*-derived toxin B-oligomer with anti-HIV-1 activity [11].

Pathogenic mechanisms in human immune deficiencies

Studies of primary immune deficiencies have improved our understanding of their origin and have contributed to unravel the physiology of the normal human immune system. In recent years, molecular characterization of several disorders, including familial haemophagocytic lymphohistiocytosis, Griscelli syndrome and Chediak-Higashi syndrome has revolutionized our understanding of inherited disorders leading to haemophagocytic lymphohistiocytosis. Geneviève de Saint Basile (Inserm, Paris, France) focused on genes associated with these inherited forms. She concluded that most of the genes (FHL2/perforin, FHL3/UNC13D, FHL4/Syntaxin 11, GS2/Rab27a, CHS/LYST) are part of the cytotoxic granule-mediated cell death pathway. Her observations shed light on a previously unsuspected role for this pathway in lymphocyte homeostasis. Murine models of these immune deficiencies have evidenced the central role of $CD8^+$ T cells and interferon (IFN)-γ production in the pathogenesis of haemophagocytic lymphohistiocytosis, strongly suggesting that neutralization of IFN-γ could represent a potential treatment in humans [12, 13].

The most common primary immunodeficiency in humans is Common Variable Immunodeficiency (CVID). With an estimated incidence of 1:10,000 to 1:50,000 in the Caucasian population and despite normal B and T cell counts, CVID is a clinically heterogeneous disorder characterized by low or absent levels of serum IgA, reduced levels of IgG (<3 g) and low or normal levels of IgM, and a defect in the final stages of B cell differentiation into plasma cells. Vassilios Lougaris (University of Brescia, Italy) detailed the underlying molecular mechanisms in a small proportion of CVID cases. Homozygous loss of ICOS, a receptor expressed on T cells, and essential for T-cell–B-cell collaboration and terminal B-cell generation, was the first genetic defect to be identified in a small number of CVID patients (<1%). Mutations in transmembrane activator and calcium-modulator and cyclophilin-ligand Interactor (TACI), a receptor for B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), were identified to be associated with CVID in 6–8% of cases [14, 15]. Whereas the homozygous mutations C104R, S144X, i.e. mutation from Serine to another amino acid at position 144, and A181E in TACI were found to be associated with CVID, variants in the heterozygous state, such as the C104R, A181E and ins204A, appear to constitute risk factors for CVID. More recently, mutations in the CD19 gene, a pan-B-cell marker, were identified in patients with hypogammaglobulinaemia (<1%), further broadening the genes involved in the pathogenesis of CVID and, at the same time, underscoring its heterogeneity. However, the variants mentioned above can account for <10% of the CVID patients, leaving the remaining patients without a definitive genetic diagnosis. Further studies are necessary to better characterize the genetic and molecular mechanisms underlying this heterogeneous and complex disorder [16, 17].

Genetics and epigenetics of autoimmunity

The aetiology of autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), remains unclear. Pinpointing susceptibility loci is being performed in a number of laboratories, and whole genome association screening and subsequent linkage disequilibrium approaches using single nucleotide polymorphisms (SNPs) are considered to be a promising strategy. Following this approach to identify RA-associated genes, Kazuhiko Yamamoto (University of Tokyo and Center for Genomic Medicine, RIKEN, Japan) reported association of functional haplotypes of *PADI4*, encoding the citrullinating enzyme peptidylarginine deiminase 4, with RA. The results suggest that the RA susceptible *PADI4* haplotype is associated with production of citrullinated peptides that act as autoantigens, resulting in a heightened risk of developing the disease. The Tokyo group also identified an RA associated SNP in the promoter region of *FCRL3*, a member of the Fc receptor-like family [18] and two functional SNPs in *CD244*, a gene encoding a receptor expressed on human NK cells, a subpopulation of T cells, basophils and monocytes [19]. Although their findings have been replicated by several large-scale studies in Japanese populations, this was not the case in the majority of studies in Caucasian populations. Naturally, these RA-associated SNPs may be ethnically specific or become important through interactions with regional environments. However, antibodies to citrullinated peptides are universally specific for RA and *PADI4* encodes for one of the enzymes responsible for protein citrullination. Thus, the RA-associated *PADI4*

