

Rat and Human Myelin Oligodendrocyte Glycoproteins Induce Experimental Autoimmune Encephalomyelitis by Different Mechanisms in C57BL/6 Mice¹

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C57BL/6 mice immunized with the extracellular Ig-like domain of rat myelin oligodendrocyte glycoprotein (MOG) developed experimental autoimmune encephalomyelitis (EAE) resembling that induced by rodent MOG 35-55 in its B cell independence and predominantly mononuclear CNS infiltrate. In contrast, human MOG protein-induced EAE was B cell dependent with polymorphonuclear leukocytes. Human MOG differs from rat MOG at several residues, including a proline for serine substitution at position 42. Human MOG 35-55 was only weakly encephalitogenic, and a proline substitution in rat MOG at position 42 severely attenuated its encephalitogenicity. However, human MOG 35-55 was immunogenic, inducing proliferation and IFN- γ and IL-3 to human, but not rodent MOG 35-55. The B cell dependence of EAE induced by human MOG protein was not due to a requirement for Ag presentation by B cells, because spleen cells from B cell-deficient mice processed and presented human and rat MOG proteins to T cells. The different pathogenic mechanisms of human and rat MOG proteins might result from different Abs induced by these proteins. However, rat and human MOG proteins induced Abs to mouse MOG that were equivalent in titer and IgG subclass. These data demonstrate that EAE can be induced in C57BL/6 mice by two mechanisms, depending on the nature of the immunogen: an encephalitogenic T cell response to rat MOG or rodent MOG 35-55, or an encephalitogenic B cell response to epitopes on human MOG protein that most likely cross-react with mouse determinants. *The Journal of Immunology*, 2003, 171: 462–468.

Considerable uncertainty exists concerning the relative roles of T cells, B cells, and cytokines in the pathogenesis of multiple sclerosis (MS).³ Several different mechanisms could be involved in the various forms of the disease, with extensive T cell and macrophage involvement in some situations, and a more prominent role for B cells and Ab in others (1). Controversy also exists with regard to the animal models of MS that are induced by immunization with myelin oligodendrocyte glycoprotein (MOG) (2–5). MOG appears to be a relevant disease Ag because MS patients exhibit Ab titers to MOG and because mice, rats, and marmosets immunized with MOG develop autoimmune, inflammatory, demyelinating lesions with paralysis and optic nerve involvement that resemble the human disease with regard to clinical signs and demyelination.

T cells, in the absence of Ab, are crucial mediators of clinical disease, inflammation, and demyelination in C57BL/6 mice immunized with rodent MOG 35-55 peptide, and Ag-activated CD4 Th1 T cells can transfer disease (6, 7). CD4 Th1 T cell-derived inflammatory cytokines such as lymphotoxin, TNF- α , and IFN- γ ,

and inflammatory chemokines such as RANTES, macrophage-inflammatory protein-1 α , and IFN- γ -inducible protein-10 are expressed in the CNS of animals immunized with rodent MOG 35-55 peptide (8, 9). Likewise, T cell-, macrophage-, and microglial-derived inflammatory cytokines or chemokines are required for the full manifestation of the disease (8, 10–12). Most importantly, disease induced in B cell-deficient C57BL/6 mice immunized with rodent MOG 35-55 is indistinguishable from that induced in wild-type (WT) mice, indicating that T cell responses to this Ag are sufficient to induce CNS inflammation and demyelination in this model (2, 3, 13).

Abs to MOG augment disease in rat and marmoset experimental autoimmune encephalomyelitis (EAE) models, and are detected in demyelinated lesions (5, 14, 15). Furthermore, B cell-deficient C57BL/6 mice immunized with human MOG protein are resistant to EAE, and transfer of activated B cells or serum from mice immunized with human MOG protein restores disease susceptibility (3, 16). However, the presence of B cells and Abs to MOG is not sufficient to cause disease in that immunization is still required, presumably to activate a T cell response (16, 17). Because B cells are not required for EAE to the rodent MOG 35-55 peptide in C57BL/6 mice (2, 3, 13), it is possible that B cells or Abs are required for EAE to a complex protein Ag, but not to a peptide. However, there are other explanations; for instance, human MOG protein differs from rodent MOG proteins at several sites. Human MOG 35-55, which has a proline instead of a serine at position 42, is a weak encephalitogen for C57BL/6 mice (18), and may not be capable of inciting an encephalitogenic T cell response within the context of human MOG protein. If this is true, B cells or Abs could be required for the encephalitogenicity of human MOG protein, but not that of rat MOG protein, which contains rodent MOG 35-55.

The goal of the present study was to determine whether the previously observed requirement for B cells for the induction of

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³ Abbreviations used in this paper: MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; Igd, Ig-like domain; LNC, lymph node cell; MOG, myelin oligodendrocyte glycoprotein; PMN, polymorphonuclear leukocyte; WT, wild type.

