



## **The 15th Rappaport Symposium Immune-Mediated Brain Injury and Repair: Mechanisms and Therapies**

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This meeting, held in Haifa, Israel, brought together neurologists, neuroimmunologists and neuroscientists from Europe, the United States and Canada to discuss basic and applied aspects of brain injury and repair. As the most notable result of this symposium, it became clear that a paradigmatic shift is occurring in a number of neurological diseases. Diseases such as Alzheimer's and Parkinson's that had previously been viewed as purely neurodegenerative are now recognized as possessing an important inflammatory component. Vice versa, in multiple sclerosis (one focus of this symposium), immunologists, neuropathologists and clinicians have begun to realize that degenerative aspects play a role, particularly during the progressive stage of the disease. Furthermore, it became clear that a growing number of researchers are not only interested in how brain tissue is destroyed in these disease but how damage can be prevented or repaired.

The first session on neuroimmune interactions addressed the role of cellular and humoral immune responses in MS as well as the contribution of immune-mediated repair mechanisms in regeneration. R. Martin (Bethesda, USA) presented a growing list of myelin proteins and peptides including myelin oligoden-

drocyte basic protein, oligodendrocyte-specific protein and  $\alpha$ B-crystallin, which were recently shown to be encephalogenic in animal models and/or immunogenic in MS patients. He argued that this heterogeneity in antigen specificities may in part explain the different clinical phenotypes and courses of MS. Dr. Kerlero de Rosbo (Rehovot, Israel) summarized current knowledge on humoral immune responses in MS and built a clear case that myelin oligodendrocyte glycoprotein-specific antibodies are an important effector mechanism determining the extent of demyelination. R. Hohlfeld (Munich, Germany) and M. Schwartz (Rehovot) presented intriguing data showing that the immune system and inflammation not only contribute to brain tissue damage but may also be crucial for the subsequent repair by secreting neural growth factors such as brain-derived neurotrophic factor. In addition to these *in vitro* data, Schwartz and colleagues examined in an *in vivo* system whether inflammation is beneficial or detrimental in optic nerve and spinal cord crush injury paradigms. Surprisingly, her elegant data demonstrate that the tissue-specific immune response after optic nerve injury will lead to improved outcomes of subsequent spinal cord trauma and, more directly, that the transfer of myelin-specific, potentially encephalitogenic, T cells can mediate this beneficial effect.

A session on brain inflammation included aspects of blood-brain barrier function and cellular transmigration as well as the heterogeneous infiltrate and mediators in immune-mediated central nervous system processes. J. Archelos (Graz, Austria) dissected the complex and coordinated contributions of integrins, selectins, immunoglobulin superfamily members and of various chemokines to the transmigration of T lymphocytes and other cellular components into the CNS and the subsequent development of tissue inflammation. While the functional role of these molecules has been documented in experimental allergic encephalomyelitis, the animal model of MS, Archelos pointed out that the treatment results in MS do not yet allow firm conclusions, and that inhibition of adhesion molecules in rare instances (anti-LFA-1) may also lead to worsening of EAE. Based on magnetic resonance imaging and computed tomography studies in patients as well as on animal experiments, H. Soreq (Jerusalem, Israel) argued that a number of factors – including stress, increased cortisol levels and elevated blood pressure – may locally and transiently compromise BBB integrity, possibly paving the way for numerous disease conditions. The central role of resident microglia and macrophages in brain

MS = multiple sclerosis

EAE = experimental allergic encephalomyelitis

inflammation both during classical neuroimmunological conditions such as MS and degenerative diseases was stressed by J. Antel (Montreal, Canada). While microglia are clearly involved in these processes, their activation state and quality of microglia-T cell interaction may lead to pro-inflammatory (CD40:CD40L, CD80:CD28 interaction; interleukin-12 secretion) or immunomodulatory (CD86:CD28 interaction; IL-10 secretion) conditions. Microglia activation can be achieved either by transmigrating T cells, e.g., during autoimmune conditions, or by infection, tissue injury or the presence of dying cells, e.g., during neurodegeneration. Activated microglia serve a central role in tissue inflammation via soluble mediators (cytokines, chemokines, proteases, excitotoxins, radicals) or by acting in concert with cells and antibodies of the adaptive immune system.