polymorphism found to be significant in Asian populations by Yamamoto and colleagues has also been, by a meta-analysis, positively associated with RA in Caucasians. Similarly, an RA-associated *FCRL3* polymorphism proved to be significant in a Canadian RA subgroup stratified according to a lack of a risk variant in *PTPN22*, a gene encoding a protein tyrosine phosphatase expressed primarily in lymphoid tissues and exhibiting an 1858T (rs2476601) non-synonymous SNP associated with a range of autoimmune disorders, including RA and SLE. As with *PTPN22*, the Tokyo group suggested that pathological pathways involving *PADI4* and *FCRL3* also play important roles in Caucasians with RA.

Whereas such observations underscore the need for global collaborations and comparisons of multiple ethnic populations, other gene hunting studies in RA and SLE studies disclosed that some susceptibility loci are not universally found in patients with different ethnic origins. One possibility discussed by Moncef Zouali (Inserm and University of Paris, Paris, France) is that epigenetic regulators play an important role in the pathogenesis of autoimmune diseases. Since epigenetics shapes development of all species studied, from the division of labour in honeybees to human behaviour, it is not surprising that immune tolerance to self-antigens requires epigenetic regulators [20]. In addition, environmental hormones, drugs and xenobiotics, known to trigger or exacerbate autoimmune disease, seem to impact epigenetic pathways. For example, investigation of the effect of hydralazine, an antihypertensive drug that triggers lupus in humans, revealed that, by altering DNA methylation and disrupting the ERK signalling pathway, hydralazine impairs B lymphocyte tolerance to self-determinants and contributes to generation of pathogenic autoreactivity [21].

Biomarker analysis in autoimmune disease

Another approach to gain insight into autoimmune pathogenesis is to analyze the implications of multiple molecules and to provide insight into the inter-relationships that define specific pathways. Such a strategy was followed by Roland Jonsson and colleagues (University of Bergen, Bergen, Norway) in Sjögren's syndrome (SS), an autoimmune disease characterized by severe impairment of exocrine gland function and focal mononuclear cell infiltrates within the salivary and lachrymal glands. Investigating the involvement of 87 proteins measured in serum and 75 proteins analyzed in saliva in the spontaneous SS model of the non-obese diabetic (NOD) mouse, as compared with control BALB/c animals, they found that the autoimmune SS manifestations were greatly independent and associated with various immunological processes. However, CD40, CD40 ligand, IL-18, granulocyte chemotactic protein-2 and anti-muscarinic M3 receptor IgG_3 could connect the different aspects of the disease. Processes related to the adaptive immune system appeared to promote SS with a strong involvement of T-helper 2-related proteins in hyposalivation [22]. This approach further established saliva as an attractive biofluid for biomarker analyses in SS and provided a basis for the comparison and selection of potential drug targets and diagnostic markers. To investigate a potential immunomodulatory effect of the 60-kDa heat-shock protein (Hsp60) on experimental SS, 7-week-old NOD mice were immunized with eukaryotic Hsp60 or an Hsp60-derived peptide (amino acid residues 437–460). Comprehensive analyses by the Bergen laboratory revealed specific biomarker signatures capable of predicting treatment outcome [23]. Molecules involved in inflammatory chemotaxis, neovascularization and regulatory pathways caused the differences displayed by the biomarker profiles.

Multiple $CD8^+$ suppressive T-cell (Ts) subtypes are now recognized as essential regulators of the immune system. They can prevent autoimmunity through secretion of multiple cytokines and the subsequent inhibition of effector lymphocyte function. As regulatory/suppressor T cells can suppress immunity against any antigen, including self-antigens, they emerge as an ideal therapeutic target. For example, in a xenograft model of human synovium, $CD8^+CD28^-CD56^+$ T cells effectively suppressed rheumatoid inflammation [24]. Margarida Souto-Carneiro (Center for Neurosciences and Cellular Biology, Coimbra, Portugal) used the arthritis K/BxN mouse model, which shares similarities with human RA, to induce amelioration in the clinical manifestations through $CD8^+$ T-cell manipulation via blockade of specific surface molecules. Arthritic K/BxN mice receiving the same therapy after total thymectomy maintained low joint scores during a 100-day follow up. Future studies should take into account the central role of $CD8^+$ T cells in anti-viral and anti-tumoral responses.

