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A Switch in Pathogenic Mechanism in Myelin Oligodendrocyte Glycoprotein-Induced Experimental Autoimmune Encephalomyelitis in IFN-γ–Inducible Lysosomal Thiol Reductase-Free Mice

Cheryl M. Bergman,* Cecilia B. Marta,†‡ Maja Maric,§ Steven E. Pfeiffer,†,2 Peter Cresswell,§ and Nancy H. Ruddle*§

IFN-γ–inducible lysosomal thiol reductase (GILT) is an enzyme located in the Lamp-2–positive compartments of APC. GILT−/− mice are phenotypically normal, but their T cells exhibit reduced proliferation to several exogenously administered Ags that include cysteine residues and disulfide bonds. We undertook the present studies to determine if GILT−/− mice would process exogenously administered myelin oligodendrocyte glycoprotein (MOG), which contains disulfide bonds, to generate experimental autoimmune encephalomyelitis (EAE) to the endogenous protein. One possibility was that MOG35–55 peptide would induce EAE, but that MOG protein would not. GILT−/− mice were relatively resistant to MOG35–55–induced EAE but slightly more susceptible to rat MOG protein–induced EAE than wild-type (WT) mice. Even though MOG35–55 was immunogenic in GILT−/− mice, GILT APCs could not generate MOG35–55 from MOG protein in vitro, suggesting that the endogenous MOG protein was not processed to the MOG35–55 peptide in vivo. Immunization of GILT−/− mice with rat MOG protein resulted in a switch in pathogenic mechanism from that seen in WT mice; the CNS infiltrate included large numbers of plasma cells; and GILT−/− T cells proliferated to peptides other than MOG35–55. In contrast to WT rat MOG–immunized mice, rat MOG–immunized GILT−/− mice generated Abs that transferred EAE to MOG35–55–primed GILT−/− mice, and these Abs bound to oligodendrocytes. These studies, demonstrating the key role of a processing enzyme in autoimmunity, indicate that subtle phenotypic changes have profound influences on pathogenic mechanisms and are directly applicable to the outbred human population. The Journal of Immunology, 2012, 188: 000–000.

The immune system loses its ability to distinguish self from nonself in autoimmune diseases. These diseases are multifactorial with influences of genetics and environment. Making them more complex is the fact that an individual disease can exhibit identical clinical manifestations, though the pathogenic mechanisms may vary widely. Such is the case in multiple sclerosis, an inflammatory demyelinating disease presenting with a variety of clinical manifestations that include relapsing-remitting, primary progressive, and secondary progressive subtypes. Even when the clinical signs are similar, several different pathogenic mechanisms have been postulated (1–4). These include: effector T cells and cytokines, Abs or B cells, and/or oligodendrocyte dystrophy. In the latter case, inflammation has been postulated to be secondary to the intrinsic tissue damage. The challenge in devising treatment for any autoimmune disease is to identify the pathogenic mechanism in the individual patient to choose the optimal therapy. This becomes even more daunting when the clinical signs are similar, but the pathogenic mechanisms differ.

In our previous studies, we have used myelin oligodendrocyte glycoprotein (MOG) induction of experimental autoimmune encephalomyelitis (EAE) in the mouse as a model of multiple sclerosis. MOG is a trans-membrane protein expressed on the cell body and processes of oligodendrocytes (OLs) and on the outermost lamellae of the myelin sheath. It is a type I integral membrane protein with a single extracellular domain (ECD). MOG is normally glycosylated at asparagine 24 and can multimerize (5). MOG35–55, the immunodominant peptide for C57BL/6 (B6) mice, lies at a dimer interface (5). Immunization of C57BL/6 mice with rodent MOG peptide35–55 (MOG35–55) results in EAE that is CD4 T cell mediated, B cell independent, and involves both Th1 and Th17 cells and their cytokines (6–8). We previously showed that immunization with the ECD of recombinant rat MOG protein (ratMOG) also induces a T cell mediated, B cell-independent EAE. However, immunization with recombinant human MOG protein (rhuMOG), even though it elicits similar clinical signs, is a B cell-dependent disease (9). This is due to a difference in
a particular amino acid residue, a proline rather than a serine at position 42 in human MOG. Immunization of wild-type (WT) mice with the ECD of human MOG results in the generation of a pathogenic Ab that recognizes glycosylated MOG on the surface of OLs (10) and induces EAE in human MOG-primed B cell-deficient mice. These data indicate that a minor switch in immunizing Ag can have a profound effect on the pathogenic mechanism in autoimmune disease, even in mice of the same genetic background.

