The Fifth International Congress of Autoimmunity

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The international meeting on autoimmunity has become a tradition that is eagerly awaited by clinicians and scientists in the field from all over the world. The 4th meeting on autoimmunity (Budapest 2004), organized by Prof. Yehuda Shoenfeld, was attended by 850 participants. The 5th meeting, the most recent (Sorrento 2006), organized by Prof. Y. Shoenfeld and co-chaired by Prof. T. Koike (Japan) and Prof. P.L. Meroni (Italy), was a further success and achieved a high attendance of 1450 participants. By virtue of having several parallel sessions, participants were exposed to a wide range of subjects and eminent scientists. In addition, one should mention the extraordinary beauty of the place and the faultless organization, including social events and marvelous food, that added to the meeting's success. Below is a summary of the main topics discussed at the meeting.

Aspects of autoimmunity

In his introductory lecture on autoimmunity, I. Cohen (Weizmann Institute of Science, Rehovot, Israel) raised the theoretical possibility that the immune system should be able to provide us with both the history and potential of its activity, which in practical terms translates into vital diagnostic and prognostic information. Traditionally, immunological diagnosis has been based on an attempt to correlate each disease with a specific immune reactivity, such as an antibody or a T cell response to a single antigen specific for the disease entity. The state of the body, however, appears to be encoded by the immune system in collectives of reactivities and not by single ones. In this regard, the use of microarray technology and informatics to develop an antigen chip capable of detecting global patterns of autoantibodies binding to hundreds of self-antigens simultaneously is suggested. The patterns fashion diagnostic signatures for particular autoimmune diseases and for cancer. This antigen chip microarray provides a novel and informative look at the immunological homunculus.

Autoantibodies against dsDNA and nucleosomes are a characteristic feature of systemic lupus erythematosus. However, purified nucleosomes by themselves are poorly immunogenic, most likely due to lack of "danger signals" that contribute to break self-tolerance. In his lecture, J.R. Kalden (University Hospital Erlangen, Nuremberg, Germany) pointed to a high mobility group box protein 1, a nuclear DNA-binding protein and a secreted pro-inflammatory mediator that could represent such an endogenous danger signal. In apoptotic cells, HMGB1 becomes tightly attached to hypoacetylated chromatin; therefore, it is neither released nor does it induce inflammation. In his lecture he raised the hypothesis that in conditions of apoptotic cell clearance deficiency, which is observed in a subset of patients with SLE, non-phagocytosed apoptotic cells may undergo secondary necrosis, thereby releasing nucleosomes with tightly attached HMGB1. Hence the endogenous adjuvant HMGB1 may foster an autoimmune response towards nucleosomes, a crucial autoantigen in SLE.

In an attempt to better understand the development of SLE, M. Herrmann (Institute for Clinical Immunology, Friedrich Alexander University of Erlangen, Germany) reported his findings of impaired clearance functions for dying cells, which lead to the accumulation of nuclear autoantigens in various tissues of some SLE patients. His data showed that in a subgroup of patients with SLE, apoptotic cells accumulated in the germinal centers of the lymph nodes. The numbers of tangible body macrophages usually containing engulfed apoptotic nuclei were significantly reduced in these patients. Also found was an accumulation, after ultraviolet exposure, of apoptotic cells in the skin of patients with cutaneous lupus. The analysis of high affinity DNA-binding immunoglobulin G autoantibodies from SLE patients revealed that those antibodies had gained their DNA reactivity in a germinal center reaction. The early recognition of dying cells requires characteristic membrane surface changes, which do not occur on normal cells, suggesting the existence of chemotactic attraction signals of phagocytes for apoptotic cells. Finally, the attraction signals of apoptotic cells were blocked by sera in approximately 30% of the SLE patients. He concluded by stating that failure of clearance in the early phases of apoptosis may lead to a secondary necrotic status of the cells, followed by the release of danger signals and the beginning of an autoimmune reaction.