Inflammatory diseases

Benign prostatic hyperplasia (BPH), a condition seen in middle-aged and elderly men, is characterized by an increase in size of the prostate and by an important inflammatory component. Luciano Adorini (Intercept Pharmaceuticals, Perugia, Italy) showed that human stromal prostate cells obtained from BPH tissues represent non-professional antigen-presenting cells able to induce and sustain chronic inflammatory processes, further supporting the relevance of inflammation in BPH pathogenesis [25]. Stimulation of BPH cells with IL-8 activates the calcium-sensitizing RhoA/Rho kinase (ROCK) pathway, as demonstrated by the increased membrane translocation of RhoA and by phosphorylation of the ROCK substrate myosin phosphatase target subunit 1 (MYPT-1). The vitamin D receptor agonist elocalcitol

significantly inhibits IL-8 production by BPH cells stimulated with inflammatory cytokines and IL-8-induced proliferation of BPH cells [26]. In addition, this vitamin D3 analogue inhibits IL-8-induced membrane translocation of RhoA and MYPT-1 phosphorylation in BPH cells and their IL-8-dependent migration. The inhibition of IL-8 production by BPH cells is accompanied by decreased COX-2 expression and PGE_2 production, and by arrest of NF-κB p65 nuclear translocation, associated with inhibition of the RhoA/ROCK pathway. The data provide a mechanistic explanation for the anti-proliferative and anti-inflammatory properties of elocalcitol in BPH cells.

Behçet's disease (BD), which affects preferentially subjects of Middle Eastern or Far Eastern ancestry, is an inflammatory disease with unknown aetiology. Its prevalence and expression vary geographically, but Turkey has the highest prevalence (80 to 370 cases per 100,000). Several reports suggested that host genetic factors play significant roles in susceptibility to BD. H. Barbaros Oral (Uludag University, Bursa, Turkey) reported data on the association of cytokine gene single nucleotide polymorphisms with the development of BD in the Turkish population. The frequencies of TGF-β1 codon 25-GG genotype and the IL-10 1082-AA genotype were significantly lower in BD patients compared with healthy controls. The IL-10 1082-GA and 592-CA genotypes were also more frequent in the BD group. Polymorphisms of TGF-β1, IFN-γ and IL-6 may be associated with some clinical features, such as skin lesions, genital ulcer and pathergy positivity, a test helpful in the diagnosis of BD. The studies suggest that a complex interplay of cytokines governed at the genetic level may be responsible for the diverse spectrum of symptoms seen in patients with BD.

Endothelial cell activation and expression of adhesion molecules play a central role in orchestrating leukocyte recruitment into tissues and in regulating the course of inflammatory responses. As most of the knowledge on the mechanisms involved in endothelial cell activation comes from *in vitro* experiments with cultured endothelial cells, there is a need to better understand how endothelium is activated *in vivo*, both in healthy and in diseased conditions. Dorian Haskard (Imperial College, London, UK) highlighted the importance of blood flow in regulating the responsiveness of endothelial cells to inflammatory activation, an effect that may account for the susceptibility of arterial branch points and curvatures to mononuclear cell infiltration and atherosclerosis. He illustrated these effects with confocal microscopic imaging data of protected versus susceptible sites in mouse aorta. Suppression of proinflammatory gene expression at protected sites was, at least partially, related to the suppression of JNK- and p38-mediated signal transduction by flow-mediated induction of MAP kinase phosphatase 1 [27]. Presenting recent unpublished data using anti-E-selectin antibody-conjugated microbubbles to image activated endothelium with ultrasound, Haskard also discussed the potential of activated endothelium as a target for clinical molecular imaging.