Protein presentation to CD4 T cells requires processing to peptides that can bind to MHC class II and be recognized by the TCR in that context. In the case of exogenously administered rratMOG to WT mice, this results in class II-associated peptides that include MOG35−55, the predominant encephalitogenic epitope in C57BL/6 mice (11, 12). IFN-γ-inducible lysosomal thiol reductase (GILT) is located in the class II-positive, Lamp-2–positive compartments of APCs (13–16). Mice deficient in GILT (GILT−/− mice) are phenotypically normal, but their T cells exhibit reduced proliferation to hen egg lysozyme (HEL), RNase A, and human Ig G, all proteins with cysteine residues and disulfide bonds (16). Such is not the case with α-casein, which has no disulfide bonds. In the case of HEL, presentation of one of the epitopes is severely reduced, whereas other cysteine-containing epitopes are not affected (16). GILT is therefore essential for facilitating processing and presentation of some, but not all, peptides in exogenously administered protein Ags containing disulfide bonds. In rat and mouse MOG, cysteine residues are located in the extracellular domain at positions 24 and 98 and form an internal disulfide bond that includes the predominant MOG35−55 epitope (Fig. 1). Additional cysteines are also located in the intracellular region at positions 177 and 198. These features suggested a potential role for GILT in the CD4 T cell response to MOG.

In this study, we evaluated the role of GILT in MOG-induced EAE, an autoimmune disease that is induced by injection of the exogenous Ag but also depends on presentation of the endogenous Ag. We hypothesized that GILT−/− mice would be protected from EAE induced by rratMOG because their APCs would be reduction deficient and therefore unable to generate the encephalitogenic peptide MOG35−55 from the immunizing protein. However, they might be susceptible to EAE when immunized with MOG35−55 that would not require processing, although the possibility remained that the endogenous MOG would need to be processed, and these mice might be resistant as well. Peptide-immunized GILT−/− mice were relatively resistant to EAE, though their T cells recognized the peptide immunogen. In contrast, rat MOG protein-immunized GILT−/− mice developed EAE. These mice demonstrated a switched from the usual effector T cell-mediated pathogenic mechanism generated by rratMOG in WT mice to one that was B cell dependent, with pathogenic Abs that recognized MOG on the surface of OLs. Furthermore, T cells isolated from rratMOG-immunized GILT−/− mice did not recognize the usually dominant epitope MOG35−55 but underwent a switch in epitope recognition to peptides that included those not dependent on breaking the disulfide bond. These studies support the concept that subtle differences in Ag processing can result in different pathogenic mechanisms and emphasize the importance of recognition of both the immunizing and endogenous Ags in autoimmunity.

Materials and Methods

Mice

C57BL/6 (B6) female mice between the ages of 6 and 10 wk were obtained from The Jackson Laboratory (Bar Harbor, ME). GILT−/− mice that had been backcrossed to B6 mice for a minimum of eight generations (16) were maintained in a colony at Yale University. B cell-deficient (μMT) mice were originally obtained from The Jackson Laboratory and maintained in a colony at Yale University. All mice were treated according to protocols approved by the Yale University Animal Care and Use Committee.

Myelin oligodendrocyte glycoprotein

MOG protein was prepared as previously reported with bacteria expressing the extracellular domains of MOG from rat, human (C. Linington; University of Aberdeen, Aberdeen, Scotland), or mouse (M. Gardiner; University of Iowa, Iowa City, IA) (17) (Fig. 1).

Rodent MOG35−55 peptide was synthesized by the W.M. Keck Biotechnology Resource Center at Yale University. The sequence of the rodent peptide (rat and mouse MOG proteins are identical in this region) is MEVGYWYRPSFRVHLYRNGK.

A series of six additional overlapping mouse MOG peptides (p1−21, p15−35, p30−70, p67−87, p85−105, and p104−117) were synthesized by the W.M. Keck Biotechnology Resource Center at Yale University.


EAE induction by active immunization

For EAE induction, female WT and GILT−/− mice were immunized by s.c. injections with either 100 μg MOG35−55 or 100 μg rMOG proteins in CFA (Difco, Detroit, MI) with 300 μg Mycobacterium tuberculosis on days 0 and 7. Mice received i.p. injections of 500 ng pertussis toxin (PTX) (List Biological Laboratories, Campbell, CA) on days 0 and 2.

