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Immunospecific approaches to therapy of autoimmune diseases: Multiple targeting of pathogenic autoreactivities

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Our major goal is to devise new immune-specific approaches to therapy of multiple sclerosis (MS), a prototypic organ-specific T-cell-mediated autoimmune disease, using a new animal model of complex MS-like disease associated with multiple pathogenic autoreactivities (maEAE). Studies from our and other laboratories have shown that T-cell autoreactivities against several myelin proteins may be implicated in the pathogenesis of MS. This multiplicity of potential target antigens imposes major difficulties in devising approaches to immune-specific therapy of MS, as suppression of a single autoreactivity is unlikely to be sufficiently effective. We aim at developing a multi-targeted approach to therapy of MS which takes into account the multiple pathogenic autoreactivities, the concept of which can be applicable to other autoimmune diseases.

Identification of MS-related target antigens/epitopes

In addition to myelin basic protein (MBP) and proteolipid protein (PLP), long investigated as potential primary targets in MS, potentially pathogenic autoreactivity against myelin oligodendrocyte glycoprotein (MOG), myelin-associated basic protein (MOBP) and oligodendrocyte-specific protein (OSP) has now been demonstrated. Our work on anti-MOG reactive T-cells in MS has identified three immunodominant epitope clusters, two of which were shown to induce EAE in non-human primates. Pathogenic autoreactivity to MOBP in two susceptible mouse strains is directed against two different epitopes, increased reactivity to which has been demonstrated in MS patients. The pathogenic autoreactivity to OSP is most unusual in that it involves T-cells with two or three-way intramolecular cross-reactivity to epitopes with no primary structural sequence homology, a feature with potentially high significance in disease pathogenesis.

Characterization of the encephalitogenic T-cells in murine EAE induced by MOG, MOBP and OSP has revealed immunodominance in epitope recognition despite a wide diversity of TCR expression, suggesting that immune-specific approaches to therapy should favour epitope-directed rather than TCR-targeted routes. Accordingly, we are studying two antigen/epitope-specific therapeutic approaches which, when combined, should lead towards the definition of new safe, highly specific therapeutic agents to mediate multi-targeted immunomodulation of autoimmune diseases.

Epitope-directed APL-mediated immunomodulation

We are investigating the potential protective and/or curative

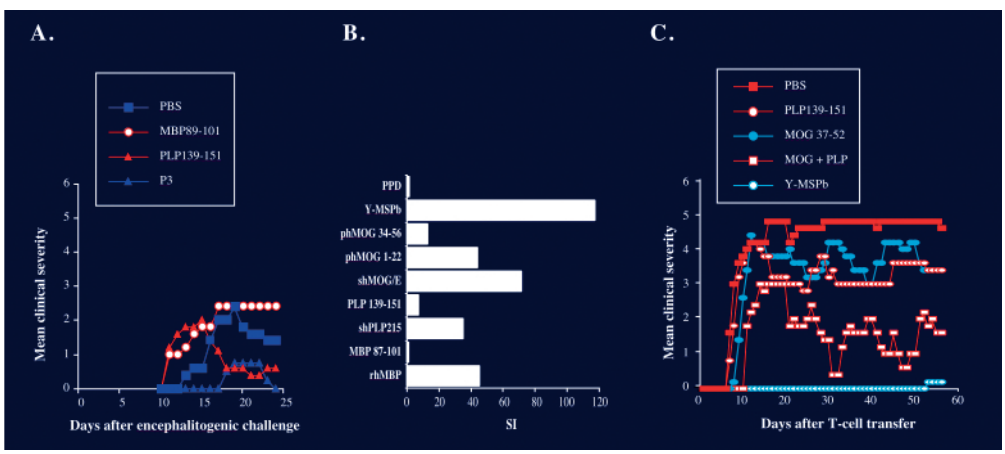

Fig. 1 *Multi-targeted immunomodulation suppresses EAE with multiple pathogenic autoreactivities. A. Single peptide administration (MBP89-101 or PLP139-151) fails to suppress EAE induced with a mixture of encephalitogenic peptides (P3); B. Y-MSP-reactive T-cells are multi-specific. C. Tolerogenic administration of Y-MSP suppresses EAE transferred with Y-MSP-reactive T-cells.*