Tight junctions create a paracellular barrier in epithelial and endothelial cells and protect them from the external environment. Together with cytoplasmic scaffolding molecules, these junctions regulate diverse physiological processes, such as proliferation, cell polarity and regulated diffusion. This regulated barrier can be disrupted in several conditions. In celiac disease, for example, expression and localization of epithelial junctional proteins are altered [28]. Sefik S. Alkan (Alba Therapeutics, Baltimore, MD, USA) showed that larazotide acetate, an 8-mer peptide, is a tight junction regulator believed to act locally by temporarily inhibiting the opening of tight junctions in the epithelial cell lining the small intestine [29]. The compound inhibited the transport of immune reactive gliadin peptides across a Caco-2 monolayer, a model for human drug intestinal permeability, as well as cytokine (TNF-α, IFN-γ and IL-1β)-induced decrease of transepithelial electrical resistance and increase of Lucifer Yellow permeability [30]. In a transgenic mouse model, oral administration of larazotide acetate prior to oral gliadin treatment blocked several intestinal changes. The results suggest that larazotide acetate can disrupt the pathogenic 'intestinal permeability-inflammatory loop' in celiac disease. In a randomized, double blind, placebo-controlled, proof-of-concept study in subjects receiving larazotide acetate before gluten challenge, overall gastrointestinal symptoms were significantly reduced. There was a 70% increase in intestinal permeability as measured by the lactulose-to-mannitol ratio in the placebo group, but no change in the larazotide acetate group. A Phase IIa study further defined the safety, efficacy and tolerability of larazotide acetate. Two additional Phase IIb studies are being conducted. Alkan suggested that larazotide acetate has a therapeutic potential in celiac disease.

NK cells mediate the early responses against virus-, intracellular bacteria- and parasite-infected cells, and modulate the activity of other effector cells of adaptive and innate immunity through production of cytokines and direct killing of transformed or infected cells. Günnur Deniz (Istanbul University, Istanbul, Turkey) presented properties of a regulatory subset of NK cells. When cultured in the presence of IL-12 or IL-4, human NK cells differentiate into cell populations with distinct patterns of cytokine secretion. The *in vivo* existence of human NK cell subsets was demonstrated in freshly isolated IFN-γ-secreting and IFN-γ-non-secreting NK cells. The former subset showed a typical cytokine pattern with predominant expression of IFN-γ, but almost no IL-4, IL-5 and IL-13. By contrast, IFN-γ-non-secreting NK

cells mainly produced IL-13 and contributed to IgE production [31]. To investigate the existence of regulatory NK cells in humans, NK cell subsets were purified and characterized. After stimulation with phytohemagglutinin and IL-2, or a combination of dexamethasone and vitamin D3, NK cells showed up to a four-fold increase in IL-10 mRNA production. IL-10-secreting NK cells expressed CD16 and CD56, activation markers (CD25, CD69, CD49d, CD45RA, CD45RO), activatory immunoglobulin-like receptors (CD94, CD161) and killer inhibitory receptors (CD158a, NKAT2, NKB1). The frequency of IL-10-secreting NK cells was significantly low compared with IFN-γ-secreting NK cells. Importantly, IL-10$^+$ NK cells significantly suppressed T-cell proliferation induced by bee venom major allergen or by a purified protein derivative of *Mycobacterium bovis*, and the effect was mediated by IL-10 [32]. For comparison, IFN-γ-secreting NK cells did not show any suppression. The findings demonstrate that a small fraction of human NK cells display regulatory functions.