EAE to human MOG protein is due to distinct pathogenic mechanisms induced by peptide and protein Ags, or to sequence differences between the rat and human MOG proteins. The encephalitogenicity of extracellular rat and human rMOG proteins, as well as their respective MOG 35-55 peptides, was examined in WT C57BL/6 mice and B cell-deficient μ MT mice on the C57BL/6 background. The results of this study demonstrate that B cells are not required for the induction of EAE to the rat protein, even though mice immunized with this protein produce Abs that bind murine MOG protein. They confirm that B cells are required for EAE to the human MOG protein, and indicate that the difference in pathogenic mechanisms is determined in large part by the difference at position 42. Even though the 35-55 region of human MOG is immunogenic and activates T cells alone and within the context of the rat protein, it is not encephalitogenic. These results suggest that the T cell responses to the 35-55 regions are responsible for the differential encephalitogenicities of rat and human MOG proteins in B cell-deficient mice, and indicate that these proteins induce EAE via distinct pathogenic mechanisms.

Materials and Methods

Expression and purification of rMOG proteins

Escherichia coli DH5 α bacteria expressing the extracellular Ig-like domain (Igd) of rat or human MOG proteins were obtained from C. Linington (Max-Planck-Institute, Martinsreid, Germany). Bacteria expressing mouse MOG Igd were obtained from M. Gardinier (University of Iowa, Iowa City, IA). The plasmid coding for rat MOG was modified by site-directed mutagenesis to change the serine at position 42 of the expressed protein to a proline (rat MOG P42). Plasmid DNA was isolated using Qiagen (Valencia, CA) Miniprep kits and modified by site-directed mutagenesis using the Stratagene (La Jolla, CA) QuikChange procedure, according to the instructions provided by the manufacturer. The forward primer sequence was GTGGGGTGGTACCGTCCTCCCTTTTCAAGAG, and the reverse primer sequence was CTCTTGAAAAGGGGAGGACGGTACCACCCAC.

The identity of all inserts was verified by DNA sequencing of purified plasmids using appropriate primers. Hexahistidine-tagged proteins were expressed and purified according to the original published protocol (19). Briefly, bacteria were grown in antibiotic-supplemented Luria-Bertani broth and lysed, and protein was purified by Ni-chelate column (Novagen, Madison, WI) chromatography under denaturing conditions, according to the instructions supplied by the manufacturer. Pooled protein-containing fractions as determined by SDS-PAGE were then dialyzed into acetate buffer (20 mM, pH 4.0) and concentrated. Protein purity and concentration were assessed by SDS-PAGE and Bio-Rad (Hercules, CA) protein assay, respectively, and proteins were stored at -80°C until use.

MOG peptides

Peptides corresponding to the published sequences of rodent and human MOG 35-55 were synthesized by the W.M. Keck Biotechnology Resource Center at Yale University. Peptides were purified by reverse-phase (C18) column HPLC using a trifluoroacetic acid/acetonitrile gradient. The sequence of the rodent peptide (rat and mouse MOG proteins are identical in this region) is MEVGWYRSPFSRVVHLYRNGK. The sequence of the human MOG 35-55 peptide is MEVGWYRPPFSRVVHLYRNGK.

A series of overlapping synthetic peptides derived from the sequence of human MOG protein was provided by C. Linington.

Animals and immunization

WT female C57BL/6 mice and B cell-deficient μ MT mice on a C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME) and were 8–12 wk old at the time of immunization. EAE was induced as previously described (11). Mice were immunized by s.c. flank injections of either 300 μg of MOG 35-55 peptides or 100 μg of rMOG proteins in CFA (Difco, Detroit, MI) with 500 μg of *Mycobacterium tuberculosis* on days 0 and 7. Mice were injected i.p. with 500 ng of pertussis toxin (List Biological Laboratories, Campbell, CA) on days 0 and 2. All animal use and husbandry protocols were approved by the Yale University Institutional Animal Care and Use Committee.

Clinical disease scoring

Mice were monitored daily for clinical signs of EAE following the booster immunization. Clinical scores were based on a scale of 0–5, with a score

of 0 indicating no disease and 5 indicating death. A score of 1 indicates limp tail, 2 indicates paresis or partial paralysis of the hind limbs, 3 indicates total hind limb paralysis, and 4 indicates hind and front limb paralysis. Data are presented as mean clinical scores for each group, with dead animals given a score of 5 on the day of death. Disease onset was calculated as the average day of appearance of clinical signs. Disease indices for each group were calculated as: ((sum of the mean clinical scores)/(day of disease onset)) \times 100.

Statistical analysis

Differences in disease onset and maximum disease scores were analyzed by Student's *t* test (two-sample, one-tailed test), using a *p* value of ≤ 0.05 as the threshold of significance.

Histopathologic examination

Mice were deeply anesthetized and perfused intracardially with cold HBSS. Brains and spinal cords were removed and fixed in zinc-buffered Formalin. Tissues were processed, sectioned, and stained with H&E by the Dermatopathology Laboratory at Yale University.