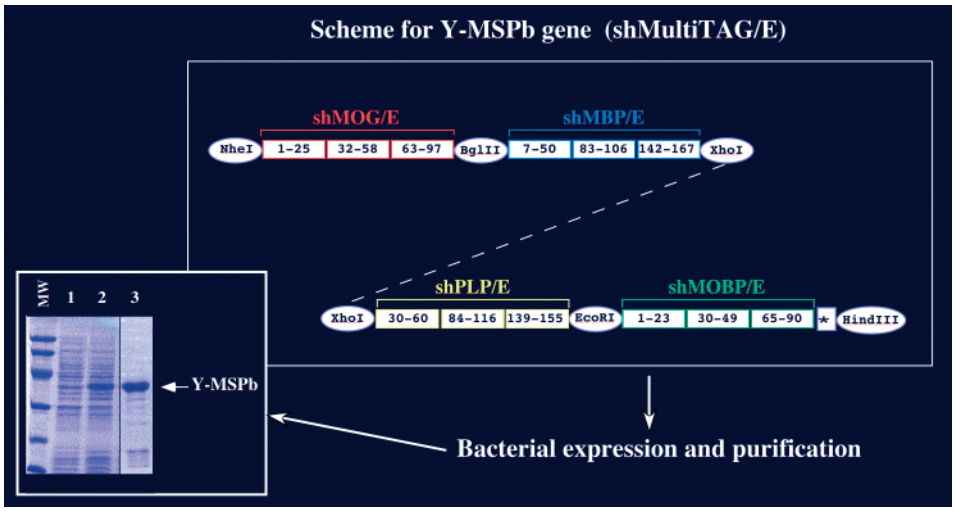

Fig. 2 *Scheme for construction of a synthetic multi-target autoantigen gene (shMultiTAG/E) coding for MS/EAE-relevant epitopes of MOG, MBP, PLP and MOBP, and SDS-gel of purified protein product Y-MSP (lane 3) isolated from IPTG-induced E. coli (lane 2); lane 1 shows uninduced bacteria.*

effect of analogs of immunodominant encephalitogenic epitopes where TCR contact residues have been substituted, and which specifically antagonize T-cell reactivity, i.e. 'altered peptide ligands' (APLs). Following our delineation of the core sequence within the encephalitogenic region of MOG in H-2^b mice, we have defined the crucial TCR contact residues for interaction with the TCR of MOG-specific T-cells. Designed APLs were effective both in vitro and in vivo in inhibiting proliferation of encephalitogenic MOG-specific T-cell clones to the native epitope, and thereby suppressing EAE.

Multi-targeted immunomodulation of maEAE

While EAE induced by a peptide representing a single epitope can be effectively suppressed by tolerogenic administration of that peptide or the relevant APL, targeting a single epitope does not inhibit complex maEAE (Fig. 1A and 1C). Hence, in view of the multiplicity of potential primary target antigens in MS, concomitant targeting of the potentially pathogenic T-cell autoreactivities to all known primary targets is likely to be a more effective therapy. Towards this goal, we have constructed synthetic genes which encode tandemly arranged disease-relevant epitope clusters of all five encephalitogenic proteins, MBP, PLP, MOG, MOBP and OSP; tolerogenic administration of the purified protein product (Y-MSPb) of a pilot synthetic gene (Fig. 2) not only suppressed EAE associated with single autoreactivities, but also fully abrogated the development of maEAE (Fig. 1C) induced by transfer of T-cells reactive against defined epitopes of MBP, PLP, MOG and MOBP (Fig. 1B). The relevant PLP or MOG peptides administered singly according to the same regimen had no effect on disease development, while a combination of MOG+PLP only marginally decreased disease severity (Fig. 1C). These data strongly emphasize the necessity to neutralize as many as possible of the relevant multiple autoreactivities for effective immunomodulation of autoimmune diseases associated with a multiplicity of potential primary target antigens. While immunomodulation with soluble autoantigen can be highly effective, the possibility of proteolytic degradation of the protein and rapid clearance may necessitate repeated treatment which, at least in the case of s.c. injections, can result in hypersensitivity reactions. In this context, we are also evaluating DNA-mediated delivery of multi-antigen/multi-epitope encoding gene products as a potential effective mechanism for immunomodulation.

To minimize the risk of T-cell stimulation by synthetic gene products encompassing native epitopes, we shall combine the APL approach with our multi-antigen/multi-epitope-directed approach for concomitant targeting of the relevant potentially pathogenic autoreactive T-cells. In view of eventual extrapolation to therapeutic approaches for MS, we aim at constructing synthetic genes coding for non-stimulatory, non-encephalitogenic APLs of the disease-relevant epitopes, which in vitro inhibit, concomitantly, myelin-reactive T-cells potentially involved in the pathogenesis of MS.

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For additional information see:

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