In line with the above, C.G.M. Kallenberg (Department of Rheumatology and Clinical Immunology, University of Croningen,

HMGB1 = high mobility group box protein 1
SLE = systemic lupus erythematosus

The Netherlands) added some information on the accumulation of apoptotic cells and their key role in the induction of autoimmunity. He evaluated the capacity of monocyte-derived macrophages from SLE patients to phagocytose apoptotic Jurkat cells and apoptotic keratinocytes. In the presence of normal serum, monocyte-derived macrophages from SLE patients did not differ from controls in their uptake of apoptotic cells. However, uptake was decreased in the presence of serum from patients with active SLE. Also, uptake was decreased in the presence of IgG from SLE patients. Binding of autoantibodies from SLE sera to apoptotic cells expressing autoantigens on their surface inhibited the uptake of these cells via an Fc gamma receptor-dependent mechanism. I*n vivo*, apoptosis of keratinocytes was induced by ultraviolet B irradiation of the skin. Clearance of apoptotic cells was studied in biopsies taken within 10 days after irradiation. Although SLE patients showed increased sensitivity to ultraviolet B, there was no difference in the number of apoptotic cells in SLE patients and controls. However, 30% of SLE patients and none of the controls developed infiltrates in the vicinity of apoptotic cells, suggesting inflammatory clearance of apoptotic cells. This study strengthened the finding that uptake of apoptotic cells in SLE patients is decreased in the presence of autoantibodies and that uptake of apoptotic cells *in vivo* may result in inflammatory responses.

The issue of DNA repair in immunoglobulin somatic hypermutation was discussed by P. Casali (Center for Immunology, University of California, Irvine, CA, USA). Somatic hypermutation underlies the maturation of antibody and autoantibody responses, as it provides the structural substrate for the selection of higher affinity mutants by antigen or self-antigen, thereby giving rise to a diverse repertoire of high affinity antibodies or autoantibodies. It is affected by a two-step process: a) DNA lesions initiated by activation-induced cytokine deaminase, and b) lesion repair by combined intervention of DNA replication and repair factors that include mismatch repair proteins and translesion DNA synthesis polymerases. AID and TLS polymerases are crucial to somatic hypermutation, namely polymerase (pol), and are induced in B cells by the stimuli that are required to trigger this process: B cell receptor cross-linking and CD40 engagement by CD154. These polymerases would assemble to form a multimolecular complex (mutasome) at the site of DNA lesions. Molecular interactions in the mutasome would result in a DNA polymerase switch.

G.R.V. Hughes (London Lupus Research Centre, London Bridge Hospital, UK) presented a brief history on the failure and success in treating lupus patients. Examples of "failure" at the individual level include the development of strokes or myocardial infarction in under-treated antiphospholipid syndrome patients, the treatment resistance of some cases of severe lupus skin disease, and the failure at present to protect against congenital heart block. On a more general level, there remains the problem of under-recognition and under-treatment on the one hand, and perversely, over-treatment and iatrogenic Cushing's syndrome on the other. It is also apparent that lupus rivals diabetes in its risk of late atherosclerotic disease, and that known risk factors such as high cholesterol and positive antiphospholipid antibody are inadequately addressed. On the bonus side, the outcome of lupus pregnancy has improved dramatically and the development of renal failure has been reduced. New drugs such as mofetil and rituximab are already extensively used. Another major advance has been the more conservative approach to steroids and cyclophosphamide treatment, and the wider use of milder agents such as hydroxychloroquine. Perhaps the single most important change in lupus has come from the recognition of the importance of APS. Many features such as stroke, migraine, atypical multiple sclerosis, seizures and myocardial infarction, previously ascribed to "vasculitis" and treated with steroids and cyclophosphamide, are recognized as more likely due to APS and treated more appropriately with antithrombotic agents.

Autoimmunity and protective regulatory cells

The issue of tolerance and autoimmunity was discussed by A.K. Abbas (Department of Pathology, UCSF, San Francisco, CA, USA). He focused on the role of T regulatory lymphocytes (Treg) in maintaining suppression of self-reactive lymphocytes, the failure of which leads to autoimmune diseases. In order to study systemic T cell tolerance, his group developed an experimental model in which CD4 T cells specific for ovalbumin (0va) encounter a transgene-encoded soluble form of secreted ova expressed as a self-antigen. In an intact antigen-expressing recipient, the T cells become tolerant and are deleted. In lymphopenic recipients that express the sOVA, tolerance fails, and pathogenic effector cells cause a severe disease resembling graft versus host disease. Tissue inflammation in this model was found to be mediated by interleukin-7-producing cells, and Th1 cells were surprisingly protective. Over time, the effector cells are replaced by CD25+FoxP3+ Treg cells, which function to control the pathologic immune response. IL-2 is required for the maintenance of Treg; in the absence of IL-2, the acute disease is less severe but the mice develop a chronic progressive disease that is not controlled. Thus, the balance between pathogenic effector T cells and protective regulatory cells is central to the choice between tolerance and autoimmunity.

