Autoimmune Demyelinating Disease of CNS; Immune-Specific Therapy and Neuronal/Myelin Repair by Adult Stem Cells

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Multiple sclerosis (MS) is a neurological autoimmune disease characterized by demyelinated lesions in the central nervous system (CNS) associated with axonal damage and neuronal loss. Spontaneous remyelination can be detected in the CNS of MS patients, but it is not sufficiently effective to repair the relatively massive demyelination that occurs upon disease progression. As the neuronal loss resulting from demyelination does not repair spontaneously, the damage caused by the autoimmune attack may result in permanent neurological impairment that can worsen with disease progression. Thus, an effective therapy of chronic MS should not only immunospecifically neutralize the pathogenic autoimmune process, but also offer means to repair the non-spontaneously reversible CNS tissue damage. Using “complex EAE” as an animal model system for MS, we are studying approaches to immune-specific therapy of MS, and investigating manageable means to repair the myelin/neuronal damage incurred as a result of the pathogenic autoimmune attack. Effective immune-specific approaches obtained from studies in EAE can be readily applied to other T-cell-mediated organ-specific autoimmune diseases, and insights into mechanisms of repair of myelin/neuronal damage in EAE should be relevant to other CNS neurodegenerative diseases.

Immune-Specific Therapy of MS-like disease (“complex EAE”) by a “Multi-targeting” synthetic gene product

Developing immune-specific approaches whereby only deleterious immune cells can be neutralized without affecting the innocent immune cells is the ultimate goal in immunotherapy of autoimmune diseases. However, the potential multiplicity of primary target antigens/epitopes in MS, the possible variability among patients and the dynamic autoimmunity by which specificity of anti-myelin pathogenic autoreactivities may shift/expand (“autoimmune spread”) in the same patient with disease progression, impose major difficulties in devising immune-specific approaches to therapy of MS. In view of such potential complexity, a multi-target-directed approach to immune-specific modulation is likely to be more effective than single antigen/epitope-directed immunomodulation of the disease. To investigate the feasibility and potential efficacy of multi-antigen/multi-epitope-directed immunomodulation, we constructed a pilot synthetic gene designed to encode in tandem EAE/MS-related epitopes of all known encephalitogens (MBP, PLP, MOG, MOBP and OSP) (Fig.1).

The protein product (designated pilotY-MSP) was immunofunctional and, upon tolerogenic administration (i.v.), fully abrogated EAE associated with multiple pathogenic autoreactivities (“complex EAE”) induced by a mixture of five encephalitogenic T-cell lines, each specific to a different encephalitogen. Such a model of EAE simulates the complexity of anti-myelin autoreactivities in MS.

EAE and disease-therapy in “HLA-humanized” transgenic (Tg) mice - In view of the potency of the multi-target-directed immunomodulatory approach demonstrated on “complex EAE”, our research is presently aimed at advancing the “multi-targeting” approach towards potential application to MS. We use “HLA-DR-humanized” Tg-mice for identifying/defining epitopes of MBP, PLP, MOG, MOBP, and OSP myelin antigens specifically relevant to MS associated with HLA-DR2 haplotype (most prevalent haplotype in MS). HLA-DR2-relevant epitopes predicted by computer modeling, using bioinformatic technologies, and authenticated by epitope mapping in HLA-DR2-Tg mice, and/or by reactivity of MS patients’ T-cells, were integrated in a new tolerogenic multi-targeting agent (Y-MSP-DR2) geared to specifically target potentially pathogenic autoreactivities in HLA-DR2 MS. Preclinical studies in mice show highly effective downregulation of multiple pathogenic anti-myelin autoreactivities relevant to MS, upon tolerogenic administration of Y-MSP-DR2.

Multi-APL approach to targeting multiple pathogenic anti-myelin autoreactivities - Although potentially highly effective, tolerogenic treatment with “multi-targeting”
agent comprised of native antigen/epitope carries an inherent potential risk of also activating the deleterious T-cells to be neutralized. Such a risk can be greatly reduced by replacing the native epitopes with altered peptide ligands (APLs). We therefore aim at defining antagonistic APLs for each myelin epitope constituting Y-MSP-DR2 towards converting the Y-MSP-DR2 into Y-MSP-DR2-APL and thereby generating a “multi-APL/multi-targeting” agent in which all the myelin epitopes will be replaced with well-characterized antagonistic APLs (non-encephalitogenic and able to neutralize relevant specific T-cells without being stimulatory). The feasibility of the therapeutic benefit of the multi-APL concept is now being assessed in a well-defined model of “complex EAE” and a well-defined “multi-APL/multi-targeting” agent. Upon demonstration of its efficacy, the benefit of Y-MSP-DR2-APL, as a safer therapeutic agent, will be assessed in “humanized complex EAE” induced in HLA-DR2-Tg mice.