However, they may also be involved in repair by secreting neurotrophins. Whether this balance of damaging and repair functions can be utilized therapeutically needs to be established. Along similar lines, L. Grimaldi (Milan, Italy) elaborated on the intriguing similarities of tissue inflammation in such diverse conditions as human immunodeficiency virus-associated encephalopathy, tertiary syphilis, MS, paraneoplastic diseases and vasculitides to neurodegenerative diseases such as Alzheimer's and Parkinson's disease or amyotrophic lateral sclerosis. While different stimuli may initiate the process, namely the deposition of intracellular (tau protein) and extracellular ( $\beta$ -amyloid protein) in Alzheimer's or an infectious organism in syphilis, similar pro-inflammatory mediators including IL-1 family members (IL-1 $\alpha$ , IL-1 $\beta$ , IL-1RA, IL-18) are secreted by microglia and found in all of them. H. Lassmann (Vienna, Austria) elegantly summarized data on the histopathological heterogeneity of MS. The qualitative and quantitative characterization of MS plaques with respect to cellular composition (T cells and macrophages, microglia activation), presence of antibodies, deposition of complement,

presence of various myelin proteins, apoptotic oligodendrocytes, and signs of demyelination, axonal loss and remyelination allows the differentiation of at least four clear subtypes of MS pathology. One is characterized by marked oligodendrocyte dystrophy and is reminiscent of virus-induced or metabolic disturbances, while others show the classical signs of T cell-mediated autoimmune pathology or a coordinated involvement of macrophages/microglia and antibodies. These data indicate that demyelination in MS is a complex event. Although the immunological mechanisms appear to differ between patients, only one pattern was observed in any given patient at different stages of the disease. Together with the increasingly recognized heterogeneity of MS with respect to clinical phenotype and MRI characteristics (see below), these data will hopefully also allow more differentiated therapies in the future.

A. Compston (Cambridge, UK) addressed the current state of affairs regarding the genetics of MS. He summarized the results from several whole-genome screens that have been conducted so far and concluded that there is now firm evidence that MS is a complex trait resulting from an interplay of multiple genetic and environmental factors. Apart from the known but weak association with the HLA-DR/DQ alleles DRB1\*1501, -B5\*0101, DQA1\*0102 and -B1\*0602 in Caucasians and the complex haplotype DRB1\*0405-DQA1\*0301, -B1\*0302 in Mediterranean MS patients, very few additional markers have been identified during recent years. Therefore, a European Initiative (GAMES = Genetic Analysis of Multiple Sclerosis in EuropeanS) plans to type pooled DNA from cases, their parents and controls by a 6,000 microsatellite marker whole-genome linkage disequilibrium screen in 20 locations. This effort is expected to provide new insights into quantitative traits as well as disease heterogeneity.

E. Levi-Lahad (Jerusalem) outlined observations on the genetics of Alzheimer's disease and neurodegeneration. Basically, the discovery of genes that are involved in familial rather than

sporadic disease opens a window of opportunity to study factors that are primary rather than secondary to the disease processes. The later onset, incomplete penetrance and variable age of onset for example in familial Alzheimer cases with the presenilin-2 mutation suggest that the effect of this gene is modified by other genetic/environmental factors in sporadic cases as well. She mentioned the modulation of apoptosis as one mechanism of presenilin function and presented novel data on the regulation of PS2 expression via two TATA-less promoters, one of which is inducible particularly in neuronal cell lines.