J.-F. Bach (INSERM U25, Immunologie Clinique, Hospital Necker, Paris, France) pointed to the existence of natural regulatory T cells by the onset of a poly-autoimmune syndrome following day 3 themectomy I BALB/c mice and its prevention by infusion of CD4+CD25+ T cells. It appears though that CD25+ T cell depletion does not markedly enhance the onset or progression of various spontaneous or experimentally induced autoimmune diseases. In these settings the responsibility of antigen-driven adaptive regulatory T cells has been fully documented. The data provide a precise determination of the various subsets of adaptive regulatory T cells, which may express CD25 but are derived from CD25-neg T cells. Most therapeutic maneuvers that prevent or cure autoimmune diseases through stimulation of regulatory T cells do so after inducing adaptive Treg cells. This is notably the case for soluble

IgG = immunoglobulin G
AID = activation-induced cytokine deaminase
TLS = translesion DNA synthesis

APS = antiphospholipid syndrome
IL-2 = interleukin-2

autoantigens, which stimulate Th2 and Th3 cells, and anti-CD3 monoclonal antibodies, which stimulate TGFβ-dependent CD62L+ regulatory T cells. The dissection of such complexity of regulatory T cells is important for a better understanding of the immunological events, which lead to distinct autoimmune diseases.

A role for B cells in the regulation of immune response was presented by S. Gupta (Cellular and Molecular Immunology Laboratories, University of California, Irvine, USA). In murine models of autoimmunity and allergic diseases as well as in adoptive transfer experiments, there is evidence showing that B cells may also regulate immune responses. Dr. Gupta provided data to support the presence of human Breg cells. These cells inhibit T cell proliferation and appear to target predominantly effector memory CD4+ and CD8+ T cells. The mechanisms by which Breg cells may possibly suppress T cell proliferation are not yet clear. Active B cells were not found to inhibit CD25 expression on regulatory T cells and they did not induce T cell apoptosis. Breg cells were shown to overexpress IL-10 and belonged more to CD5+ B cells. Thus, their suppressive function could be mediated by IL-10. More studies are needed to better characterize these cells.

A role for B cells in autoimmunity

In his lecture P. Youinou (Medical School Hospital, Brest, France) described B cells as the conductor of the lymphocyte orchestra in autoimmunity. The relevance of B cells is currently being emphasized and new insights into their functions are being revealed. Beyond the paradigm that T lymphocytes maintain strict control over B cells, the latter cells are now acknowledged to solicit their own help from the former cells to release a flurry of interleukins, and to act as antigen-presenting cells. Increased levels of the B cell-activating factor may be responsible for qualitative anomalies, such as high numbers of circulating Bm2 cells and accumulation of marginal zone-like B cells in solid tissues. BAFF is also associated with functional abnormalities of B cells, such as aberrant production of BAFF, or increased secretion of IL-6 – both of which act in an autocrine manner. Thus, it is no surprise that B cell ablative treatment has proven remarkably efficacious in rheumatoid arthritis, Sjogren's syndrome, and SLE.

In an attempt to better understand the development of autoreactive B cells, C. Jamin (Laboratory of Immunology, Brest University Medical School, Brest, France) reported on the association between CD5 and the expression of RAG in mature B cells outside germinal centers. Tonsillar B cells were purified and stained with anti-IgD, anti-CD38 and anti-CD5 antibodies to identify the different mature B cell subpopulations. The expression of RAG1 and RAG2 mRNA and protein were also performed. Activated mature CD5-pos IgD+CD38+ B cells co-express both RAG1 and RAG2 mRNA and protein, and display DNA cleavage resulting from their recombinant activity. Furthermore, *in vitro* activation of CD5-neg naïve mature B cells by immunoglobulin receptor and CD40 cross-linking induces expression of CD5 on a subset of cells, and leads to the up-regulation of RAG1 and RAG2 only in cells turned positive for CD5. Thus, RAG gene expression is closely related to CD5 expression outside germinal centers. CD5 is associated with receptor revision in activated mature B cells and is likely to promote expression of a suitable immunoglobulin receptor capable of initiating the germinal center reaction. Defaults in the control of this process may contribute to the generation of autoreactive B cells.

