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

Department of
Immunology

Prof. Avraham Ben-Nun

Dr. Rina Zilkha-Falb,
Dr. Nathali Kaushansky,
Dr. Molly Dayan, Neta Madar-Kella,
Maya Sherf, Joey Oved

☎ 972 8 934 4558
FAX 972 8 934 2991
@ Avraham.ben-nun@weizmann.ac.il
www.weizmann.ac.il

Multiple sclerosis (MS) is a neurological autoimmune disease which is believed to result from an abnormal activation of pathogenic autoimmune T cells reactive against myelin or neuronal components of the central nervous system (CNS). What triggers the activation of autoimmune T-cells to become pathogenic and cause MS is not yet known. Upon progression, the disease is characterized by demyelinated lesions associated with axonal damage and neuronal loss in the CNS. A spontaneous remyelination process could be detected in the CNS of MS patients. However, the spontaneous remyelination processes observed in MS lesions are not sufficiently effective for repairing the relatively massive demyelination that occurs upon disease progression, and the resulting neuronal loss cannot be spontaneously repaired. Therefore, 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 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 associated with multiple pathogenic anti-myelin autoimmune reactivities, reminiscent of the complex anti-myelin autoimmunity in MS, 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 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 to neighboring myelin proteins (a phenomenon called "spread of autoimmunity") 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 of the pathogenic autoimmunity, 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 active immunization with a mixture of encephalitogenic myelin peptides (Fig. 1c), or passively transferred by a mixture of five encephalitogenic T-cell lines, each specific to different encephalitogen. Such a model of EAE simulates the complexity of anti-myelin autoreactivities in MS. Moreover, and most relevant to therapy of MS, administration of Y-MSP to mice with chronic EAE displaying tail and hind leg paralysis, resulted in immediate improvement in clinical manifestation to a complete recovery (Fig. 1d).

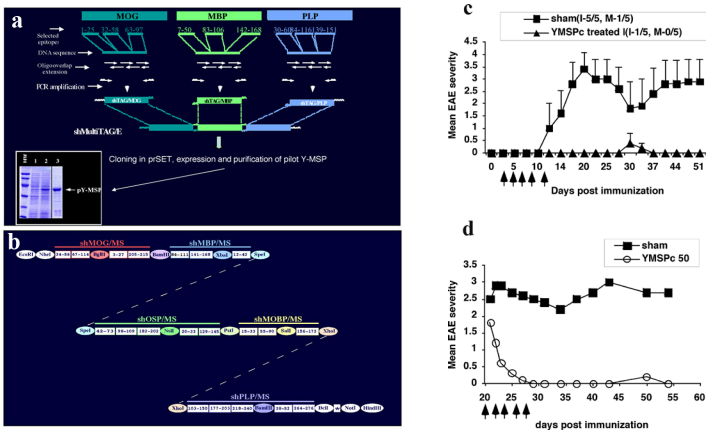

Fig. 1 *The feasibility and potential efficacy of the multi-antigen/multi-epitope-directed approach to immune-specific therapy of MS-like disease (EAE). a. The strategy for the construction of MS-related synthetic human multi-target autoantigen gene; b. The scheme of the protein product (pilotY-MSP); c. Tolerogenic administration of Y-MSP to mice induced to develop EAE, suppress the development of EAE when given before disease onset, or reverse clinical signs of ongoing EAE when administered after disease onset (d).*

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 advancingthe"multi-targeting"approach towards potential application to MS.

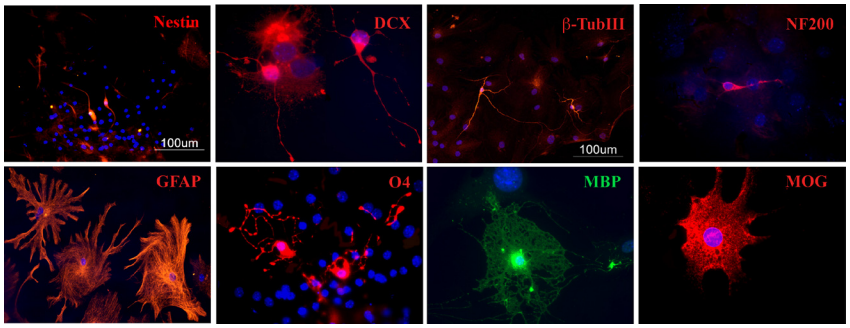

Fig. 2 *SVZ-derived neuronal stem/progenitor cells (Nestin) that were differentiate in-vitro into astrocytes (GFAP), oligodendrocytes (O4; MBP; MOG) and neurons (DCX; β-Tubulin; NF200). Nuclei stained by DAPI (blue).*