Following the discussion on predisposing genetic factors, D. Offen (Tel Aviv) asserted that apoptotic cell death contributes not only to physiological tissue differentiation, but is observed during different pathological events including immune-mediated diseases such as EAE or MS as well as in neurodegenerative processes. The presence in MS tissue of cell death-related proteins, increases in DNA oxidation and DNA fragmentation as well as the demonstration of mediators in the CSF that are capable of inducing axonal damage and apoptosis of neurons in culture, all indicate that apoptosis is an important aspect of tissue damage in the CNS. Offen therefore pointed out that future therapeutic approaches should emphasize neuroprotection and inhibition of apoptosis.

Another session focused on selected aspects of biomarkers for monitoring brain inflammation. A. Miller (Haifa, Israel) summarized data on matrix metalloproteinases and their tissue inhibitors. Besides their physiological role in extracellular matrix and tissue remodeling during wound healing and repair, they are involved in tumor invasion, metastasis, inflammation and cellular transmigration (through the BBB), as well as in arteriosclerosis and autoimmune diseases. MMP2 and MMP9 levels in the peripheral blood of MS patients were shown to correlate with disease activity in MS and in experimental stroke models. Although not yet entirely

BBB = blood-brain barrier  
IL = interleukin

PS2 = presenilin 2

clear, the activity of MMP and TIMPs may also be involved in amyloid deposition in Alzheimer's disease. Miller provided evidence for diverse MMP/TIMP profiles in relapsing vs. progressive MS, and for modulation of MMPs/TIMPs by interferon- $\beta$  treatment in MS. Additionally, elevation of MMPs in the CNS seem to correlate with brain edema following stroke. Thus, MMPs appear to be promising candidates as biomarkers for inflammatory activity, BBB permeability and for the response to anti-inflammatory treatments. Better understanding of their structure and function may ultimately be exploitable in therapies for the above conditions.

J. Whitaker (Birmingham, USA) reported on longstanding efforts to develop a disease biomarker in MS based on detecting myelin basic protein and its degradation products in CSF, blood and urine. He previously showed that urinary MBP-like material, a cryptic epitope of MBP 83-89 of human MBP, can be determined by radioimmunoassay. It is present in normal adults, remains at similar levels in RR-MS, but is elevated in primary and secondary progressive MS, and its level correlates with the extent of black-hole volume on T1-weighted MRI scans in SP-MS. Using a fascinating array of biochemical techniques, Whitaker demonstrated that p-cresol sulfate is the dominant component of MBPLM. Since urine MBPLM and p-CS correlate highly, Whitaker proposes that the role of p-CS in diagnostics and treatment of MS should be examined further.

Most impressive progress in the field of biomarkers is evident regarding brain imaging and the use of MRI in the diagnosis and treatment of MS. During recent years coordinated efforts of many groups have made MRI a validated biomarker that is now used to address pathogenetic questions, diagnose MS and monitor treatment efficacy in phase II and III trials. M. Fillippi (Milan), one of the pioneers in this field, presented an

impressive array of different MRI techniques that are suitable to visualize various aspects of tissue inflammation and BBB permeability (by Gd-enhancing T1-weighted MRI), and more permanent tissue damage with demyelination and axonal loss (by T1-hole volume, reduced magnetization transfer ratio, spectroscopy) or atrophy (by brain parenchymal fraction determination). There is currently a strong interest in refining these techniques and correlating them carefully with clinical and pathological disease phenotypes.