D. Melamed (Dept. of Immunology, Faculty of Medicine, Technion, Haifa, Israel) presented his research on the activation of developing B cells by CpG-DNA as a novel pathway for the breakdown of self-tolerance. Because the major part of the B cell repertoire during bone marrow development is self-reactive, the basic immunological dogma excludes these cells from an immune response. Hence it is thought that B cell autoimmunity exclusively reflects the breakdown of peripheral tolerance. His study was designed to test whether developing bone marrow B cells are capable of mounting an immune response and breaking tolerance upon Toll-like receptor stimulation. He found that developing B cells express Toll-like receptor-9 and undergo a robust polyclonal response to CpG-DNA, as reflected by proliferation, secretion of antibodies and formation of antibody-producing plasma cells. Strikingly, he found that CpG-DNA stimulation protects precursor B cells from negative selection, imposed by apoptosis and receptor editing, resulting in the production of autoantibodies. This suggests a novel pathway whereby developing non-tolerant B cells can be driven by mitogenic stimuli to initiate an autoimmune response.

Antiphospholipid syndrome

T. Koike (Dept. of Medicine II, Hokkaido University School of Medicine, Sapporo, Japan) pointed to the importance of tissue factor in the initiation of the coagulation system. He stated that TF is induced on monocytes by aPL *in vitro*, explaining, in part, the pathophysiology of APS. He investigated aPL-induced genes in peripheral blood mononuclear cells using the cDNA array system and reverse transcriptase-polymerase chain reaction. He showed that the mitogen-activated protein kinase pathway was related to TF expression when PBMCs were treated in the presence of beta 2-glycoprotein 1 with human monoclonal anti-2GP1 antibodies. Western blotting studies using monocyte cell line demonstrated that p38 MAPK protein was phosphorylated with nuclear factor B activation by monoclonal anticardiolipin antibody/2GP1 treatment, and that SB203580, a specific p38 MAPK inhibitor, decreased the aCL/2GP1-induced TF mRNA expression. These results demonstrated that the p38 MAPK signaling pathway plays an important role in aPL-induced TF expression on monocytes and suggest that the p38 MAPK may be a possible therapeutic target to modify a prothrombotic state in patients with APS.

P.L. Meroni (Dept. of Internal Medicine, University of Milan, Milan, Italy) discussed the important issue of APS being an

TGFβ = transforming growth factor-beta
BAFF = B cell-activating factor

TF = tissue factor
aPL = antiphospholipid antibody
PBMCs = peripheral blood mononuclear cells
2GP1 = 2-glycoprotein 1
MAPK = mitogen-activated protein kinase
aCL = anticardiolipin antibody

inflammatory disease. I*n vivo* experimental models showed that aPL thrombogenic activity is associated with a pro-inflammatory endothelial phenotype (increased adhesion molecule expression and white blood cell adhesion) in addition to a procoagulant one (TF expression). Mice deficient in ADM or treated with anti-ADM blocking Abs were found to be protected against the endothelial cell thrombogenic effect of aPL. This is in line with the *in vitro* aPL ability to trigger intracellular signaling and to up-regulate endothelial ADM, TF and pro-inflammatory cytokine/chemokine expression. Increased plasma levels of soluble ADM isoforms, circulating ECs, and impaired endothelium-dependent flow-mediated vasodilation are found in primary APS, as described in other chronic systemic inflammatory diseases. In addition, complement activation is required by aPL to display their thrombogenic activity in *in vivo* models. Interestingly, complement activation blocking as well as tumor necrosis factor-alpha neutralization protect animals from aPL-induced fetal losses. Chemokines were also found in his studies to be involved in aPL-mediated fetal loss in mice. These findings support the role of inflammation in APS. However, a localized rather than a systemic inflammation seems to be a contributing factor with the exception of the APS catastrophic variant.