Proliferation assays

Spleens were removed from WT or μ MT mice; the dispersed, irradiated (2000 rad) cells were used as a source of APC. T-MOG cells were a line established from C57BL/6 mice immunized with rodent MOG-35-55 (a kind gift of T. Spahn (Westfälische Wilhelms-Universität, Münster, Germany) (20)). These cells have been maintained in long-term culture by regular administration of human rIL-2, rodent MOG 35-55, and irradiated C57BL/6 spleen cells as feeder cells. Cell cultures contained 1.5×10^6 APC and 5×10^5 T-MOG cells per well in a volume of 200 μl in 96-well flat-bottom plates, in addition to MOG proteins (120 $\mu\text{g}/\text{ml}$) or peptides (20 $\mu\text{g}/\text{ml}$), as indicated, in RPMI supplemented with penicillin/streptomycin, fungizone, 10% FBS, and 0.05 μM 2-ME. Cultures were pulsed with 1.0 μCi of [^3H]TdR at 48 h, and harvested 24 h later with a Tomtec (Hamden, CT) harvester. Thymidine incorporation was measured in a Wallac (Gaithersburg, MD) Microbeta 1450 liquid scintillation counter.

Lymph node cells (LNC) were isolated from the periaortic and inguinal lymph nodes of mice immunized 10 days previously with a single injection of 300 μg of MOG peptide or 100 μg of MOG protein in CFA in a total volume of 200 μl distributed in the rear flanks and the base of the tail. Cells were cultured in 200 μl in triplicate at 2×10^5 cells/well for proliferation assays and 8×10^5 cells/well for cytokine assays.

Cytokine ELISA

Cells were cultured in 96-well plates with 50 $\mu\text{g}/\text{ml}$ of MOG peptide or 10 $\mu\text{g}/\text{ml}$ of MOG protein. Supernatants were harvested after 72 h of culture, centrifuged to remove cellular debris, and frozen until used. Capture and detecting Abs and cytokine standards for IFN- γ , IL-4, and IL-10 were purchased from BD PharMingen (San Diego, CA). Nunc Maxisorp 96-well plates (Macalaster Bicknell, New Haven, CT) were coated overnight at room temperature with capture Abs diluted in 0.05 M carbonate buffer, pH 9.6. Standards, samples, and biotin-labeled detecting Abs were diluted in PBS containing 10% FBS. Reagents for IL-13 assays were purchased from R&D Systems (Minneapolis, MN); assays were performed according to instructions provided by the manufacturer. Plates were developed using streptavidin-HRP and tetramethylbenzidine substrate (both from Sigma-Aldrich, St. Louis, MO), and absorbance was read at 405 nm on a Molecular Devices (Sunnyvale, CA) Vmax plate reader using SoftMax software. Cytokine concentrations were determined by interpolation from a standard curve.

Ab ELISA

Microtiter plates (Costar, Cambridge, MA) were coated overnight at room temperature with 100 $\mu\text{l}/\text{well}$ of extracellular mouse rMOG protein at a concentration of 10 $\mu\text{g}/\text{ml}$ in PBS. Plates were washed with PBS containing 0.05% Tween 20 and blocked with PBS containing 1% BSA. After washing, serum samples diluted in PBS containing 1% BSA were applied to the wells. Secondary Abs were HRP-labeled Abs to mouse IgG, IgG1, or IgG2a purchased from BD PharMingen. Plates were developed using tetramethylbenzidine substrate.

Results

B cells are required for EAE to human, but not rat MOG protein

To evaluate the role of B cells in disease induced by a known encephalitogenic peptide, WT and B cell-deficient μ MT mice were

immunized with rodent MOG 35-55. As we have reported previously (2), the clinical disease courses were similar in these groups of animals, as were the cumulative disease indices calculated on day 40 (447 for WT, 518 for μ MT). These data, in agreement with the results of previous studies, demonstrate that B cells are not required for the induction of EAE to this peptide. To test the role of B cells in EAE induced by a protein Ag, WT and μ MT mice were immunized with rat MOG Igd, which includes the encephalitogenic 35-55 peptide. This protein induced disease in WT and μ MT mice with similar kinetics (Fig. 1A) and disease indices (370 for WT, 351 for μ MT). In addition, there were no statistically significant differences between WT and μ MT mice with regard to disease onset or maximum score ($p = 0.17$ and 0.31 , respectively). These results indicate that B cells are not required for the induction of EAE to this encephalitogenic protein Ag.