The second day of the conference dealt with immunomodulation in neuroinflammatory diseases as well as with repair and future strategies. E. Shohami (Jerusalem) discussed a controversial aspect of brain inflammation – namely, the damaging or beneficial role of tumor necrosis factor- $\alpha$  and other pro-inflammatory cytokines. She demonstrated in a brain trauma paradigm that TNF- $\alpha$  levels increase early in both the damaged and the contralateral hemisphere. Data from this or a middle cerebral artery occlusion model indicate that TNF- $\alpha$  has beneficial roles. TNF- $\alpha$  wildtype mice initially show a poorer outcome compared to TNF- $\alpha$  knockout mice; however, this trend later reverses and overall recovery is better in wildtype mice. Furthermore, the lack of a direct toxic effect of TNF- $\alpha$  *in vitro* for neurons, astrocytes and endothelial cells prompted the investigators to speculate that additional factors contribute to toxicity. Reactive oxygen species were later identified to act in concert with TNF- $\alpha$  signaling pathways ultimately via NF $\kappa$ B translocation to the nucleus. TNF- $\alpha$  potentiates the effect of ROS, and elegant experiments have established that heat acclimatization may be exploited as protection from their combined damage.

M. Youdim (Haifa) used a well-defined system, the induction of Parkinson's disease by MPTP or 6-hydroxydopamine in rats, to introduce pharmacogenomics for future research in neurodegenerative and inflammatory diseases as well as for rational drug development. Using large-scale cDNA expression arrays in the above models, he examined not only the damaging

mechanisms such as iron deposition, the consecutive generation of ROS, excitatory neurotransmitters (glutamate) and TNF- $\alpha$ , but also protection from these effects by either apomorphine, iron chelators, the monoamine-oxidase B inhibitor Rasagiline, or naturally occurring flavonoides such as found in green tea. His data suggest complex effects of both damaging and protective agents with multiple and functionally distinct genes acting in concert. These included apoptosis-related genes, the up-regulation of numerous neurotrophic factors as well as entirely unsuspected genes such as those involved in cell-cycle control. Youdim stressed that other techniques such as *in situ* hybridization or quantitative polymerase chain reaction should be performed to verify these data, but is optimistic that these novel tools will lead to a better understanding of drug action and allow the development of rationally combined therapies for these neurological diseases.

The above theme was continued by H. Weiner (Boston, USA). While it is accepted that MS is a T cell-mediated autoimmune disease, Weiner contended that it is becoming increasingly clear that the disease is heterogeneous in many respects including response to treatment. The currently available medications, i.e., interferon-beta and glatiramer acetate, are an important step forward. However, they are only partially effective, and while some patients respond very well others do not. This may be due to differences in the underlying immune mechanisms such as a more chronic immune activation with strong IL-12 secretion and persistent Th1 bias in longstanding disease. Weiner further presented data on the use of chemokine receptors such as CCR5 expression of IFN- $\gamma$ -secreting cells as an indicator of disease activity, but stressed that better biomarkers are urgently needed for disease monitoring. After discussing the putative mechanism of action of a series of drugs, he concluded that therapeutic strategies should be combined in order to block the multifactorial disease process and that it will become imperative to treat the disease early to prevent long-

MMP = matrix metalloproteinase

TIMP = tissue inhibitor of MMP

MBP = myelin basic protein

SP-MS = secondary progressive multiple sclerosis

MBPLM = myelin basic protein-like material

p-CS = p-cresol sulfate

ROS = reactive oxygen species

term CNS damage. To this goal, even more aggressive intervention, for example the use of immunosuppressive drugs such as cyclophosphamide, should be considered.

N. Karin (Haifa) introduced DNA vaccine strategies for EAE. Based on the rationale that the blockade of pro-inflammatory chemokines such as MIP-1 $\alpha$  and MCP-1 should prevent migration of T cells and monocytes into the brain, he designed naked DNA constructs encoding these chemokines together with immunostimulatory sequences. This strategy blocked EAE development probably as a result of the development of antibodies against the DNA-encoded chemokines and consequently by inhibiting chemotaxis. The protective nature of the antibodies can be demonstrated by antibody transfer into naïve animals. Similar results were obtained with DNA vaccines encoding TNF- $\alpha$  and Fas-L. The level of antibody production paralleled the disease activity, and future research will try to establish which combinations are most effective to prevent inflammation and demyelination.