Passive transfer of EAE with serum

A previously described protocol for passive transfer with serum was used (10). GILT−/− or μMT mice were immunized with a single injection of MOG35−55 or rhMOG in CFA on day 0 and injected with PTX on days 0 and 2. These manipulations do not induce EAE in these particular mouse strains, but do prime T cells, which is a necessity for the success of this protocol (18). Mice received four i.v. injections of 150 μl pooled serum beginning at day 0 and at 3-d intervals for a total of 600 μl. The donor antisera were obtained from GILT−/− or C57BL/6 mice 14 d after immunization with rratMOG or rhMOG in CFA. Control serum was obtained from nonimmunized GILT−/− mice.

Clinical disease scoring

Mice were monitored daily for clinical signs of EAE. Clinical scores were based on a scale of 0–5, with a score of 0 indicating no disease and a score of 5 indicating death. A score of 1 indicates a limp tail, 2 indicates paresis or partial paralysis of the hind limbs, 3 indicates total hind limb paralysis, and 4 indicates fore and hind limb paralysis. Data are presented as the 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]/[mean day of disease onset]) × 100.

Histopathologic examination

The cellular composition of the infiltrates in the CNS of WT and GILT−/− mice on day 20 after EAE induction with rratMOG was evaluated. Mice were deeply anesthetized and intracardiac perfused with cold PBS. Spinal cords were removed and fixed in zinc-buffered formalin. Tissues were paraffin embedded, sectioned, and stained with H&E by the Dermatopathology Laboratory at Yale University. Four slides were prepared of axial cords. Each slide held sections 100 μm apart, along the length of spinal cord. A 100× oil immersion objective and scaling software were used to outline four distinct 50-μm2 regions per section. A total of 16 regions were evaluated per mouse spinal cord. The number of cells with the appearance of small mononuclear cells or plasma cells was counted and compared between WT and GILT−/− mice.

Statistical analysis

Differences in disease onset and maximum disease scores and the cellular composition of the CNS were analyzed by Student t test using a p value of ≤0.05 as the threshold of significance.

Immunofluorescence

For immunofluorescence, spinal cords were removed and immediately frozen in OCT compound (Sakura Finetek, Torrance, CA) using a dry ice/
methylbutane bath. Sections of 7 μm were cut onto poly-l-lysine–coated glass slides (Sigma-Aldrich, St. Louis, MO), fixed in 100% cold acetone, and stored at −70°C. Sections were blocked with 5% mouse serum/5% BSA in PBS (pH 7.4). Allophycocyanin-conjugated rat anti-mouse CD138 (BD Pharmingen, San Diego, CA) was diluted 1:250 in blocking buffer and incubated on sections for 1.5 h. Slides were washed three times in PBS and then incubated with PE-conjugated rat anti-mouse CD38 (BD Pharmingen) diluted 1:250 in blocking buffer and incubated on sections for 1.5 h. Slides were washed three times in PBS, and then sections were counterstained with DAPI (Sigma-Aldrich) and mounted with Fluorosave (Calbiochem, San Diego, CA). Tissue sections were analyzed by fluorescence microscopy using a Carl Zeiss Microimaging Axioskop microscope (Carl Zeiss).

Proliferation assays

Spleens were removed from GILT+/− or C57BL/6 mice. The dispersed, irradiated (2000 rad) cells were used as a source of APC. The Ag-specific T cell line was established from the lymph node (LN) cells of C57BL/6 mice immunized with MOG35–55. These cells have been maintained in long-term culture with biweekly administration of recombinant human IL-2, MOG35–55, and irradiated C57BL/6 spleen cells as APC. LN cells were isolated from the periaortic and inguinal LN of GILT−/− mice immunized 10 d previously with a single injection of 100 μg MOG protein in CFA in a total volume of 200 μl distributed in the rear flanks and at the base of the tail. Assay cultures contained 2 × 10³ T cells and 6 × 10³ APC/well, in triplicate, in a volume of 200 μl in 96-well flat-bottom plates in the presence of ratMOG (40 μg/ml) or peptides (20 μg/ml), as indicated, in RPMI supplemented with penicillin/streptomycin, fungizone, 10% FCS, and absorbance read at 450 nm on a PowerWave XS plate reader (Bio-Tek Instruments, Winooski, VT). Titers were determined with dilutions that resulted in 2 SDs above the highest reading of the negative control.

IgG preparation and purification

IgG was purified from the sera of the following groups of mice: C57BL/6, GILT−/−, or GILT−/− immunized with ratMOG or GILT−/− immunized with rhuMOG. IgG from sera pooled from at least 10 mice collected 14 or 21 d after immunization were purified by protein G-Sepharose (Sigma-Aldrich) chromatography; purity and titer were assayed by SDS-PAGE and ELISA. Three independent IgG preparations were used for each experiment.

