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Clonal T-cell Cross-reactivity Between Myelin Antigens MBP and PLP and Human Respiratory Coronaviruses in Multiple Sclerosis

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Viruses could be involved in the induction of immunopathological events associated with multiple sclerosis (MS). For instance, molecular mimicry between viruses and myelin antigens could mediate cross-reactive immune responses leading to autoimmune disease. We previously showed that a human respiratory coronavirus (229E), and myelin basic protein (MBP) activated a large proportion of T-cell lines established from MS patients by *in vitro* selection with either 229E or MBP. We now report the generation and maintenance of T-cell clones specific for MBP, proteolipid protein (PLP) and the two known strains of human coronavirus (229E and OC43). Some of these clones were activated by both coronavirus and myelin antigens, which is consistent with molecular mimicry at the single T-cell level. The observation and further characterization of such T-cell clones will bring us closer to an understanding of their potential relevance in MS pathogenesis. (Supported by the Multiple Sclerosis Society of Canada)

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Immunodominance of Encephalitogenic Peptide Specific Responses in Lewis, DA, and (Lew x DA)F1 Rats

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Guinea pig MBP-CFA injection leads to the development of EAE in Lewis, DA and (Lew x DA)F1 rats. Repeated culture stimulation of Lewis-derived GPMBP-immune cells with GPMBP results in the development of a population of encephalitogenic cells that are selected for the 72-84 determinant. Repeated culture stimulation of DA-derived GPMBP-immune cells with GPMBP results in the development of a population of encephalitogenic cells that are selected for the 42-55 determinant. Studies with (Lew x DA)F1-derived GPMBP-immune cells show that repeated stimulation in culture with GPMBP results in the development of an encephalitogenic population that contains 42-55, 72-84 and 85-97-specific subsets. Proliferation assay data suggests that the 85-97-specific response of the (Lew x DA)F1-derived T-cell line is immunodominant. This multi-specific T-cell line successfully vaccinates (Lew x DA)F1 recipients against GPMBP-CFA induced EAE. We have initiated studies to select GPMBP-immune (Lew x DA)F1 cells specifically responsive to 42-55, 72-84 or 85-97 and to date, we have determined that although an encephalitogenic 85-97 specific (Lew x DA)F1-derived T-cell line vaccinates (Lew x DA)F1 recipients against 85-97-CFA induced disease, this cell line does not vaccinate (Lew x DA)F1 recipients against GPMBP-CFA induced EAE. We are continuing these studies in order to determine the efficacy of vaccination induced resistance where the potential to respond to an encephalitogen is influenced by more than a single parental haplotype.

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Epitope Specificity of Demyelinating MOG-specific Autoantibody Responses

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Demyelination in rat and primate models of MOG-induced EAE is antibody mediated, but the identity of the target B cell epitopes is obscure. Using a panel of MOG-peptide specific rat antisera and monoclonal antibodies together with human MOG (hMOG) transfected fibroblasts as an *in vitro* target, we demonstrate that the dominant **pathogenic,** demyelinating autoantibody response to MOG is conformation dependent. A minor pathogenic B cell epitope was also detected within the peptide sequence a.a. 89-113. In contrast antibodies to the dominant linear MOG B cell epitope (aa1-20) failed to bind to hMOG expressed at the cell surface and were therefore unable to mediate complement dependent lysis of the target. This transfectant-based assay may be useful to identify/quantitate pathogenic anti-MOG antibody responses in patients with MS.

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Expansion by Self Antigen is Necessary for the Induction of Experimental Autoimmune Encephalomyelitis by T Cells Primed with a Cross-reactive Environmental Antigen

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Cross-reactivity with environmental antigens has been postulated as a mechanism responsible for the induction of autoimmune disease. Experimental autoimmune encephalomyelitis (EAE) is a T cell mediated autoimmune disease model inducible in susceptible strains of laboratory animals by immunization with protein constituents of myelin. We used myelin proteolipid protein (PLP) peptide 139-151 and its analogs to define motifs to search a protein database for structural homologues of PLP 139-151 and identified five peptides derived from microbial antigens that elicit immune responses that cross-react with this self-peptide. Exposure of naive SJL mice to the cross-reactive environmental peptides alone was insufficient to induce autoimmune disease even when animals were treated with antigen non-specific stimuli (superantigen, LPS). However, immunization of SJL mice with suboptimal doses of PLP 139-151 peptide after priming with cross-reactive environmental peptides consistently induced EAE. These data suggest that expansion by self-antigen is required to break the threshold to autoimmune disease in animals primed with cross-reactive peptides.

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Binding Stretches of Myelin Basic Protein Define Encephalitogenic T Cell Epitopes for RT1.B and RT1.D Alleles of Four Different Rat Haplotypes

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We have measured the apparent affinity of overlapping 13 mer myelin basic protein (MBP) peptides in the region comprising amino acids 63-105 of MBP for the affinity purified rat MHC class II molecules RT1.B (DQ) and RT1.D (DR) of four different haplotypes (a, l, n, u) with a competitive peptide binding ELISA based on europium fluorescence. Subsequently, we correlated protective or disease promoting effects of certain MBP peptides as previously published to binding affinity of these peptides for the corresponding MHC class II molecules. We demonstrate that immunogenic peptides were invariably capable of binding to the MHC class II molecules with high, intermediate or low affinity. None of the immunogenic peptides exhibited IC_{50} values higher than 100 μM, the limit of our assay. In the four RT1.D molecules studied, the MBP binding stretches were largely overlapping and binding peptides were found almost exclusively in the region surrounding the MBP 85-99 epitope, which has also been shown to be of importance in humans. In contrast to the RT1.D molecules, each of the RT1.B molecules exhibited a very distinct MBP peptide binding profile. In conclusion, our results demonstrate that in MBP induced EAE in rats, encephalitogenic stretches of autoantigen generally comprise good MHC binding peptides and underscore the importance of the peptide/ MHC interaction in activation and expansion of autoreactive T cells, leading to subsequent tissue damage.

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T-Cell Responses to Myelin Oligodendrocyte Glycoprotein (MOG) in Multiple Sclerosis

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T-CELL RESPONSES TO MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG) IN MULTIPLE SCLEROSIS Christine Ewing, Margo Honeyman*, Leonard C. Harrison*, Jayaram Bettadapura, Krishna Menon, and Claude C.A. Bernard. Neuroimmunology Laboratory, La Trobe University, Bundoora, 3083; *Autoimmunity and Transplantation Division, Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, 3050. AUSTRALIA MS is a chronic inflammatory disease of the central nervous system, characterized by cellular infiltration and demyelination. MS is thought to be mediated primarily by T-cells reactive to myelin proteins such as MBP and PLP. However autoimmune recognition of quantitatively minor CNS-specific myelin components such as MOG may be highly relevant in the initiation of demyelination. We previously showed that PBMC's from a cohort of Israeli MS patients had significantly greater proliferative responses to MOG than to MBP, PLP and MAG. In this present study, PBMC's from MS subjects, healthy non-HLA matched and HLA-DR2 homozygous controls were tested in proliferation assays with native and recombinant MOG preparations and with overlapping MOG peptides. 50% of MS patients and non-HLA matched controls showed positive T-cell responses to MOG (SI > 3). Diverse peptide epitopes were recognized by MS and controls. Interestingly the mean T-cell response of the homozygous HLA-DR2 controls was significantly greater than the responses of either the MS group or non-HLA-matched controls. In some cases, SI's were of the order of 80. We are presently investigating the fine specificity of these responses to MOG as well as determining motifs for binding of MOG peptides to HLA-DR2 molecules in vitro.