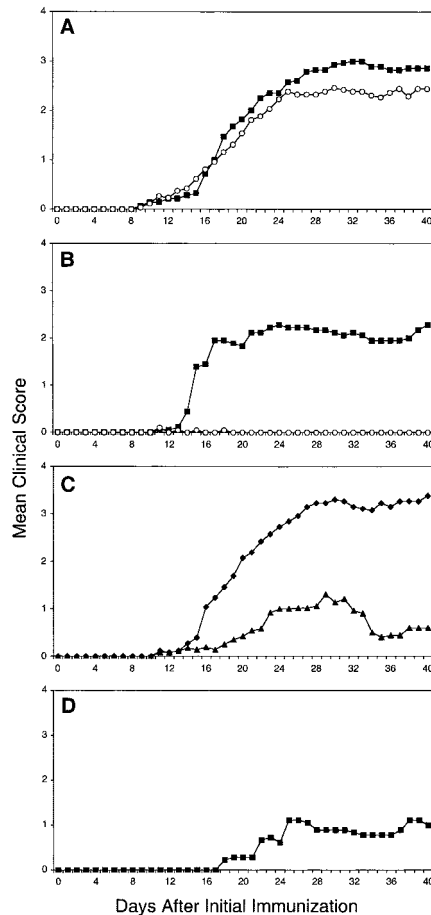


FIGURE 1. Clinical responses to MOG proteins and peptides. Data represent mean clinical scores derived from daily observation. *A*, B cells are not required for EAE to rat MOG protein. WT (■) and μ MT (○) mice ($n = 13$ of each) were immunized with rat rMOG protein. Cumulative day 40 disease indices were 370 for WT and 351 for μ MT. *B*, B cells are required for EAE to human MOG protein. WT (■) and μ MT (○) mice ($n = 12$ WT, 14 μ MT) were immunized with human rMOG protein. Day 40 disease indices were 361 for WT and 8 for μ MT groups. *C*, Human MOG 35-55 is weakly encephalitogenic relative to rodent MOG 35-55. WT mice were immunized with either rodent (◆, $n = 13$) or human (▲, $n = 13$) MOG 35-55 peptides. Day 40 disease indices were 447 for rodent peptide and 103 for human peptide. *D*, The 35-55 region is critical to encephalitogenicity of rat MOG protein. WT mice ($n = 8$) were immunized with rat rMOG P42, which differs from rat MOG protein by a proline at position 42 instead of the normal serine. Day 40 disease index was 69. Compare with unmodified rat MOG protein in *A*.

To determine whether B cells are required for disease induction by human MOG protein, WT and μ MT mice were immunized with human MOG Igd. Disease was induced in WT, but not μ MT mice by this Ag (Fig. 1B). Day 40 disease indices were 361 for WT mice and 8 for μ MT mice. This finding, in agreement with a previous study (3), indicates that B cells are required for the induction of EAE to this Ag. Thus, the previous controversy regarding a role for B cells in MOG-induced EAE in C57BL/6 mice is explained by the finding that the crucial difference lies in the origin of the protein.

The cellular composition of CNS infiltrates differs in mice immunized with rat and human MOG proteins

Immunization with human MOG protein results in a shift from a B cell-independent to a B cell-dependent pathogenic mechanism. This indicates that the disease induced by the individual proteins, although indistinguishable clinically, arises through different mechanisms. The possibility that these differences would be reflected in the cellular infiltrate was evaluated by histologic examination of CNS tissue from mice immunized with MOG peptides or proteins. The infiltrates of WT and μ MT mice evaluated at different times after immunization with rodent MOG 35-55 or rat MOG protein were predominantly mononuclear with activated microglia (Fig. 2A), whereas those of WT mice immunized with human MOG protein had more macrophages, fewer activated microglia, and many polymorphonuclear leukocytes (PMN) (Fig. 2B). The extent of demyelination appeared to be comparable in mice

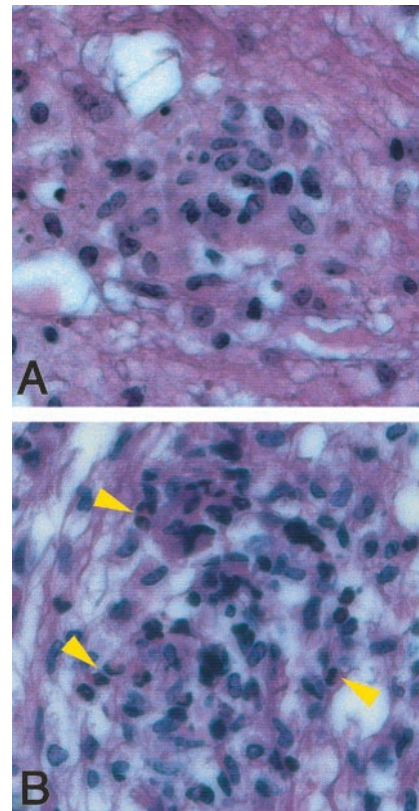


FIGURE 2. Rat and human MOG proteins induce distinct infiltrates in spinal cords of immunized mice. WT mice were immunized with rat MOG protein (*A*) or human MOG protein (*B*), and spinal cords were removed on day 20, at 4 or 5 days after disease onset. Immunization with rat MOG induced a mostly mononuclear infiltrate (*A*), whereas many PMN (representative cells indicated by arrows) were present in the infiltrates of mice immunized with human MOG protein (*B*).

Table I. *The 35-55 region is crucial to the encephalitogenicity of rat MOG protein^a*

Immunogen	Incidence	Mean Day of Onset (range)	Mean Maximum Disease Score (range)	<i>p</i>	Day 40 Disease Index
Rodent MOG 35-55	13/13	16 (11–23)	3.7 (2.5–5)		518
Human MOG 35-55	11/13	18 (11–28)	1.7 (0.5–2.5)	<0.001	103
Rat MOG P42	7/9	26 (18–38)	2.5 (2–3)	<0.001	69

^a C57BL/6 mice were immunized with either rodent or human MOG 35-55 peptides, or rat MOG P42 protein and observed daily for signs of clinical disease. Onset and maximum disease scores were calculated for mice that developed clinical signs. Human MOG 35-55 is only weakly encephalitogenic relative to the rodent peptide. Substitution of the serine at position 42 of rat MOG protein with the proline characteristic of human MOG severely diminishes the encephalitogenicity of the protein.

immunized with human or rat MOG. There was little or no inflammation in the CNS of μ MT mice immunized with human MOG protein (data not shown), as was expected based on the clinical observations. These data suggest that the dependence or not on B cells influences the composition of the infiltrate, resulting in tissue damage mediated by different cell types.