We are investigating the T- and B-cell autoimmunity in Tg mice expressing the HLA-DR2 molecules (HLA –DR2 haplotype confers a 2-3 fold increase in the risk of developing MS) [HLA-DR15 (DRA1*0101;DRB1*1501) and the HLA-DQ6 (DQA1*0102;DQB1*0602)-Tg mice (MHC-II$^{-/-}$) as well as in the (HLA-DR15 x HLA-DQ6)F1 double Tg-mice. We use the "HLA-DR2-humanized" Tg-mice for identifying/defining the epitopes of MBP, PLP, MOG, MOBP, and OSP myelin antigens, which are pathogenic and most specifically relevant to MS associated with HLA-DR2 haplotype (the 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 the "humanized" mice are now in progress to show 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). APL is a peptide epitope (which in a complex with MHC molecule is a TCR-ligand) in which the TCR-contact residues of the native epitope is mutated and thereby converting the peptide from an agonist to a partial agonist or antagonist, which paralyze/neutralize rather than activating the specific T-cells. We therefore aim at defining antagonistic APLs for each of the major MS-relevant myelin epitopes constituting the 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 (can neutralize relevant specific T-cells without being stimulatory). The feasibility of the therapeutic benefit of the multi-APL concept has recently been confirmed in our laboratory in wild-type mice, by the demonstration of effective downmodulation of the disease in a well-defined model of "complex EAE" with an appropriate well-defined "multi-APL/multi-targeting" agent. Upon demonstration its efficacy in wild-type mice, 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. Towards this goal, we are now defining effective APLs for the HLA-DR2-relevnat myelin epitopes pathogenic for the "HLA-DR2-humanized" Tg-mice.

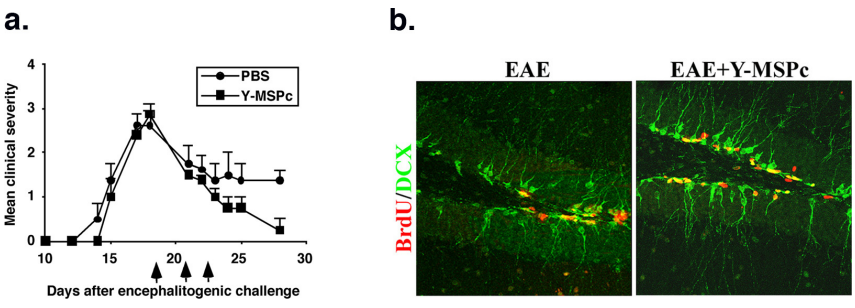

Fig. 3 *Amelioration of ongoing EAE upon treatment with Y-MSPc is associated with enhanced neurogenesis. a. Reversal of clinical manifestations of EAE by Y-MSPc. b. Confocal micrographs showing newly formed BrdU+ cells co-expressing the neuronal cytoplasmatic marker DCX in the dentate gyrus.*

In-vitro and *in-vivo* analysis of mechanisms down-regulating pathogenic autoimmune T-cells by Multi-APL artificial proteins

The autoimmune pathogenic T-cells are pro-inflammatory TH1/TH17 T-cells that can be regulated by anti-inflammatory Th2 T-cells and T-regulatory cells. Each type of T-cells is characterized by a specific pattern of secretion/expression of cytokines, chemokines and other function-related molecules. Our studies show that the Multi-APL artificial protein is significantly more effective than a single relevant APL in the downregulation of the epitope-specific pathogenic T-cells, both *in-vitro* and *in-vivo*. Moreover, the Multi-APL was more effective in converting the Th1- into Th2-cells, and in the induction of T-regulatory cells. Tg-mice expressing TCRMOG transgene of pathogenic T-cells specific for MOG35-55, the major encephalitogenic epitope of MOG, is used to monitor the *in-vivo* and *in-vitro* effect (on apoptosis, anergy, cytokine shift, and regulatory T-cells) by the multi-APL agent on pathogenic T-cells, and its mechanism

of action. Foxp3-GFP Tg-mice, and (TCRMOG x Foxp3-GFP)F1 double Tg-mice and DEREG Tg-mice (which genetically express conditional Foxp3-GFP that can be specifically deleted in the presence of diphtheria toxin) are being utilized for these studies. Mechanisms are also investigated on myelin-specific pathogenic T-cell clones that we have isolated, towards deciphering signaling pathways.