Preventing graft rejections

Improvements in matching patients in new immunosuppressive drugs and in the clinical management of transplant recipients have greatly improved graft survival. However, two problems limit the success of organ transplantation: the shortage of donor organs and chronic rejection. In addition, most transplant recipients are on life-long medication that can have serious side effects and leave recipients at risk of infection and neoplastic disease. One solution to both chronic rejection and the need for long-term immunosuppression would be to induce tolerance to the donor organ. Andrew J.T. George (Imperial College, London, UK) discussed gene-based approaches to prevent graft rejection. The first approach developed by his group is to protect the graft itself by genetic modification of the endothelial cells that line the posterior surface of the cornea. Genetic modification of the cornea itself can be performed using viral (adenovirus or lentivirus being the most common) vectors or with non-viral vectors. To prolong graft survival, the London group developed a range of non-viral vectors and transduced the cornea with a variety of genes, including the gene encoding the enzyme indoleamine 2,3 dioxygenase. The results showed that over-expression of indoleamine 2,3 dioxygenase in the cornea can lead to considerable prolongation of graft survival [33]. The second approach is to induce tolerance by pretreatment of recipients with tolerogenic dendritic cells by 'knocking out' CD80/86 expression on target cells – by expressing a fusion protein that consists of an alternative ligand for CD80/86 (CTLA4) fused to the endoplasmic reticulum retention peptide (KDEL) [34]. As a result, cells expressing CTLA4-KDEL did not express surface CD80/86. CTLA4-KDEL dendritic cells were then administered to animals prior to receiving a corneal graft. When the dendritic cells were identical to the donor graft, there was a mild, but significant prolongation of graft survival. However, when the dendritic cells are (donor × recipient)F_1, there was considerable prolongation of survival, with the majority of grafts surviving >100 days, suggesting that corneal rejection is because of an indirect pathway of allorecognition, as only the F_1 dendritic cells will be capable of presenting donor antigen in the context of recipient MHC. In the long term, it is likely that both strategies will be effective in preventing graft loss. According to George, this strategy may have potential applications not only in the context of corneal transplantation but also in other forms of allotransplant as well as autoimmune disease.

Surrogate biomarkers for immunosuppression

In the past 20 years, the short-term outcomes of kidney transplant recipients have improved dramatically, in large part because of the availability of more potent immunosuppressive drugs capable of preventing or treating acute allograft rejection. However, adverse effects persist and cancer is a major cause of morbidity and mortality in renal transplant recipients. Thomas Giese (University of Heidelberg, Heidelberg, Germany) described correlations between the incidence of malignancies in kidney-transplanted patients and the individual degree of immunosuppression after cyclosporine A treatment. Almost 90% of all patients who developed such complications showed a residual (<15%) NFAT-regulated gene expression, underlying a stronger immunosuppression, and had significantly more malignancies compared to patients with residual NFAT activity above 15%. Thirty-six months after kidney transplantation, 14 of 55 patients aged over 60 years developed non-melanoma skin cancers and had significantly lower NFAT-regulated gene expression. Therefore, assessment of cyclosporine A-induced gene expression seems to reflect more accurately the patient's response to this immunosuppressive agent. The lower NFAT-regulated gene expression may represent a surrogate biomarker for a higher degree of functional immunosuppression. The availability of a quantitative, quick laboratory test to assess the functional activity of immunocompetent cells that are crucial for transplant rejection, defence against viral infection and tumour surveillance, along with the ability to adjust doses of immunosuppression, may have applications in transplantation medicine and in attempts to individualize treatments in transplanted patients [35].

Immune evasion during tumour progression

A hallmark of tumours is that they deploy strategies to evade immune responses. For example, during melanoma

progression, patients develop anti-tumour immunity that includes the production of anti-tumour antibodies. However, although the strategies developed by malignant cells to escape anti-tumour cellular immunity have been extensively investigated, little is known about tumour resistance to humoral immunity. Catherine Sautes-Fridman (Inserm & University Pierre et Marie Curie, Paris, France) reported that some human metastatic melanoma cells express ectopically the FcγRIIB1, an inhibitory isoform of FcγR. Analysis of a large panel of different types of human primary and metastatic solid tumours showed that expression of FcγRIIB is restricted to melanoma and is acquired during tumour progression. Importantly, the expression of FcγRIIB prevents the lysis of human metastatic melanoma cells by antibody-dependant cell cytotoxicity, *in vitro* and *in vivo,* in immunocompetent mice. Therefore, FcγRIIB is a marker that human metastatic melanoma may acquire during tumour progression to escape FcγR-dependent effector responses [36]. Other unpublished studies by Sautes-Fridman and co-workers were devoted to lung tumours. In this organ, the respiratory epithelium is continuously exposed to a broad range of infectious agents. Investigation of human lung tumour cells *in situ* and *ex vivo,* and of tumour cell lines revealed the expression of TLR-7 and -8, natural innate receptors for single-stranded RNA. Stimulation by TLR agonists led to increased tumour cell survival and chemoresistance and transcriptional analysis suggested that TLR chronic stimulation of tumour cells is taking place *in situ*.