The 35-55 region is crucial to the encephalitogenicity of rat MOG protein

Human and rat MOG proteins differ at several amino acids. To determine whether the differences in mechanism could be due to differences in the 35-55 peptides, mice were immunized with human MOG 35-55, which has a proline in place of the serine found at position 42 in the rodent peptide. Human MOG 35-55 induced minimal clinical signs of EAE relative to the rodent peptide (Fig. 1C, Table I). We next examined the ability of the weakly encephalitogenic human 35-55 to induce EAE within the context of a B cell-independent encephalitogenic protein. The plasmid coding for rat MOG was modified by site-directed mutagenesis so that the expressed protein contained a proline at position 42. The resultant protein (rat MOG P42) was only weakly encephalitogenic (Fig. 1D, Table I), reminiscent of human MOG 35-55. Thus, human MOG 35-55, which is only weakly encephalitogenic on its own, severely attenuated the encephalitogenicity of rat MOG protein. This result indicates that the strong encephalitogenicity of rat MOG is dependent on the presence of the rodent MOG 35-55 sequence, and confirms that the T cell response to rodent MOG 35-55, independent of B cell responses, is responsible for the encephalitogenicity of rat MOG protein.

Table II. *Human MOG 35-55 peptide and protein are immunogenic^a*

Immunizing Ag	Culture Ag	Mean cpm	Stimulation Index
Human MOG 35-55	Nothing	359	
	Human MOG 35-55	16,159	45
	Rodent MOG 35-55	2,840	8
Rodent MOG 35-55	Nothing	317	
	Rodent MOG 35-55	7,630	24
	Human MOG 35-55	3,231	10
Human MOG Protein	Nothing	102	
	Human MOG Protein	10,864	106
	Mouse MOG Protein	2,277	22
Rat MOG Protein	Nothing	204	
	Rat MOG Protein	14,157	71
	Mouse MOG Protein	6,171	30

^a C57BL/6 mice were immunized with MOG peptides or proteins emulsified in CFA; LNC were removed 10 days later and cultured with MOG peptides or proteins. [³H]TdR (1 μ Ci/well) was added for the final 24 h of the 72-h culture period. Representative data from at least three experiments are presented as mean cpm of triplicate cultures and stimulation indices (stimulated/unstimulated). Human MOG 35-55 is immunogenic, but primes poorly for the endogenous encephalitogenic peptide. Rodent MOG 35-55 also primes for strong response to the cognate, but not the opposite peptide. Immunization with either human or rat MOG proteins primes for strong response to mouse MOG protein.

Human MOG 35-55 is a strong immunogen

We next investigated whether human MOG 35-55, although weakly encephalitogenic, was immunogenic, as it was possible that this peptide did not induce EAE in C57BL/6 mice because it did not bind H-2^b class II MHC or because of a deficient T cell repertoire. To address this issue, LNC from mice immunized 10 days previously with human MOG 35-55 were cultured with this peptide. Robust T cell proliferation to human MOG 35-55 was observed, indicating that this peptide is immunogenic (Table II). T cells from mice immunized with human MOG 35-55 proliferated weakly when cultured with rodent MOG 35-55, suggesting that human MOG 35-55-specific T cells were only weakly responsive to the endogenous encephalitogenic epitope (Table II). Likewise, LNC from mice immunized with rodent MOG 35-55 proliferated much more vigorously to the cognate than the noncognate peptide (Table II). In contrast, LNC from mice immunized with human or rat MOG proteins proliferated when cultured with either the immunizing Ag or mouse MOG protein (Table II). As was the case with human MOG 35-55, the weakly encephalitogenic rat MOG P42 was immunogenic, as LNC from immunized mice proliferated vigorously when cultured with the protein (stimulation index of 130).

LNC from mice immunized with human MOG protein were tested against a panel of overlapping human MOG peptides to identify immunogenic epitopes. Proliferation was induced only to human MOG 35-55 or peptides including this sequence (Fig. 3). This indicates that human MOG 35-55, even though nonencephalitogenic, is the major T cell epitope within human MOG protein in C57BL/6 mice. Thus, even though T cells from C57BL/6 mice respond to human MOG 35-55 peptide alone and within the context of the protein, this response is not sufficient to induce EAE.

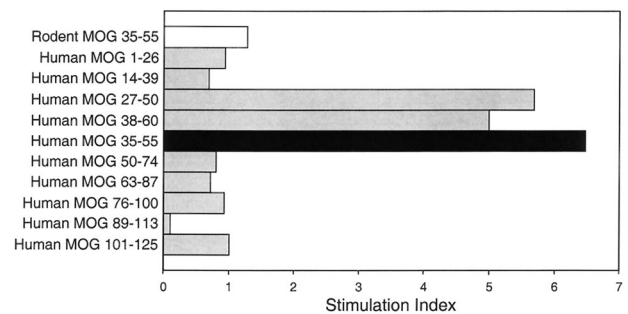


FIGURE 3. MOG 35-55 is the immunodominant T cell epitope on human MOG protein. LNC taken from WT mice 10 days after immunization were cultured with overlapping peptides from the Igd of human MOG or with rodent or human MOG 35-55. LNC proliferated most strongly to human MOG 35-55 and peptides containing these amino acids.

