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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 CNS associated with axonal damage and neuronal loss. As remyelination processes observed in MS lesions are not sufficiently effective for repair of a relatively massive demyelination, and neuronal loss cannot be spontaneously repaired, the damage caused by the autoimmune attack may result in permanent neurological impairment that can worsen with disease progression. Thus, effective therapy of chronic MS not only should 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, we are studying approaches to immune-specific therapy of MS, as well as investigating a manageable means to repair of myelin/neuronal damage incurred by the pathogenic autoimmune mechanisms. Effective immune-specific approaches obtained from studies in EAE can be readily applied to other T-cell-mediated organ-specific autoimmune diseases, and insights and mechanisms of myelin/ neuronal repair in EAE should be relevant to other CNS neurodegenerative diseases.

Immune-Specific Therapy of " complex EAE", as a model for MS. 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 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 immonomodulation 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, fully abrogated EAE associated with multiple pathogenic autoreactivities ("complex EAE") induced by a mixture of five encephalitogenic T-cell lines, each specific to

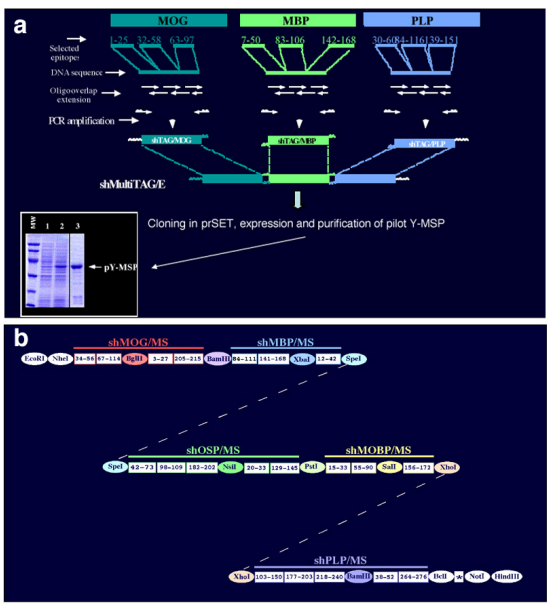

Fig. 1 *The strategy for the construction of MS-related synthetic human multi-target autoantigen gene (A) and a scheme (B) of the protein product (pilotY-MSP).*

different encephalitogen.

Our research is presently aimed at advancing the "multi-targeting" approach towards potential application to MS. We use HLA-humanized mice for defining myelin epitopes specifically relevant to MS associated with HLA-DR2 haplotye (most prevalent haplotype in MS), towards generating a new tolerogenic agent (Y-MSP-DR2) geared to specifically target potentially pathogenic autoreactivities in HLA-DR2 MS.

Tolerogenic treatment with "multi-targeting" agent comprised of native antigen/epitope carries an inherent 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 define antagonistic APL 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 antagonistic APLs. The therapeutic benefit of Y-MSP-DR2-APL will be assessed in "humanized complex EAE" induced in HLA-DR2-Tg mice.

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 could not be spontaneously repaired, remain persistent regardless of how effective is the immune-specific therapy in eliminating and/or neutralizing the pathogenic T-cells. In such a chronic model of EAE, 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 loss. On the other hand, any mechanisms of neurological repair, including stem cells, without neutralization of the autoimmune pathogenic mechanisms will result in recurrent damage and are doomed to fail.

Most neurons in the adult CNS are terminally differentiated and are not replaced when they die. However, evidence exist that small proportion 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).

Although disputed on the basis of possible fusion of transplanted cells with resident cells in the brain, accumulating evidence suggest that the adult bone marrow (BM) stem cells transplantated into the brain tissue can transdifferentiate into neuronal cells. The potential repair of CNS tissue damage with adult stem cells should have important advantages over the use of embryonal stem cells. The possibility that adult BM stem cells can differentiate to neuronal cells provides an accessible source of adult stem cells and makes the use of adult stem cells for neuronal repair a lot more practical.

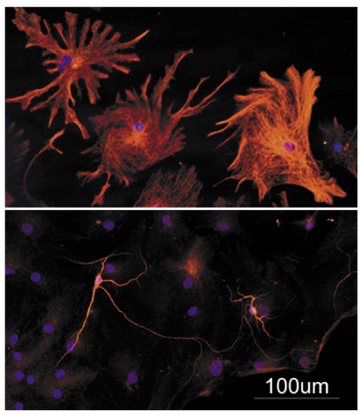

Fig.2 *Differentiation of adult NSC into astrocytes & neurons.*

Towards investigating myelin/neuronal repair mechanisms by adult stem cells, we isolated and cultured adult SVZ-NSC and adult BM stem/progenitor cells from transgenic GFP-mice and investigated their *in vitro* and in-vivo differentiation into astrocytes, oligodendrocytes and neurons (Fig. 2). Studies are now in progress to in-vivo demonstrate: migration of SVZ-NSC and adult BM stem/progenitor cells into areas of demylinated EAE-lesions; study their ability to remyelinate axons; demonstrate newly formed neurons; and assess their therapeutic effect.

Our ultimate goal is to achieve a manageable means for a complete recovery from chronic "complex EAE", as a model for MS, with full reversal of severe neurological impairment, using our "multi-targeting" agent for neutralization of pathogenic T-cells, in combination with adult BM stem/progenitor cells for effective myelin/neuronal repair.

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