Vaccine strategies against tumours

The utility of human self/tumour antigens as candidates for cancer vaccines was discussed by Olivera J. Finn (University of Pittsburgh, USA) who showed data in support of a new hypothesis that immune responses against such antigens found in cancer patients are in fact memory responses generated against the same antigens early in life during various viral infections. In particular, she showed that healthy individuals who have experienced various infections and other inflammatory conditions that affect tissues of origin of various tumours, have immune responses against two known tumour antigens, MUC1 and Cyclin B1, and consequently, a reduced cancer risk [37]. She proposed that these infections and inflammations lead to abnormal expression of these antigens and an immune response. Later in life, subsequent encounter with these antigens abnormally expressed on tumour cells could lead to a memory response and successful tumour immunosurveillance.

Tumours positive for HER-2/neu proto-oncogene account for approximately 20% of all breast cancer that carry poor prognosis. Constantin N. Baxevanis (Cancer Immunology and Immonotherapy Center, Athens, Greece) discussed the immunogenicity of HER-2/neu and its potential use in peptide-based anti-tumour vaccination protocols. He presented data on the identification and characterization of a novel HER-2/neu decamer, HER-2/neu(85-94), with potent *in vitro* and *in vivo* anti-tumour activity [38]. Importantly, patients with breast cancer (5 of 16) displayed increased *ex vivo* frequencies of peripheral blood $CD8^+$ T cells with specificity for this peptide. When combined with a helper epitope from HER-2/neu [i.e. peptide HER-2/neu(776–790)], this single peptide was capable of breaking tolerance against HER-2/neu in triple transgenic HLA-A2.1/HLA-DR1/neuT mice, allowing these animals to reject transplantable and spontaneously arising HER-2/neu^+ tumours.

Lack of space meant that we could not highlight all contributions. Despite these limitations, it is evident from the range of topics addressed herein that the conference has reflected progress in a variety of human immune

Table 1 A highlight of the most novel and important findings.

Investigation of vaccination strategies against *Mycobacterium tuberculosis* provided improved understanding of what constitutes a protective immune response. It also suggested that a prime–boost scheme comprising priming with improved recombinant BCG and boosting with the most efficacious subunit vaccine candidate would provide optimal protection.
Studies of *Bacillus anthracis* led to identification of a novel subversion mechanism whereby the bacterium impacts the innate immune branch of host defence. Specifically, *B. anthracis* represses expression of the anthracidal enzyme type-IIA secreted phospholipase A2 produced by alveolar macrophages and IL-8 secreted by endothelial cells. These observations provide new molecular targets for future intervention against this deadly pathogen.
Observations made in patients affected with autoimmune diseases and in animal models further underlined the key roles of both genetic and epigenetic factors in their pathogenesis. For instance, the RA susceptible PADI4 haplotype was found to be associated with production of citrullinated peptides that act as autoantigens, resulting in a heightened risk of developing the disease.
Specific biomarker signatures capable of predicting treatment outcome have been discovered. In Sjögren syndrome, saliva was found to represent an attractive biofluid for biomarker analyses in this systemic autoimmune disease.
Studies of human immune deficiencies revealed new genetic and molecular deficits. For example, mutations in the CD19 gene, expressed in all B lymphocyte subsets, were identified in patients with hypogammaglobulinaemia (<1%).
A subset of NK cells was found to display regulatory functions in humans, an observation that may contribute to a better understanding of the role of human NK cells in viral infections, autoimmunity, pregnancy, cancer, bone marrow transplantation and allergy.
In tumour immunology, candidate antigens for cancer vaccines have been identified and some of them are entering clinical trials. For instance, a novel HER-2/neu decamer, with potent *in vitro* and *in vivo* anti-tumour activity, was tested in patients with breast cancer. It led to increased *ex vivo* frequencies of peripheral blood $CD8^+$ T cells with specificity for this peptide.

disorders (Table 1). As discussed elsewhere, the moderately warm Mediterranean climate of Antalya was optimal to foster additional debates devoted to other issues, including functional imaging in the immune system [39], further reflecting progress in several facets of clinical immunology. We hope that this clinical immunology tradition will continue around the Mediterranean to provide further productive and rewarding understanding of disease pathogenesis and therapeutic interventions.