Myelin purification

Myelin was purified from postnatal day 35 C57BL/6 mouse brains (23).

MOG cross-linking

Rat OL cultures were incubated for 30 min at 37°C with the different purified IgGs (100 μg/ml). MOG-anti-MOG complexes were then cross-linked with goat anti-mouse IgG as previously described (24, 25).

Ab binding

To estimate Ab binding ability, live OLs were incubated with 100 μg/ml of the different purified IgGs followed by fluorescently labeled secondary Abs. To examine changes in morphology, mature OLs were stained live with O4 Ab as described (24). The diameter of randomly chosen cells (100 cells/experiment) was determined by using a calibrated microscopic grid.

Cell lystate preparation and detergent extraction

OLs were scraped into 150 mM NaCl, 5 mM EDTA, 25 mM Tris-Cl buffer (pH 7.5) containing 1 mM PMSE, 10 g/ml leupeptin-aprotinin, 50 mM NaF, 10 mM NaPO₄, 1 mM Na β-Vanadate, and 1% Triton X-100, centrifuged to separate them into detergent insoluble pellet and detergent-soluble supernatant fractions, and processed for SDS-PAGE (24).

SDS-PAGE and immunoblot analysis

Samples of purified myelin (10 μg) or equal volumes of soluble and insoluble fractions of detergent extracts were solubilized in 50 mM Tris HCl (pH 6.8), 2% glycerol, 5% SDS, 4 M urea, 0.01% bromophenol blue, and 10 mM DTT for SDS-PAGE (24) and immunoblotted with 2 μg/ml purified IgGs, followed by incubation with HRP-labeled secondary Ab.

Results

GILT−/− mice are relatively resistant to MOG35–55-induced EAE but are more susceptible than WT mice to rat MOG protein-induced EAE

We hypothesized that GILT−/− mice with defective Ag processing would be unable to process MOG protein to MOG35–55 and would thus be resistant to EAE after injection of ratMOG. We also hypothesized that GILT−/− mice immunized with MOG35–55 peptide (Fig. 1), which does not require processing, would be able to respond to the encephalitogenic peptide and might develop EAE. The opposite occurred. GILT−/− mice were nearly completely resistant to EAE after injection of MOG35–55 but were slightly more susceptible than WT (C57BL/6 or GILT+/+ littermates) mice to EAE induced by the extracellular domain of ratMOG (Figs. 1, 2, Table I). After immunization with MOG35–55-fewer GILT−/− mice developed clinical disease, none died, and the mean maximum disease score was lower in GILT−/− mice (0.87) than in WT (3.25) (p < 0.007), as was the disease index (69.4 versus 445.6). Histological analysis of spinal cords at 40 d postimmunization confirmed these observations. As expected from the clinical scores, there was more intense inflammation in...
the spinal cords of WT mice immunized with the peptide than those of GILT−/− mice (data not shown). These data indicate that MOG35–55 is relatively poorly encephalitogenic in GILT−/− mice.

WT mice that had been injected with rratMOG had a disease index of 541.7 and a mean maximum disease score of 3.25, similar to our previous studies (9). In contrast, identically injected GILT−/− mice had a disease index of 774 with a mean maximum disease score of 4.25 (p, 0.022), indicating a somewhat more severe disease. At day 20, there was a striking difference in the histological appearance of the infiltrates (Fig. 3). Those from WT mice (Fig. 3A, 3C) had the previously described typical appearance of an intense mononuclear infiltrate with lymphocytes, macrophages, activated microglia, T cells, and very few B cells (9), whereas the CNS of GILT−/− mice immunized with rratMOG had fewer (Fig. 3B, 3D) and larger infiltrating cells. Most of those cells had the large clock-faced appearance of plasma cells (Fig. 3D), cells rarely found in the CNS of MOG 35–55-immunized WT mice.