Myelin/neuronal repair by adult stem cells

In an advanced 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. This chronic model of "complex EAE" associated with myelin loss and extensive neuronal damage is used as an *in-vivo* model for investigating neuronal and myelin repair in the CNS, as well as immunospecific therapy. In this model, which is 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 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 neural stem cell (NSC). Our aim is to investigate molecular and cellular means for enhancing the spontaneous neurogeneration and remyelination, and means 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 suggest that the adult bone marrow (BM) stem cells transplanted into the brain tissue can transdifferentiate into neural cells. Furthermore, adult multipotent stem cells isolated from skin, nasal and inner ear have been shown to have the potential to differentiate into neural cells. The potential repair of CNS tissue damage with adult neural stem/progenitor cells should have important advantages over the use of embryonic stem cells. The possibility that adult BM stem cells can differentiate to neural cells may provide an accessible source of adult neural stem/progenitor cells and makes the use of adult stem cells for neuronal repair a lot more practical.

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 investigate *in-vivo*: promotion of migration of SVZ-NSC and adult BM stem/progenitor cells as well as of endogenous de novo neural progenitors into areas of demyelinated EAE-lesions; their ability to remyelinate axons; promotion of generation of *de novo* neural progenitors (Fig.2); 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 "multi-targeting" 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.

Immunomodulatory effects of adult stem cells on T-cell function

It has been shown that injection (i.v.) of adult stem cells into mice with EAE ameliorates the clinical expression of the disease. Later, although some injected stem cells find their way to the CNS, it has been shown that the injected stem cells have an immunomodulatory effect on T-cells. Our studies show that both adult BM stem cells and adult NSC have strong *in-vitro* and *in-vivo* immunomodulatory functions. In view of the fact that the injected adult stem cells first encounter the immune system, we are now extensively investigating in our laboratory mechanisms by which adult stem cells affect the immune cells *in-vitro/in-vivo*.

Enhancement of endogenous neuro/oligodendrogenesis

The artificial multi-epitope targeting protein Y-MSP is highly effective in the treatment of chronic EAE. As shown in Fig. 3, preliminary studies suggest that the recovery from ongoing EAE upon treatment with Y-MSP results also in enhanced induction of de novo neuro/oligodendrogenic progenitor cells in the CNS of treated mice. We, therefore, investigate the efficacy of multi-epitope targeting agents in disease amelioration as well as in the induction of potential *de novo* neuro/oligodendrogenesis, examine the relationship between these two functions upon immune-specific treatment of chronic EAE with myelin/neuronal loss, and elucidate the mechanisms associated with both functions. Our major efforts are presently directed towards correlating the induction of de novo neuro/oligodendrogenic progenitor cells with immune-regulatory mechanisms/molecules.

Recent publications

Zhong, M.C., Kerlero De Rosbo, N. and Ben-Nun, A. (2002) Multiantigen/multiepitope-directed immune-specific suppression of "complex autoimmune encephalomyelitis" by a novel protein product of a synthetic gene. J. Clin. Invest. 110:81-90.

Evaluated by Faculty of 1000: evaluations for Zhong MC et al J Clin Invest 2002 Jul 110 (1) :81-90 http://www.f1000biology.com/article/12093891/evaluation

Kipnis, J., Yoles, E., Mizrahi, T., Ben-Nun, A. and Schwartz, M. (2002) Myelin specific Th1 cells are necessary for post-traumatic protective autoimmunity. J. Neuroimmunol. 130:78-85.

Rivera A, Chen CC, Dougherty JP, Ben-Nun A, Ron Y. (2003) Host stem cells can selectively reconstitute missing lymphoid lineages in irradiation bone marrow chimeras. Blood 101: 4347-4354.

Bank I, Cohen L, Kneller A, Kerlero de Rosbo N, Book M, Ben-Nun A. (2003) Aberrant T-cell receptor signalling of interferon-gamma- and tumour necrosis factor-alpha-producing cytotoxic CD8+ Vdelta1/Vbeta16 T cells in a patient with chronic neutropenia. Scand. J. Immunol. 58: 89-98.