Acknowledgment

The meeting was organized by the Turkish Society of Immunology and financially supported by the European Federation of Immunological Societies (EFIS), EJI, the International Union of Immunological Societies (IUIS) and the Scientific and Technological Research Council of Turkey (TUBİTAK). We also thank Dr Sefik Sanal Alkan for a special grant in the name of his father, Mr. Hakkı Alkan, for supporting the attendance of young scientists. We would like to apologize to the many speakers whose excellent contributions could not be mentioned here because of space limitations.

References

1 Winau F, Weber S, Sad S *et al.* Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 2006;24:105–17.
2 Ulrichs T, Kosmiadi GA, Trusov V *et al.* Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J Pathol* 2004;204:217–28.
3 Baumann S, Nasser Eddine A, Kaufmann SH. Progress in tuberculosis vaccine development. *Curr Opin Immunol* 2006;18:438–48.
4 Piris-Gimenez A, Paya M, Lambeau G *et al.* In vivo protective role of human group IIa phospholipase A2 against experimental anthrax. *J Immunol* 2005;175:6786–91.
5 Raymond B, Leduc D, Ravaux L *et al.* Edema toxin impairs anthracidal phospholipase A2 expression by alveolar macrophages. *PLoS Pathog* 2007;3:e187.
6 Rehr M, Cahenzli J, Haas A *et al.* Emergence of polyfunctional CD8 + T cells after prolonged suppression of human immunodeficiency virus replication by antiretroviral therapy. *J Virol* 2008; 82:3391–404.
7 Martin MP, Qi Y, Gao X *et al.* Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 2007;39:733–40.
8 Majumder B, Venkatachari NJ, O'Leary S, Ayyavoo V. Infection with Vpr-positive human immunodeficiency virus type 1 impairs NK cell function indirectly through cytokine dysregulation of infected target cells. *J Virol* 2008;82:7189–200.
9 Arthos J, Cicala C, Martinelli E *et al.* HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol* 2008;9:301–9.
10 Brooks DG, Hamer DH, Arlen PA *et al.* Molecular characterization, reactivation, and depletion of latent HIV. *Immunity* 2003;19: 413–23.
11 Lapenta C, Spada M, Santini SM *et al.* Pertussis toxin B-oligomer inhibits HIV infection and replication in hu-PBL-SCID mice. *Int Immunol* 2005;17:469–75.
12 Feldmann J, Callebaut I, Raposo G *et al.* Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* 2003;115:461–73.
13 Menasche G, Feldmann J, Fischer A, de Saint Basile G. Primary hemophagocytic syndromes point to a direct link between lymphocyte cytotoxicity and homeostasis. *Immunol Rev* 2005;203:165–79.
14 Castigli E, Wilson SA, Garibyan L *et al.* TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet* 2005;37:829–34.
15 Salzer U, Chapel HM, Webster AD *et al.* Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat Genet* 2005;37:820–8.
16 Schaffer AA, Salzer U, Hammarstrom L, Grimbacher B. Deconstructing common variable immunodeficiency by genetic analysis. *Curr Opin Genet Dev* 2007;17:201–12.
17 Park MA, Li JT, Hagan JB, Maddox DE, Abraham RS. Common variable immunodeficiency: a new look at an old disease. *Lancet* 2008;372:489–502.
18 Kochi Y, Yamada R, Suzuki A *et al.* A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. *Nat Genet* 2005;37:478–85.
19 Suzuki A, Yamada R, Kochi Y *et al.* Functional SNPs in CD244 increase the risk of rheumatoid arthritis in a Japanese population. *Nat Genet* 2008;40:1224–9.
20 Zouali M, ed. *The Epigenetics of Autoimmune Diseases*. Wiley-Blackwell (an imprint of John Wiley & Sons Ltd, West Sussex, USA) (April 3 2009), 2009; 480 pp.
21 Mazari L, Ouarzane M, Zouali M. Subversion of B lymphocyte tolerance by hydralazine, a potential mechanism for drug-induced lupus. *Proc Natl Acad Sci U S A* 2007;104:6317–22.
22 Delaleu N, Immervoll H, Cornelius J, Jonsson R. Biomarker profiles in serum and saliva of experimental Sjogren's syndrome: associations with specific autoimmune manifestations. *Arthritis Res Ther* 2008;10:R22.
23 Delaleu N, Madureira AC, Immervoll H, Jonsson R. Inhibition of experimental Sjogren's syndrome through immunization with HSP60 and its peptide amino acids 437–460. *Arthritis Rheum* 2008;58:2318–28.
24 Suzuki M, Konya C, Goronzy JJ, Weyand CM. Inhibitory CD8 + T cells in autoimmune disease. *Hum Immunol* 2008;69:781–9.
25 Penna G, Mondaini N, Amuchastegui S *et al.* Seminal plasma cytokines and chemokines in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. *Eur Urol* 2007;51:524–33; discussion 33.
26 Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol* 2008;4:404–12.
27 Zakkar M, Chaudhury H, Sandvik G *et al.* Increased endothelial mitogen-activated protein kinase phosphatase-1 expression suppresses proinflammatory activation at sites that are resistant to atherosclerosis. *Circ Res* 2008;103:726–32.
28 Ciccocioppo R, Finamore A, Ara C, Di Sabatino A, Mengheri E, Corazza GR. Altered expression, localization, and phosphorylation of epithelial junctional proteins in celiac disease. *Am J Clin Pathol* 2006;125:502–11.
29 Li M, Oliver E, Kitchens KM, Vere J, Alkan SS, Tamiz AP. Structure-activity relationship studies of permeability modulating peptide AT-1002. *Bioorg Med Chem Lett* 2008;18:4584–6.
30 Gopalakrishnan S, Pandey N, Tamiz AP *et al.* Mechanism of action of ZOT-derived peptide AT-1002, a tight junction regulator and absorption enhancer. *Int J Pharm* 2009;365:121–30.
31 Aktas E, Akdis M, Bilgic S *et al.* Different natural killer (NK) receptor expression and immunoglobulin E (IgE) regulation by NK1 and NK2 cells. *Clin Exp Immunol* 2005;140:301–9.
32 Deniz G, Erten G, Kucuksezer UC *et al.* Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 2008;180:850–7.