Reactivity with Abs to the plasma cell markers CD38 and CD138 (Fig. 3F) confirmed their identity. A measurement of the mean number of infiltrating cells in a 50-μm² section of spinal cord confirmed significantly fewer mononuclear cells (GILT−/− = 0.88 ± 1.1 versus WT = 7 ± 3.32; p < 0.001) and significantly more plasma cells (GILT−/− = 3 ± 1.1 versus WT = 0.44 ± 0.63; p < 0.001) in GILT−/− mice compared with WT mice (Fig. 4). These

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<th>Immunogen</th>
<th>Incidence</th>
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<th>Mean Day of Onset (Range)</th>
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<tr>
<td>Rodent MOG 35–55</td>
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<td>18 (13–24)</td>
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<tr>
<td>Rat MOG protein</td>
<td>8/8</td>
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<td>4/8</td>
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There is a 50% reduction in incidence, 0% mortality, increased time before onset of symptoms, and a significant decrease in mean maximum disease score and clinical disease index in GILT−/− mice immunized with peptide compared with WT. In contrast, GILT−/− mice are equivalent to, or more susceptible than, WT mice to rratMOG-induced EAE.
data suggested that the mechanism of EAE in rratMOG-immunized GILT<sup>−/−</sup> mice might differ from that of WT mice.

**Exogenous MOG<sub>35-55</sub> is immunogenic in GILT<sup>−/−</sup> mice but GILT<sup>−/−</sup> APCs cannot generate MOG<sub>35-55</sub> peptide from rat MOG protein**

We have previously shown that WT C57BL/6 mice immunized with MOG<sub>35-55</sub> develop EAE, and their LN cells proliferate to the peptide (9) in confirmation of data from other investigators (11). We also showed that LN cells from rratMOG-immunized WT mice proliferate in response to that protein and to mouse MOG (9). Even though MOG<sub>35-55</sub> was only weakly encephalitogenic in GILT<sup>−/−</sup> mice, it was still possible that the peptide was immunogenic in these mice. To test this hypothesis, WT and GILT<sup>−/−</sup> mice were immunized with MOG<sub>35-55</sub>, and after 10 d, their spleen cells were re-exposed in vitro to that peptide. As shown in Fig. 5, GILT<sup>−/−</sup> spleen cells (Fig. 5B), like those from WT mice (Fig. 5A), which include T cells and APCs, proliferated under these conditions, suggesting that in GILT<sup>−/−</sup> mice, MOG<sub>35-55</sub> is immunogenic even though in these mice, it is very weakly encephalitogenic. However, in contrast to WT mice, spleen cells from GILT<sup>−/−</sup> mice immunized with rratMOG did not proliferate when cultured with MOG<sub>35-55</sub> in vitro (Fig. 5A, 5B). This suggested that GILT<sup>−/−</sup> APC could not process rratMOG to MOG<sub>35-55</sub>. Evaluating the ability of GILT<sup>−/−</sup> spleen cells to present rratMOG to a WT T cell line specific for MOG<sub>35-55</sub> tested this possibility. Irradiated GILT<sup>−/−</sup> spleen cells were incubated with T cells in the presence of rratMOG. Whereas APCs from WT mice induced a striking proliferative response to Ag (cpm 3546 ± 745; stimulation index = 74), those from GILT<sup>−/−</sup> mice effectcd a response only slightly higher than background (cpm 128 ± 89; stimulation index = 3) (Fig. 6). These data indicate that GILT<sup>−/−</sup> APCs cannot process the exogenous MOG protein to a peptide recognized by T cells specific for MOG<sub>35-55</sub>, whereas they are capable of presenting the processed peptide. These data provide a possible explanation for the inability of MOG<sub>35-55</sub> immunization to induce EAE in these mice because it is likely that they cannot process the endogenous MOG protein to MOG<sub>35-55</sub>, a requirement for recognition by the immunized T cells.

**A switch in the dominant epitope occurs in GILT<sup>−/−</sup> mice immunized with rat MOG protein**

Next, we tested whether T cells from GILT<sup>−/−</sup> mice recognized the same peptides as WT T cells after MOG immunization. In this study, we used mouse peptides because we were particularly interested in the response to the endogenous MOG. As indicated in Fig. 1, rat and mouse peptide 35–55 are identical, with only a total of six residue differences between the ECD of mouse and rat MOG protein. LN cells isolated from WT mice 10 d after immunization with rratMOG proliferated in vitro when exposed to rratMOG and to MOG<sub>35-55</sub>, but not to other mouse MOG peptides (Fig. 7A). In contrast, LN cells isolated from rratMOG-immunized GILT<sup>−/−</sup> mice proliferated to rratMOG as expected, though not when exposed to MOG<sub>35-55</sub> (Fig. 7B). However, they did proliferate variably to several other mouse peptides, including peptides 67–87, 85–105, and 104–117. The response was most marked to peptide 104–117. Thus, there is an epitope switch from the normally encephalitogenic peptide 35–55 to other peptides that are not usually recognized by WT T cells in the context of MOG protein immunization (9, 12). These data suggest that GILT<sup>−/−</sup> mice, even though they cannot process MOG to generate peptide 35–55, can generate and respond to other epitopes outside the internal disulfide bond.