Kerlero de Rosbo N., Kaye J. F., Eisenstein M., Mendel I., Hoeftberger R.,Lassmann H., Milo R., Ben-Nun A. (2004) The Myelin-Associated Oligodendrocytic Basic Protein Region MOBP15-36 Encompasses the Immunodominant Major Encephalitogenic Epitope(s) for SJL/J Mice and Predicted Epitope(s) for Multiple Sclerosis-Associated HLA-DRB1*1501. J. Immunol. 173: 1426-1435.

Mendel, I., Natarajan, K., Ben-Nun, A., Shevach, E. M. (2004) A novel protective model against experimental allergic encephalomyelitis in mice expressing a transgenic TCR-specific for myelin oligodendrocyte glycoprotein. J. Neuroimmunol. 149:10-21.

Montero, E., Nussbaum, G., Kaye, J. F., Perez, R., Lage, A., Ben-Nun, A., Cohen, I. R. (2004) Regulation of experimental autoimmune encephalomyelitis by CD4(+), CD25(+) and CD8(+) T cells: analysis using depleting antibodies. J. Autoimmun. 23:1-7.

Ben-Nun, A., Kerlero de Rosbo, N., Kaushansky N., Eisenstein, M., Cohen, L., Kaye, F. J., Mendel, I., (2006) Anatomy of T-cell autoimmunity to myelin oligodendrocyte glycoprotein (MOG): Primary role of MOG44F in the selection and control of MOG-reactive T-cells in H-2b mice. Eur. J. Immunol. 36: 478-93.

Lewitus, M. G., Kipnis, J., Avidan, H., Ben-Nun, A., and Schwartz, M. (2006) Mucosal vaccination as a neuroprotective therapy: strain related variations. J. Neuroimmunol. 175:31-8

Kaushansky, N., Zhong, M.-C., Kerlero de Rosbo, N., Hoeftberger, R., Lassmann, H., and Ben-Nun A. (2006) Epitope-specificity of autoreactive T- and B-cells associated with experimental autoimmune encephalomyelitis and optic neuritis induced by oligodendrocyte-specific protein (OSP) in SJL/J mice. J. Immunol. 177:7364-76.

Brok, HP., Boven, L., van Meurs, M., Kerlero de Rosbo, N., Celebi-Paul, L., Kap, Y.S., Jagessar, A., Hintzen, R.Q., Keir, G., Bajramovic, J., Ben-Nun, A., Bauer, J., Laman, J.D., Amor, S., and 't Hart, B.A. (2007) The human CMV-UL86 peptide 981-1003 shares a crossreactive T-cell epitope with the encephalitogenic MOG peptide 34-56, but lacks the capacity to induce EAE in rhesus monkeys. J. Neuroimmunol. 182:135-52.

Kaushansky, N., Hemo, R., and Ben-Nun, A. (2007). OSP/claudin-11-induced EAE in mice is mediated by pathogenic T cells primarily governed by OSP192Y residue of major encephalitogenic region OSP179-207. Eur. J. Immunol. 37:2018-31.

Kaushansky, K., Zilkha-Falb, R., Hemo, R., Lassman, H., Eisenstein, M., Sas, A, and Ben-Nun, A. (2007). Pathogenic T-cells in myelin-associated oligodendrocytic basic protein (MOBP)-induced EAE in mice are predominantly focused to recognition of MOBP21F and MOBP27P epitopic residues. Eur. J. Immunol. In Press.

Kerlero de Rosbo, N., Kaushansky, N,. Madar-Kela, N., Zilkha-Falb, R., Hemo, R., Freimann, S,. Cohen, L., and Ben-Nun, A. (2007). Synthetic gene product designed to concomitantly downregulate multiple MS-relevant anti-myelin autoreactivities reverses "complex" EAE. Submitted for publication.

Kaushansky, N., Eisenstein, M., and Ben Nun, A. (2007). Activation and control of pathogenic T-cells in oligodendrocyte-specific protein/Claudin-11-induced EAE in SJL/J mice are dominated by their focused recognition of a single epitopic residue (OSP58M). Submitted for publication

Acknowledgements

Incumbent of The Marcia and Eugene Appelbaum Profrssorial 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

INTERNAL support

Helen and Martin Kimmel Stem Cells Research Institute

Nella and Leon Benoziyo Center for Neurological Diseases