The issue of renal manifestations in antiphospholipid syndrome was discussed by M. Blank (Dept. of Medicine B and Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel). Whereas renal manifestations of APS include renal artery stenosis, renal infarctions, thrombotic microangiopathy, and/or malignant hypertension, non-thrombotic conditions like glomerulonephritis have also been reported. She reported on the involvement of the actin-binding protein transgelin in the renal pathology of APS. By means of the proteomics approach, IgG from an APS patient with renal involvement was subjected to 2D-electrophoresis and mass-spectroscopy. Recombinant transgelin was expressed in a bacterial system and used to screen sera from APS patients and immunization of BALB/c mice. Transgelin was the major antigen detected in APS patients. Raised titers of anti-transgelin antibodies were detected in 24% of patients with nephritis and a background of APS. Immunization of naïve mice with recombinant transgelin induced glomerulonephritis. Thus, transgelin over-expression is involved in the kidney pathology in APS. Preliminary data show that circulating anti-transgelin antibodies may have predictive diagnostic potential in APS. In addition, M. Blank presented her data on the efficacy of specific intravenous immunoglobulin in APS. The binding to pathogenic autoantibodies and hence inhibition of binding to the corresponding antigens is one of the mechanisms of the beneficial effect of IVIg. In this study, anti-2GP1 antibodies were fractionated from commercial IVIg and applied as a treatment for an experimental APS mouse model. The effect of sIVIg *in vitro* was tested in a trophoblast and choriocarcinoma matrigel/invasion assay (i.e., proliferation and matrix metalloproteinase-2/MMP9 expression) and *in vivo* in a fetal loss model of APS. Anti-2GP1 antibodies inhibited human trophoblast cell invasion *in vitro*. APS-specific IVIg fraction reduced human trophoblast invasion *in vitro* 560 times more than the whole IVIg compound and elevated the MMP2/MMP9 production by trophoblasts. sIVIg significantly improved (200 times) the pregnancy outcome in BALB/c mice passively infused with anti-2GP1 antibodies compared to the treatment with IVIg ($P < 0.02$). In conclusion, specific APS-sIVIg may be considered an effective and safe compound for treating patients with APS-related early fetal loss.

ADM = adhesion molecule
EC = endothelial cells
IVIG = intravenous immunoglobulin
MMP = matrix metalloproteinase

Autoimmunity and atherosclerosis

B.H. Hahn (UCLA School of Medicine, Los Angeles, USA) discussed the issue of accelerated atherosclerosis in SLE and rheumatoid arthritis. Risk for atherosclerosis is increased five- to sevenfold in SLE and two- to threefold in RA. Although standard factors such as age, hypertension, diabetes and metabolic syndrome contribute to risk in SLE and RA, each disease is itself an independent risk factor for atherosclerosis. It is likely that chronic vascular damage occurs from years of exposure to circulating immune complexes, antibodies to endothelium and phospholipids, and the products of chronic inflammation and chronic oxidative damage that activate endothelial cells and block the normal clearance of harmful oxidized molecules such as oxidized low density lipoproteins. Promising lipid biomarkers for increased risk of atherosclerosis in SLE patients include elevated levels of antibodies to lipoprotein lipase, elevated levels of oxLDL, IgG antibodies to oxLDL, and pro-inflammatory high density lipoproteins. Since SLE is associated with chronic inflammation and oxidative damage, piHDL probably result from oxidative damage to normal HDL, with impairment of cholesterol-transporting apolipoproteins. Such piHDL can be detected *in vitro* by their failure to release fluorochrome from a molecule (DCFH) which fluoresces as it is oxidized by oxLDL. In this respect, 154 SLE patients, 45 RA patients and 72 healthy controls were tested using the DCFH assay, which showed that 45% of SLE and 20% of RA patients had piHDL, compared to 5% of normals. In addition, piHDL were found in all patients with coronary artery disease in the SLE group, and in those with carotid artery plaques. As expected, high levels of piHDL were correlated with high levels of oxLDL. It is likely that an algorithm can be constructed, consisting of a combination of a patient's standard risks, disease state and levels of pathogenic lipids. This should inform us which patients need to be protected with preventive therapies, such as statins.