Multimeric MHC/peptide complexes (HLA-DR2/myelin-peptide tetramers and monomers) for diagnosis and therapy - The recent development of multimeric MHC/peptide complexes with the capacity to bind T-cell receptors specifically has provided an important tool to study antigen-specific T-cell responses directly ex vivo. The generation of MHC class II tetramers specific for autoimmune pathogenic T-cells is now being established in our lab. Multimeric Human HLA-DR2/myelin-epitopes as well as mouse I-As/myelin-epitopes complexes are extremely useful reagents for investigating mechanisms leading to the development and activation of EAE and MS, for monitoring disease progression, and for disease diagnosis.

Myelin/neuronal repair by adult stem cells

In a more chronic EAE, neurological impairment incurred by severely damaged myelin/axons or by neuronal loss, that does not repair spontaneously, persists regardless of how effective the immune-specific therapy is in eliminating and/or neutralizing the pathogenic T-cells. This chronic model of “complex EAE” is used as an in-vivo model for investigating neuronal and myelin repair in the CNS, as well as immunospecific therapy. In this model, highly reminiscent of MS, effective immunospecific therapy would have to be complemented with mechanisms that can repair the non-spontaneously recovering myelin/axonal damage and neuronal death. On the other hand, mechanisms of neurological repair, including stem cells, will result in recurrent damage and are doomed to fail if the autoimmune pathogenic mechanisms are not also neutralized.

Most neurons in the adult CNS are terminally differentiated and are not replaced when they die. However, evidence exists that small proportions of neurons continue to be generated in the adult ventricular zone, olfactory system and hippocampus. The forebrain subventricular zone (SVZ) and the dentate gyrus are considered to be the major source of adult self-renewing multipotent neuronal stem cell (NSC). Our aim is to investigate molecular and cellular means for enhancing the spontaneous neurogenesis and remyelination, and for supplementing external adult neural stem/progenitor cells to the damaged area.

Although disputed on the basis of possible fusion of transplanted cells with resident cells in the brain, accumulating evidence suggests that the adult bone marrow (BM) stem cells transplanted into the brain tissue can transdifferentiate into neuronal cells. Furthermore, adult multipotent stem cells isolated from skin, nasal cavity and inner ear have been shown to have the potential to differentiate into neuronal cells. The possibility that adult BM stem cells can differentiate to neuronal cells may provide an accessible source of adult neural stem/progenitor cells, rendering the use of adult stem cells for neuronal repair more applicable.

Towards investigating myelin/neuronal repair mechanisms by adult stem cells, we isolated and cultured mouse adult SVZ-NSC and adult BM stem/progenitor cells and investigated their in vitro and in-vivo differentiation into astrocytes, oligodendrocytes and neurons (Fig. 2).

Studies are now in progress to demonstrate in-vivo: mechanisms of migration of SVZ-NSC and adult BM stem/progenitor cells into areas of demyelinated EAE-lesions; their ability to remyelinate axons; generation of newly formed neurons; immunomodulation of EAE by adult neural stem/progenitor cells, and their effects on T-cells, and assess their therapeutic
potential. Our ultimate goal is to achieve a manageable means for a complete recovery from chronic “complex EAE” with full reversal of severe neurological impairment, using our “multitargeting” agent for neutralization of pathogenic T-cells, in combination with adult BM stem/progenitor cells for effective myelin/neuronal repair. The benefit of this combination treatment has been evaluated in our lab, and the underlying mechanisms are now being investigated.

Acknowledgements
Incumbent of The Marcia and Eugene Appelbaum Professorial chair in Immunology. Supported in part by National Multiple Sclerosis Society (NMSS, N.Y), Israel Academy of Science and Humanities (ISF), Israel Ministry of Health; William Sham Foundation, Stem cells center (Mrs. Ruth Ziegler), Estate of the Late Florence Blau, Nella and Leon Benoziyo Center for Neurological Diseases