Rodent and human MOG peptides induce IFN- γ and IL-13

Because rodent MOG 35-55 is encephalitogenic and the corresponding human peptide is not, it is possible that the human peptide, although immunogenic, does not generate an inflammatory cytokine response to the endogenous peptide. LNC from mice immunized with human MOG 35-55 produced large amounts of IFN- γ when cultured with human MOG 35-55 (~20,000 pg/ml), but not when cultured with rodent MOG 35-55 (~2,000 pg/ml) (Fig. 4A). Likewise, LNC from mice immunized with rodent MOG 35-55 produced large amounts of IFN- γ (~17,000 pg/ml) when cultured with the rodent peptide, but not when cultured with human MOG 35-55 (~2,000 pg/ml) (Fig. 4A). The possibility that human MOG 35-55-primed T cells produced Th2 cytokines was also tested. IL-13 was produced by human MOG 35-55-primed cells cultured with human MOG 35-55, but not to the rodent peptide (Fig. 4B). Likewise, cells from mice primed with rodent MOG 35-55 made IL-13 in response to rodent MOG 35-55, but not to the human peptide (Fig. 4B). Neither IL-4 nor IL-10 was detected in cultures of cells primed or cultured with either peptide (data not shown). These results indicate that T cells from mice immunized with human MOG 35-55 produce a Th1 cytokine in response to the immunizing peptide, but not to the endogenous encephalitogenic peptide. This finding, taken together with the results of prolifera-

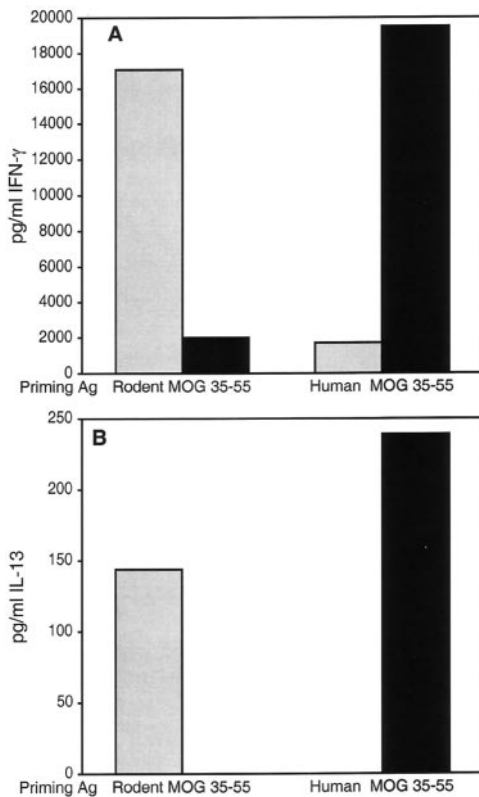


FIGURE 4. Human MOG 35-55 primes weakly for cytokine responses to the endogenous encephalitogenic peptide. LNC from WT mice primed with either human or rodent MOG 35-55 peptides were tested for production of either IFN- γ (A) or IL-13 (B) to human MOG 35-55 (filled bars) or rodent MOG 35-55 (shaded bars). Human MOG 35-55-primed T cells produced large amounts of IFN- γ when cultured with the human peptide, but not when cultured with rodent MOG 35-55. These cells also produced IL-13 when cultured with human, but not rodent MOG 35-55 peptides. Likewise, rodent MOG 35-55, but not the human peptide, was most effective at eliciting IFN- γ and IL-13 secretion from rodent MOG 35-55-primed cells. Cells cultured without Ag produced undetectable levels of cytokines.

tion assays (see Table II), may explain the weak encephalitogenicity of the human MOG 35-55 peptide.

B cells are not required to process and present MOG proteins

One explanation for the inability of human MOG protein to induce EAE in B cell-deficient mice is that B cells play a role in the processing and presentation of the intact protein. To test the ability of APC from B cell-deficient mice to present the encephalitogenic epitope from an intact protein, APC from WT and μ MT mice were cultured with the rodent MOG 35-55-specific T cell line T-MOG along with rat or mouse MOG protein. T-MOG cells proliferated vigorously when cultured with rat or mouse MOG proteins in the presence of spleen cells from WT or μ MT mice (Fig. 5A). These results indicate that B cells are not required to process rat or mouse MOG protein and present the encephalitogenic 35-55 peptide. To test whether B cells were required to process and present human MOG protein, LNC taken from WT or μ MT mice 10 days after immunization with human MOG protein were cultured with the protein. T cells from WT and μ MT mice proliferated to a similar extent, demonstrating that B cells are not required to process human MOG protein and present peptides from it to T cells (Fig. 5B).