The involvement of antiphospholipid antibodies in atherosclerosis in APS patients was summarized by K. Kobayashi (Dept. of Cell Chemistry, Okayama University Graduate School of Medicine, Okayama, Japan). Macrophage uptake of oxLDL is considered to play a critical role in the early stage of atherosclerosis. He previously reported that oxLDL forms stable complexes with β2GP1 and that the complexes are detected in the sera of patients with SLE and/or APS. oxLDL/β2GP1 complexes were shown to be an antigenic target of autoantibodies present in APS. To understand the role of autoantibodies in accelerated atherosclerosis with SLE and APS,

oxLDL = oxidized low density lipoproteins
piHDL = pro-inflammatory high density lipoproteins

he investigated the binding characteristics of β2GP1 and oxLDL to mouse macrophages J774, and the effect of anti-β2GP1 autoantibodies and anti-oxLDL autoantibodies on them. 1251-labeled β2GP1 and 1251-oxLDL were used to analyze their binding characteristics to J774 macrophages. Binding of oxLDL/β2GP1 complexes to macrophages was relatively lower than that of oxLDL. IgM anti-oxLDL antibodies partly inhibited oxLDL binding to macrophages. β2GP1 alone showed very weak binding to the macrophages, but β2GP1 showed stronger binding as complexes with oxLDL and strongest together with IgG as immune complexes. These results suggest that IgG anti-β2GP1 antibodies present in APS are pro-atherogenic. The possibility that oxLDL/β2GP1 complexes are involved in antigen presentation of β2GP1 as an autoantigen is suggested.

E. Matsura (Dept. of Cell Chemistry, Okayama University, Japan) and Y. Shoenfeld (Sheba Medical Center, Tel Hashomer, Israel) summarized new aspects in autoimmunity, infections, and atherosclerosis. Their recent findings further suggested that oxLDL forms complexes with β2GP1 and/or C-reactive protein in the intima of atherosclerotic lesions. Autoantibodies against oxLDL/β2GP1 complexes occur in patients with SLE and/or APS and significantly correlate with arterial thrombosis. IgG with such specificity emerged spontaneously in non-immunized NZWxBXSB F1 mice, an animal model of APS, and significantly increased the *in vitro* uptake of oxLDL/β2GP1 complexes by macrophages. This observation strongly suggests that such IgG autoantibodies are pro-atherogenic. In contrast, IgM anti-oxLDL natural antibodies found in atherosclerosis-prone mice (ApoE-1-and LDL-R-1-mice) have been proposed to be protective. In their most recent study, they found that infection with H*elicobacter pylori* significantly accelerates the progression of atherosclerosis. In these atherosclerosis-prone mice, inducing heat shock protein 60-specific Th1 responses and active or passive immunization could down-regulate the Th1 response, thereby preventing the progression of atherosclerosis.

Autoimmunity and the central nervous system

R. Arnon (Dept. of Immunology, Weizmann Institute of Science, Rehovot, Israel) discussed autoimmune inflammatory mechanisms in the central nervous system and the compensatory CNS resources that play a role in the development of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis. Current treatments for MS are effective in ameliorating the autoimmune inflammatory process, but their ability to enhance the intrinsic CNS repair mechanism has not been shown. Glatiramer acetate, an approved drug that was developed at Weizmann for MS treatment, exerts a marked suppressive effect on EAE. The immunomodulatory effect of GA was attributed to its ability to induce Th2/Th3 cells, which secrete high levels of anti-inflammatory cytokines. Her group showed that these cells cross the blood-brain barrier, accumulate in the CNS and express *in situ* IL-10, TGFβ, as well as brain-derived neurotrophic factor. It was demonstrated that in EAE-inflicted mice, parallel to its effect on the autoimmune reactivity, GA treatment at various stages of the disease led to sustained reduction in the neuronal/axonal damage. This suggests a direct linkage between immunomodulation, namely the dampening of the autoimmune reactivity, neuroprotection and neurogenesis.