33 Beutelspacher SC, Pillai R, Watson MP *et al.* Function of indoleamine 2,3-dioxygenase in corneal allograft rejection and prolongation of allograft survival by over-expression. *Eur J Immunol* 2006;36:690–700.

34 Tan PH, Beutelspacher SC, Xue SA *et al.* Modulation of human dendritic-cell function following transduction with viral vectors: implications for gene therapy. *Blood* 2005;105:3824–32.

35 Sommerer C, Hartschuh W, Enk A, Meuer S, Zeier M, Giese T. Pharmacodynamic immune monitoring of NFAT-regulated genes predicts skin cancer in elderly long-term renal transplant recipients. *Clin Transplant* 2008;22:549–54.

36 Cassard L, Cohen-Solal JF, Fournier EM *et al.* Selective expression of inhibitory Fcgamma receptor by metastatic melanoma impairs tumor susceptibility to IgG-dependent cellular response. *Int J Cancer* 2008;123:2832–9.

37 Cramer DW, Titus-Ernstoff L, McKolanis JR *et al.* Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1125–31.

38 Gritzapis AD, Voutsas IF, Lekka E *et al.* Identification of a novel immunogenic HLA-A*0201-binding epitope of HER-2/neu with potent antitumor properties. *J Immunol* 2008;181:146–54.

39 Oral HB, Deniz G, Zouali M. Functional imaging in the immune system. *Eur J Immunol* 2009;39:7–10.