Human and rat MOG proteins induce Ab responses to mouse MOG that are equivalent in titer and IgG subclass

Another possible explanation for the differential B cell dependence of rat and human MOG proteins is that immunization with human

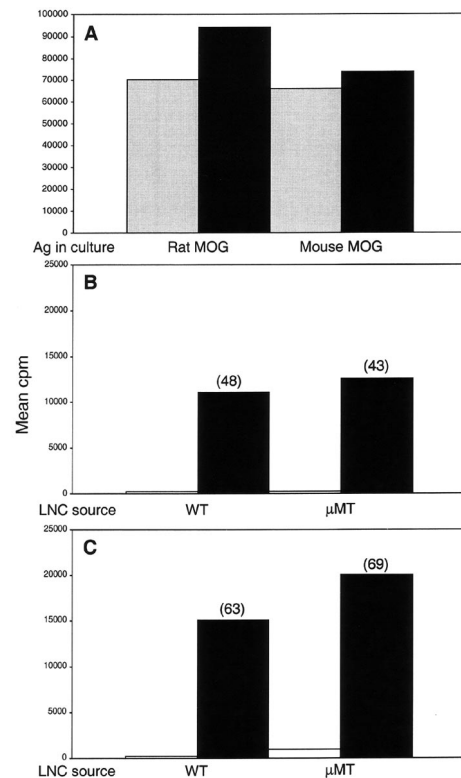


FIGURE 5. B cells are not required to process and present MOG epitopes to T cells. Data represent mean cpm of triplicate wells, representative of at least three experiments. A, T-MOG, a T cell line raised against rodent MOG 35-55, proliferates vigorously when the encephalitogenic epitope from rat and mouse MOG proteins is presented by spleen cells from WT (shaded bars) or μ MT (filled bars) mice. LNC from WT and μ MT mice primed with either rat MOG (B) or human MOG (C) proteins proliferate when cultured with the respective proteins. Open bars represent control unstimulated cultures; filled bars are Ag-stimulated cultures. Numbers in parentheses are stimulation indices.

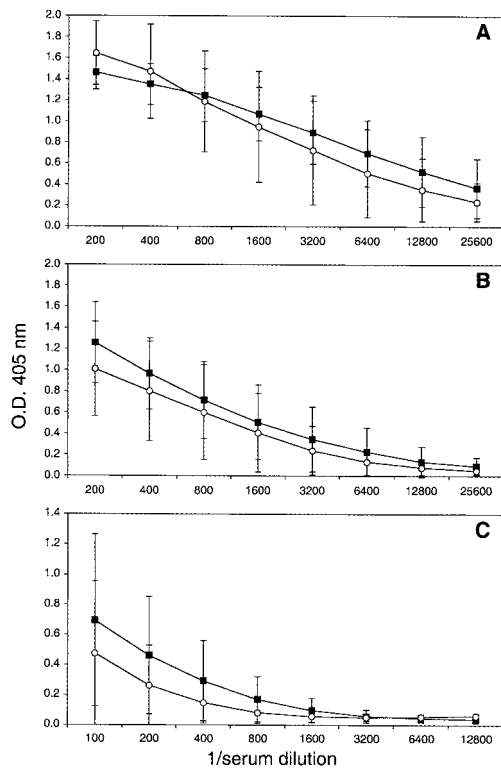


FIGURE 6. Mice immunized with rat or human MOG proteins make Ab responses that bind mouse MOG. Serum drawn at day 30 after immunization with either rat MOG protein (○, $n = 5$) or human MOG protein (■, $n = 5$) was assayed by ELISA for total IgG (A), IgG1 (B), or IgG2a (C) responses to mouse MOG protein. Error bars are SD. No MOG-specific Abs were detected in samples from unimmunized mice. Note the different scale of C.

MOG results in a higher Ab titer or a different IgG subclass response than does immunization with rat MOG. In fact, although there was considerable variation between individual mice, the mean anti-mouse MOG IgG titers were similar in the two groups, as were the titers of IgG1 and IgG2a Ab (Fig. 6). These results indicate that the different mechanisms of disease are not due to the titer or subclass of the IgG responses induced by the different proteins, but may be due to the induction of an encephalitogenic Ab by human MOG protein.

Discussion

The results of this study demonstrate that rat and human MOG proteins induce EAE in C57BL/6 mice by different mechanisms. B cells are required for EAE to human MOG protein (3) (this study), whereas they are not required for EAE to rat MOG protein. EAE induced by human and rat MOG proteins was associated with distinct CNS infiltrates, with a largely mononuclear infiltrate in mice immunized with rat MOG protein and prominent PMN in the infiltrates of mice immunized with human MOG protein. The difference at position 42 appears to be responsible for the different pathogenic mechanisms induced by rat and human MOG proteins.

The presence of PMN in the CNS of WT mice immunized with MOG has not been noted previously. Previous analyses suggested that the CNS infiltrate in mice immunized with human MOG protein was predominantly mononuclear (3, 21), but it is difficult to determine from the published data whether PMN were present in these lesions. In the experiments presented in this study with mice immunized with human MOG protein, it is likely that PMN were attracted to the CNS by as yet unidentified chemotactic factors.

PMN have been noted in a hyperacute form of EAE in rats immunized with aqueous spinal cord homogenate (22) and in a rotatory form of EAE in C3H/HeJ mice immunized with proteolipid protein in CFA (23). These models differ significantly from the human MOG model described in this study, particularly in time course, as the tissues in the present study were examined at least 4 days after onset of disease. It is unlikely that these PMN are the result of an infection, because they were observed only in WT mice immunized with human MOG protein, and because sentinel mice were free of infections. CNS tissue from mice immunized with rodent MOG 35-55 contains mRNA for RANTES, monocyte chemoattractant protein-1, and IFN- γ -inducible protein-10 (9). Because the CNS lesions in mice immunized with rat MOG resemble those induced by the rodent MOG 35-55 peptide, the chemokine profile is likely to be similar.