Under the title "To smell autoimmunity," Y. Shoenfeld (Sheba Medical Center, Israel) discussed the important issue of CNS involvement in SLE (CNS-SLE). He mentioned that CNS-SLE is associated with more than 20 different autoantibodies, the most remarkable of which are the anti-P-ribosomal antibodies. These antibodies are capable of penetrating live cells and inducing apoptotic changes, leading to inhibition of specific cytokine secretion. Anti-P-Abs were first reported to associate and correlate with CNS-SLE and especially with psychosis in 1987. When purified anti-P-Abs were injected directly into the brain ventricles of Balb/c mice, compared to irrelevant immunoglobulins, the mice clearly expressed depressive behavior, characterized mainly by a floating pattern, rather than swimming, in an enforced swimming test. This floating pattern could be reversed by a specific therapy with a monoclonal anti-idiotypic drug. The human purified Anti-P-Abs were shown to bind to CNS structures including the smell apparatus. Smell defects characterize bulbectomized depressive mice, as they do also in depressive women, and can be overcome by exposure to citrus fragrance – in both mice and women. These studies show, for the first time, the active induction of a psychiatric condition by means of a specific antibody, and the induction of a smell defect. These data may pave the way to a novel approach to depression in CNS-SLE.

The role of anti-P-Abs in inducing neuropsychiatric manifestations and autoimmune depression in animal models was further discussed by A. Katzav (Dept. of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Israel). The study was designed to address the question of how specific brain-binding autoantibodies induce well-defined psychiatric disorders. In this study (A. Katzav, J. Chapman. and Y. Shoenfeld, Israel) found that injecting mice intracerebro-ventricularly with affinity-purified human anti-P-Abs induced depressive-like behavior in a forced swim test. The anti-P-induced depressive-like behavior was significantly blocked by a specific anti-idiotypic antibody and by long-term antidepressant therapy (fluoxetine). Anti-P-Abs specifically stained neurons in the hippocampus, cingulated cortex and the primary olfactory piriform cortex, compatible with binding to previously described membrane-bound ribosomal protein PO. This is the first report of an experimental depression induced by a specific autoantibody that implicates olfactory and limbic areas in the pathogenesis of CNS dysfunction in SLE.

New aspects in the treatment of autoimmunity

The beneficial effect of mycophenolate mofetil in SLE was presented by A. Doria (Division of Rheumatology, Dept. of Clinical and Experimental Medicine, Medical Institute, Padova, Italy). Over the last few years MMF has emerged as an alternative regimen mainly

CNS = central nervous system
MS = multiple sclerosis
EAE = experimental autoimmune encephalomyelitis
GA = glatiramer acetate

Anti-P-Abs = anti-P-ribosomal antibodies
MMF = mycophenolate mofetil

for patients with SLE manifestations refractory to other therapies. To date, four randomized controlled studies on the use of MMF in the treatment of lupus glomerulonephritis have been published, demonstrating that MMF is well tolerated and effective in induction and maintenance therapy. In one of these studies MMF was even more effective than cyclophosphamide in inducing remission of lupus glomerulonephritis. Atherosclerosis is accelerated in SLE patients; and MMF is an attractive candidate for the modulation of inflammatory activation in atherogenesis due to down-regulation of the expression of adhesion molecules and attenuation of macrophage responses. A further potential effect of MMF is that of blocking the evolution from mild to severe disease, which is a very important determinant of poor long-term survival in SLE patients.

The beneficial role of rituximab in treating patients with SLE and other autoimmune diseases was discussed by C. Galarza (Unidal De Enfermedades Reumaticas Y autoimmunes UNEDA, Hospital Monte Sinai, Guenca, Guayaquil, Ecuador). He presented the results of a retrospective study conducted in four referral centers in Colombia and Ecuador. Indications for treatment were a refractory character of the disease and inefficacy of other immunosuppressors. Of 105 patients 74 met all the inclusion criteria for review. Forty-three patients had SLE, 21 had RA, 8 had Sjogren's syndrome and 2 had Takayasu's arteritis. Rituximab was well tolerated in 66 (89%), but in 8 patients side effects necessitated discontinuation of the medication. The mean follow-up period was 12 ± 7.8 months. The efficacy of rituximab was noted in 58/66 patients (87%), of whom 36 (83%) had SLE and 18/21 (85%) had RA. The mean time of efficacy was 6.3 ± 5.1 weeks. A significant steroid-sparing effect was recorded in 50% of patients. These results add further evidence supporting the use of rituximab in SLE and RA.