**Rat MOG-immunized GILT<sup>−/−</sup> mice generate pathogenic Abs that transfer EAE**

In our previous studies, we demonstrated that WT mice immunized with recombinant rat or human MOG (rhuMOG) generated Abs that bound MOG. However, only Abs from rhuMOG-immunized mice induced EAE upon transfer to rhuMOG-immunized B cell-deficient μMT mice that do not normally develop EAE. These experiments also indicate that rhuMOG immunization does not generate reactivity to the predominant mouse MOG<sub>35-55</sub> epitope, but that priming of T cells does occur, resulting in opening the blood–brain barrier to the pathogenic Ab. Even though WT mice immunized with MOG<sub>35-55</sub> or rratMOG generate anti-MOG Ab, their sera are nonpathogenic, in that they do not transfer EAE into
primed μMT mice (10). This is because the Abs in these sera only recognize rMOG and not native glycosylated MOG. The interpretation of the previous studies was: if pathogenic CD4 T cells cannot recognize a T cell encephalitogenic epitope, a switch in pathogenic mechanism occurs, resulting in a B cell-dependent, Ab-mediated disease. In this study, we asked whether such a switch in pathogenic mechanism, from a predominant inflammatory T cell-mediated disease to an Ab-mediated disease, occurred in GILT−/− mice immunized with rratMOG. First, we measured the titer of Abs to mouse MOG in sera from rratMOG-immunized WT and GILT−/− mice. Both WT and GILT−/− mice produced high titer anti-mouse MOG Ab after immunization with rratMOG (Fig. 8). Pooled sera from rratMOG-immunized WT and GILT−/− mice each revealed a titer of >1:25,600 when tested against mouse MOG protein. Nonimmune serum was negative for anti-MOG Ab.

We employed the same logic as in our previous experiments (10). That is, we hypothesized that the mechanism of EAE in rratMOG-immunized GILT−/− mice was Ab mediated and that sera from such mice should induce EAE in mice primed in such a way that they would develop only minimal EAE. Thus, we transferred serum from GILT−/− or WT mice immunized with rratMOG to MOG35–55-primed GILT−/− mice (Fig. 9A). Sera from rratMOG-immunized GILT−/− mice transferred EAE to MOG35–55-primed GILT−/− mice, but sera from rratMOG-immunized WT mice did not (Fig. 9B). We also transferred serum from rratMOG-immunized GILT−/− mice to rhuMOG-primed B cell-deficient μMT mice. As reported in our previous studies, sera from WT mice immunized with rratMOG did not transfer EAE to primed μMT mice deficient in B cells (data not shown). However, sera from GILT−/− mice immunized with rratMOG transferred EAE to two out of five rhuMOG-primed μMT mice (Supplemental Table 1). These data, taken together, indicate that immunization with rratMOG induces a pathogenic Ab in GILT−/− mice.

Serum from rat MOG-immunized GILT−/− mice recognizes native MOG

We have published several papers concerning the ability of some Abs to bind to OLs. Anti-MAG Abs bind to OLs, leading to re-partitioning into a detergent insoluble fraction, signaling changes, but no modifications in cell morphology (26). In contrast, we noted properties of pathogenic Abs from WT rhuMOG-immunized mice that were not exhibited by sera from WT mice immunized with rratMOG. These included binding to native glycosylated MOG revealed by Western blots, binding to cell-surface MOG, and after cross-linking, the induction of morphological changes in the OLs and re-partitioning of MOG into lipid rafts as indicated by a switch from a soluble to an insoluble (pellet) form (10). In this study, we evaluated whether sera from GILT−/− mice immunized with

![FIGURE 5.](image1)

**FIGURE 5.** Rat MOG protein does not prime GILT−/− mice for MOG35–55 recall. Spleen cells from WT (A) and GILT−/− (B) mice immunized 10 d previously with either MOG35–55 peptide or rratMOG in CFA were cultured with increasing concentrations of MOG35–55 for 72 h. Total of 1 μCi of [3H]Tdr was added to the culture for the final 24 h prior to harvest. Data are representative of two independent experiments.

![FIGURE 6.](image2)

**FIGURE 6.** GILT−/− APC cannot process rat MOG protein to MOG35–55. Cells from an established rodent MOG35–55-specific T cell line were cultured for 72 h with rratMOG and irradiated GILT+/+ or GILT−/− spleen cell APC. Total of 1 μCi of [3H]Tdr was added to the culture for the final 24 h prior to harvest. Data represent the mean cpm ± SEM (***p = 0.005 versus GILT+/+ APC). Data are representative of two independent experiments.