An inflammatory T cell response to the endogenous rodent MOG 35-55 peptide, the dominant encephalitogenic MOG epitope in H-2^b mice (24), may be sufficient to induce CNS inflammation and demyelination (2). Rodent MOG 35-55 (2, 3, 13) and rat MOG protein, which contains the encephalitogenic rodent peptide, induce EAE in the absence of B cells. Lymphocytes from mice immunized with rodent MOG 35-55, but not the human peptide, proliferated vigorously and produced large amounts of IFN- γ in response to the endogenous encephalitogenic peptide. In contrast, cells from mice immunized with human MOG protein proliferated weakly and produced very little IFN- γ in response to rodent MOG 35-55 (3). Although human MOG 35-55 is not encephalitogenic (18) (this study), it is immunogenic, as shown in this study, in C57BL/6 mice and also in C3H.SW (H-2^b) mice (25). T cells raised against this peptide proliferated and produced cytokines in response to the immunizing peptide; it is in fact the dominant T cell epitope of human MOG protein in C57BL/6 mice. Human MOG 35-55 is thus bound by H-2^b class II MHC and recognized by T cells. However, this peptide, when substituted for the corresponding rodent peptide in rat MOG protein, severely attenuated the encephalitogenicity of the protein. These results suggest that T cells responding to human MOG 35-55, either alone or within the context of a MOG protein, do not recognize the endogenous 35-55 peptide in the CNS. Thus, rat MOG protein, which contains the encephalitogenic T cell epitope, induces EAE in B cell-deficient mice, whereas human MOG protein, absent this epitope, requires a B cell response for disease induction.

B cells could contribute to the pathogenesis of EAE by surface Ig-facilitated Ag presentation and/or production of pathogenic Abs. Two lines of evidence suggest that B cells are not critical APCs in MOG protein-induced EAE. B cell-deficient spleen cells cultured with rat MOG protein efficiently presented the encephalitogenic rodent MOG 35-55 peptide to a peptide-specific T cell line, and spleen cells and LNC from B cell-deficient mice efficiently presented human and rat MOG protein epitopes to primed T cells (3) (this work). Recent studies provide evidence for a role of Abs in MOG-induced EAE. Serum from WT mice immunized with human MOG protein induces EAE in B cell-deficient mice (16). However, the presence of Abs to MOG is not sufficient to induce EAE in this situation or in mice transgenic for anti-MOG Ig H chain. Immunization with MOG is still required, presumably to initiate T cell responses (16, 17). In our studies, immunization with rat and human MOG proteins generated Abs that bound mouse MOG protein and were indistinguishable with regard to IgG subclass and titer. These results suggest that Abs raised against human (but not rat) MOG protein recognize endogenous MOG determinants that contribute to EAE.

The finding that rat and human MOG proteins induce EAE by different mechanisms indicates that there are determinants on these

proteins that activate T cells and/or B cells in different ways. Th1 cytokine-secreting T cells responding to rodent MOG 35-55 peptide are sufficient to cause EAE in the absence of B cells or Abs (2, 3, 13). Although rat MOG induces EAE in the absence of B cells, it clearly contains B cell determinants and induces Abs that bind mouse MOG. The encephalitogenic determinants within human MOG protein have not been determined and are likely to be conformational. The human MOG 35-55 peptide, although immunogenic and immunodominant for T cells, is poorly encephalitogenic, and induces only a weak T cell response to the corresponding mouse peptide. It is possible that IFN- γ induced by human MOG protein activates B cells that produce pathogenic Abs. It is clear that human MOG protein contains encephalitogenic determinants that are not present on rat MOG because rat MOG P42 protein was only weakly encephalitogenic, even though it contains the immunodominant T cell epitope from human MOG. We favor the hypothesis that human MOG protein induces an Ab response to a conformational encephalitogenic determinant, whereas rat MOG either does not induce such an Ab or the encephalitogenic T cell response makes the Ab response irrelevant. Recent data from Bourquin et al.⁴ support the hypothesis that C57BL/6 mice do not produce an encephalitogenic Ab when immunized with rat MOG. Our data, taken together with a recent publication (16), indicate that these mice do make an encephalitogenic Ab when immunized with human MOG. These results indicate that, in the absence of a strongly encephalitogenic T cell epitope, the contribution of B cells to the encephalitogenicity of the human MOG protein becomes critical. A minor change in an inciting peptide (alone or within the context of an encephalitogenic protein) thus results in the induction of EAE (and possibly MS) via different mechanisms. Of particular interest in this regard is the use of altered peptide ligands in the treatment of MS. The data presented in this work suggest that caution is warranted in this approach, as it could contribute to MS by activation of a different, but equally pathogenic mechanism. Taken together, the data suggest that treatments aimed at one or both arms of the immune response (inflammatory cytokines and Ab) may be warranted in different situations, but it is important that the mechanism be carefully analyzed and understood in individual cases.

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