E. Toubi (Division of Clinical Immunology, Bnai Zion Medical Center, Haifa, Israel) reported on the association between rituximab-related cell depletion and increased BAFF and IL-10 nRNA expression in macrophages of patients with RA. In this study the authors sought to correlate the effect of rituximab B cell depletion in RA patients with relevant specific autoantibody attenuation and with function changes of macrophages, at baseline and 4 months following rituximab initiation. Ten patients with active RA were treated with rituximab and assessed for clinical outcome, B cell count, total serum IgG, the presence of rheumatoid factor, and anti-cyclic citrulinated protein antibodies (anti-CCP). In addition, the expression of mRNA BAFF and IL-10 in macrophages of these patients was tested. During B cell depletion, RF titers had declined in three patients and disappeared in another two, in correlation with clinical response. However, anti-CCP antibodies disappeared in only one patient and remained unchanged in nine. Of interest is the finding that both BAFF and IL-10 mRNA expression were significantly increased in macrophages following B cell depletion compared to baseline levels. The increase in mRNA BAFF expression in macrophages is part of a compensatory effect that is responsible for B cell repopulation and survival. On the other hand, increased IL-10 expression reflects a shift in the balance of cytokines towards a Th2 response. These results point to the complexity of mechanisms that underlie the beneficial outcome of rituximab in RA.

A role for humanized monoclonal antibody therapy for RA was suggested by I. Golan and D. Naor (MaimonixDex RA Ltd., Tel Aviv, and The Hebrew University, Jerusalem, Israel). Unusual alternative CD44 mRNA splicing in inflammatory synovial fluid cells of RA patients leads to the production of a modified disease-specific protein designated CD44vRA. This disease-specific protein can therapeutically be targeted by monoclonal antibodies, resulting in selective destruction of joint-infiltrating cells. Monoclonal antibodies specifically target this modified CD44 protein without affecting cells engaged in normal function and express the normal form of CD44. With regard to status of development: Firstly, proof of concept has been achieved: a) these CD44vRA-specific mAbs display limited cross-reactivity with wt CD44; b) efficacy in a murine model of human RA has been demonstrated; and c) *ex vivo* experiments with synovial fluid cells from RA patients showed apoptotic effect of CD44vRA-specific mAbs. Secondly, two major mAbs are in the preclinical testing stage. This is an additional potential therapy for RA.

G. Zandman-Goddard (Dept. of Medicine C, Wolfson Medical Center, Holon, Israel) and Y. Shoenfeld (Sheba Medical Center, Israel) reported on the steroid-sparing effect of intravenous immunoglobulin therapy in patients with autoimmune diseases during the period 2000–2005. These included 17 patients with evidence of active disease: 11 with SLE and 6 with other autoimmune diseases such as RA, polyarteritis nodosa, Sjogren's syndrome with neuropsychiatric system involvement and dermatomyositis, who were treated with high dose IVIg (2 g/kg for a 5 day course) and high dose prednisone. Altogether, 134 courses were given. Mild adverse effects including headache, fatigue and nausea were present in 59% of patients who received two or more IVIg courses. There were three thromboembolic events in patients while on treatment: pulmonary embolism in a patient with SLE and APS, an episode of grand-mal type seizures in a patient with SLE and APS, and acute myocardial infarct in a patient with systemic sclerosis. The average prednisone consumption decreased by 11.25 mg/day in eight patients, and was discontinued in two patients. Thus, IVIg side effects were predominantly mild and transient. The use of IVIg is additionally beneficial due to its steroid-sparing effect.

In conclusion, the meeting was extremely successful. Not only did it cover most of the 'hot' issues in autoimmunity, it was also a good opportunity for physicians and researchers to set a basis for future collaboration. All participants expressed their wish to participate in the next meeting, which will be held in Porto, Portugal in 2008.

RF = rheumatoid factor
mAb = monoclonal antibodies

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