![FIGURE 7.](image3)

**FIGURE 7.** A switch in predominant epitope occurs in rat MOG protein-immunized GILT−/− LN Cells. WT (A) and GILT−/− (B) mice were immunized with rratMOG as described in Materials and Methods. To determine epitope recognition, 10 d after immunization, LN cells were cultured with rratMOG or a series of overlapping mouse MOG peptides. Data are presented as stimulation indices (stimulated/ unstimulated). For cultures with no added peptide, values were 45 cpm for cells from WT mice and 125 cpm for cells from GILT−/− mice. Data are representative of two independent experiments.
FIGURE 8. GILT\textsuperscript{−/−} and WT mice produce high-titer anti-mouse MOG Ab after immunization with rat MOG protein. Pooled sera from WT or GILT\textsuperscript{−/−} mice 14 d after immunization with ratMOG were analyzed by ELISA for total IgG and maintained a titer of Ab when diluted >1:25,000. No MOG-specific Abs were detected in samples from unimmunized mice.

rratMOG also possessed these properties. First, we evaluated IgG in Western blots to determine if sera from rratMOG-immunized mice bound to native mouse myelin. As indicated in Fig. 10A, IgG from immunized WT (B6 or GILT\textsuperscript{+/−} eighth backcross littermates) did not bind to native mouse myelin (Fig. 10Ai, ii). However, IgG from WT mice immunized with rhuMOG did bind to this native MOG (Fig. 10Aiiv), as expected from our previous studies (10). Serum from rratMOG-immunized GILT\textsuperscript{−/−} mice also bound native MOG (Fig. 10Aiii) in contrast to the results with sera from WT mice. We next tested whether these Abs could bind to OLs. Only IgG reactive to native mouse MOG bound to the cell surface of OLs. These binding sera were from WT mice immunized with rhuMOG (Fig. 10Biv) or from GILT\textsuperscript{−/−} mice immunized with rratMOG (Fig. 10Biii). As in our previous studies, when cross-linked with Ab to mouse IgG, these sera induced a switch in the location of MOG from the soluble to the pellet form (Fig. 11A), (10). Furthermore, changes in the morphology of OLs were apparent with sera from GILT\textsuperscript{−/−} mice immunized with rratMOG (Fig. 11Biii). However, sera from rratMOG-immunized WT mice did not bind native MOG (Fig. 10Ai, ii), bind OLs (Fig. 10Bi, ii), induce cross-linking and repartitioning into lipid rafts (Fig. 11A), or effect morphological changes in OLs (Fig. 11Bi, ii), all of which had been possible with sera from WT mice immunized with human MOG (10). These data, taken together, indicate that rratMOG immunization of GILT\textsuperscript{−/−} mice, but not WT mice, generates Abs that have properties similar to those from WT mice immunized with human MOG, including transfer of EAE, ability to bind to glycosylated MOG, ability to bind to the surface of OLs, and, when cross-linked, induce changes in partitioning of MOG and shape changes in OLs.

Discussion

We show in this study that a defect in a processing enzyme can result in either a diminished clinical disease after immunization with a peptide or a switch in pathogenic mechanism to an Ab-mediated disease after immunization with a protein. The data in this paper address two basic issues in autoimmunity: 1) What are the Ag processing requirements for an endogenous Ag when immunizing with an exogenous Ag? and 2) What are the consequences of immunization with an exogenous Ag that cannot be processed to an encephalitogenic peptide? We have addressed these issues experimentally and shown that 1) the endogenous Ag most likely also needs to be processed to yield an encephalitogenic peptide and 2) an individual is capable of recognizing new peptides within the protein and switching to an entirely different pathogenic mechanism. We believe that these results have profound implications for understanding any autoimmune disease in the highly outbred human population.

We have investigated the role of GILT in the context of exogenously administered and endogenously processed Ags and analyzed an autoimmune disease that requires recognition of an endogenous CNS Ag. The data indicating that immunization of GILT\textsuperscript{−/−} mice with MOG\textsubscript{35−55} does not induce a robust EAE are consistent with the concept that processing of the endogenous MOG protein is defective in these mice that are unable to reduce thiol bonds. Even though the original experiments defining the role of GILT in processing and presentation of disulphide linked proteins were carried out with exogenously administered Ags than had no endogenous counterpart (16), later experiments did evaluate the role of GILT in presentation of endogenous Ags. Our data confirm the observation that the provision of GILT to melanoma cells allowed them to present endogenous Ags (27), indicating that GILT is not only involved with processing of exogenous proteins.