M. Rodrigues (Rochester, USA) took up an earlier theme of the symposium, namely, the protective or repair properties, of immune mediators. Based on earlier observations that myelin-specific antibodies may foster remyelination, he looked for human monoclonal antibodies with these properties, which might be useful for treating MS patients. Through elaborate screening of monoclonal IgM fractions from Morbus Waldenström patients, he and his group identified a few such monoclonal IgM antibodies that promoted remyelination in virus-induced, autoimmune and toxic models of demyelination in mice. Sequencing of the light and heavy chains revealed that these antibodies express  $\lambda$  light chains and are very similar to germline-encoded antibodies. Only a fraction of antibodies that bound to as yet unknown oligodendrocyte surface molecules were also promoting remyelination, and Rodriguez speculates that their remyelination activity may either be mediated via inducing signaling events, i.e., similar to a hormone or growth factor, or via opsonization and removal of myelin debris.

T. Ben-Hur (Jerusalem) gave a concise overview on the possibilities and problems of the emerging stem cell transplantation approaches in demyelinating and degenerative disorders. While multiple cell types including progenitor cells, embryonic stem cells, Schwann cells, oligodendrocyte lineage precursors and olfactory bulb-ensheathing cells are currently being explored for repair strategies in demyelinating and dysmyelinating conditions, and the first results are promising, many questions need to be addressed. These include the inability of surviving oligodendrocytes to myelinate nude axons, the limited migratory capacity of transplanted cells, the depletion of progenitors, the narrow time window for remyelination strategies, the combination with growth factors, the prevention of graft rejection and the influence of axonal pathology. He demonstrated impressive results in an EAE model, where the intraventricular transplantation of precursor cell spheres appeared to provide a stable supply of precursors that are capable of migrating into active lesions and attenuate disease. Given the many open questions, Ben-Hur stressed that these approaches, attractive as they may appear, should not be started too early in human conditions.

Another fascinating treatment modality was discussed by G. Martino (Milan). He reported on their first successful phase I/II study in rhesus monkey EAE in which non-replicative herpes simplex type-1 derived viral vector encoding IL-4 was administered cisternally and via the lumbar route. This treatment induced neither an immune response in the CNS nor changes in the periphery. The vector showed widespread CNS distribution, improved the clinical disease course and led to up-regulation of IL-4 and transforming growth factor- $\beta$  and decrease of TNF- $\alpha$  and MCP-1. Additional experiments with a human cytomegalovirus-derived vector encoding basic fibroblast growth factor led to reduced axonal loss, inflammation and demyelination in a different EAE model in C57/Bl6 mice. While the above strategy offers advantages over other approaches – namely, the high cytokine levels in the CNS and the absence of peripheral side effects to

the immune system – cytokine production so far is of short duration after single vector administration and improvements are therefore clearly necessary.

The symposium concluded with a lecture by M. Ravel (Rehovot), co-discoverer of IFN- $\beta$ . Ravel presented novel observations with a chimeric construct of IL-6 and the IL-6R. This molecule acts via binding to the homodimer of gp130 and subsequent signaling, and in an EAE model increased neuronal survival and outgrowth and supported oligodendrocyte survival and remyelination.

### In Summary: The Symposium Highlights

Suggesting the need to rethink some of our traditional concepts in neuroimmunology, the symposium emphasized the following:

- In addition to the classical inflammatory CNS diseases such as infectious (meningoencephalitis) and autoimmune (multiple sclerosis), a prominent immune response is manifested following brain trauma, vascular insult (stroke) and in neurodegenerative diseases (Alzheimer's, Parkinson's disease, etc.)
- The importance of disease heterogeneity in immune-mediated brain processes, which may be examined by promising new approaches and new markers.
- The beneficial aspects of immune responses and autoimmunity as part of endogenous mechanisms promoting CNS repair.
- The need for early intervention aimed at preventing irreversible damage and accumulating disability, utilizing, most likely, combined therapies that block inflammation and axonal/neuronal damage and efficiently support repair.

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