Our studies revealed that MOG\textsubscript{35−55} is immunogenic but not encephalitogenic in GILT\textsuperscript{−/−} mice. It is likely that the initial presentation of the peptide occurs through binding to MHC class II on an APC in the LN, likely a dendritic cell (DC) or macrophage, and would not require further processing. However, administration of the peptide in a normally encephalitogenic form

FIGURE 9. Serum from rat MOG protein-immunized GILT\textsuperscript{−/−} mice transfers EAE to GILT\textsuperscript{−/−} mice. (A) Diagram of protocol for anti-rat MOG serum preparation in GILT\textsuperscript{−/−} and WT mice and for peptide priming and serum transfer to GILT\textsuperscript{−/−} mice. (B) Average daily clinical scores for GILT\textsuperscript{−/−} mice that received four injections of 150 µl anti-rat MOG serum (GILT\textsuperscript{−/−} antiserum [■], nonimmune serum [●], or WT antiserum [○]) at 3-d intervals (days 0, 3, 6, and 9), MOG\textsubscript{35−55} in CFA (day 0), and two injections of PTX (days 0 and 2). Clinical signs were observed for 40 d. Mean maximum score (p = 0.001). These data are the average of two independent experiments.
Another difference was the loss of recognition of the dominant mice, serving as a site of B cell activation in germinal centers. that the CNS is an ectopic or tertiary lymphoid organ (30) in these cells. The function of these cells has not been completely evaluated, but it is possible that the CNS is an ectopic or tertiary lymphoid organ (30) in these mice. These differences included the cellular composition of the CNS infiltrates, epitope recognition, and pathogenic mechanism. The cellular composition in the CNS of GILT−/− mice was different from that seen after rratMOG or MOG35–55 immunization of WT mice. These differences were noted that the response to a cysteine-containing epitope by GILT−/− mice, there was no sign of CNS infiltration, suggesting the necessity for processing of the endogenous MOG protein, likely in the CNS.

In contrast to the results obtained with MOG35–55 immunization, GILT−/− mice immunized with rat MOG exhibited a robust EAE. However, there were three important differences in this disease from that seen after rratMOG or MOG35–55 immunization of WT mice. These differences included the cellular composition of the CNS infiltrates, epitope recognition, and pathogenic mechanism. The cellular composition in the CNS of GILT−/−, but not WT, mice included many cells likely to be plasma cells. The function of these cells has not been completely evaluated, but it is possible that the CNS is an ectopic or tertiary lymphoid organ (30) in these mice, serving as a site of B cell activation in germinal centers. Another difference was the loss of recognition of the dominant MOG35–55 and the generation of recognition of other epitopes. One of these (104–117) is completely outside the region defined by the disulphide bond, another (85–105) encompasses the bond, and the third (67–87) is actually within the area. It is possible that fine structure mapping of the 85–105 epitope will reveal that the class II binding sequences are actually outside the disulphide bond. Additional investigation is necessary to determine how the epitope defined by 67–87 is available in the absence of disruption of the disulphide bond. It is possible that the three amino acid differences between mouse and rat MOG in this region influence this event. Thus, the ability to respond, or not, to these new epitopes is neither negatively nor positively correlated with amino acid differences between mouse and rat peptides. It should be noted that the response to a cysteine-containing epitope by GILT−/− T cells is not unprecedented. Two cysteine-containing epitopes of HEL were recognized by T cells from GILT−/− mice, indicating that some epitopes exist that do not require reduction and refolding before proteolytic processing (16). Our data are consistent with the previous demonstration that the CD4+ T cell response to the immunodominant TRP-1 epitope is diminished when GILT−/− DCs are used as APC to present this endogenous Ag (27). Mass spectrometry analysis of MHC class II associated self-peptides from GILT−/− and WT splenocytes suggested that self-peptides are more abundantly expressed by GILT−/− splenocytes and that the diversity of the MHC class II-bound self-peptides is moderately affected by the absence of GILT (31). We assume that...
that case and the present situation, the endogenous MOG35–55 rhuMOG with its proline rather than serine at position 42 (10). In similar phenomenon is observed in WT mice immunized with the initial presentation of both MOG35–55 and rratMOG normally the myelin-axolemmal complex: biochemical dissection of its many cellular processes. J. Biol. Chem. 276: 19775–19783.

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