Targeting ganglioside epitope 3G11 on the surface of CD4+ T cells suppresses EAE by altering the Treg/T$_{h}$17 cell balance

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Abstract

Loss of expression of the 3G11 epitope, present on disialoceramide that is predominantly found on CD4+ T cells, has been associated with a regulatory T cell (Treg) phenotype and tolerance induction in experimental autoimmune encephalomyelitis (EAE). Here we report that treatment with anti-3G11 mAb shifts the immune response from pro-inflammatory to tolerogenic and suppresses both chronic-progressive and relapsing-remitting EAE. This therapeutic effect can be achieved at different stages of EAE. Treatment with anti-3G11 mAb increased the proportion of Foxp3+CD25+CD4+ Tregs and IL-10 production while inhibiting production of pro-inflammatory cytokines and responsiveness to IL-2 and decreasing the proportion of Th17 cells. The effect of anti-3G11 mAb was diminished in IL-10-/- mice, indicating that this cytokine mediates some of its effects. As 3G11 belongs to the ganglioside family, which is expressed on the surface of both murine and human CD4+ T cells, targeting this class of molecules may provide a novel approach for treating autoimmune diseases.

Keywords: apoptosis, EAE, 3G11, T$_{h}$17, Treg

Introduction

Cell surface glycosphingolipids may serve as differentiation or activation markers for leukocytes (1). mAb SM3G11 (IgM) recognizes a sialylated carbohydrate epitope, 3G11, present on disialoganglioside GD1c, which is predominantly expressed on CD4+ T cells (2, 3). The 3G11 epitope has been used as a marker to distinguish naive and memory T cells, as 3G11 is irreversibly down-regulated upon activation of CD4+ T cells (4, 5). The biological function of this molecule is not known. 3G11+ T cells stimulated by mitogen produce a large amount of IL-2, while the 3G11- population does not, implying that this molecule may be involved in T cell activation and proliferation (4, 5).

CD4+ regulatory T cells (Tregs) inhibit immunopathology and prevent autoimmune disease. Bluestone and Abbas (6) proposed the existence of two subsets of Treg cells, which differ in origin, specificity and effector mechanisms. ‘Natural’ Treg cells develop during the normal process of T cell maturation in the thymus; they are antigen specific and survive as a long-lived population in the periphery. The second subset is ‘adaptive’ Treg cells that develop from mature T cells in the periphery under suboptimal activating and/or co-stimulating conditions. Natural and adaptive subsets of Treg cells differ in their mechanism of action. Adaptive Treg cells mediate their inhibitory activities by producing immunosuppressive cytokines, such as transforming growth factor-β (7) and IL-10 (8). However, the adaptive Treg cell subset, although it suppresses in a cytokine-dependent manner, might still require direct cell–cell contact to initiate the suppressive cascade. In contrast, natural Treg cells, at least in vitro, function by a contact-dependent and cytokine-independent mechanism, which presumably involves direct interactions with T cells or antigen-presenting cells (APC) (9).

T$_{h}$17 cells have been implicated in the pathogenesis of experimental autoimmune encephalomyelitis (EAE) and they also appear to be highly relevant to human multiple sclerosis (MS). Kebir et al. (10) showed the presence of memory T cells that produced IL-17 and IL-22 within MS lesions and also showed that T$_{h}$17 cells were neurotoxic in vitro. Furthermore, Tzartos et al. (11) also showed a high proportion of T$_{h}$17 cells in active MS lesions.

The balance between Treg and effector T cells (Teff) is crucial to the maintenance of immune tolerance and prevention of autoimmune diseases (12). This has clearly been shown in EAE (13). Recently, we have found that tolerance in EAE mice induced by intravenous (i.v.) injection of myelin peptide is associated with the loss of the 3G11 molecule on the surface of...
CD4+ T cells. These 3G11+CD4+ T cells produce low levels of IL-2 and high levels of IL-10 and suppress MBP-reactive T cell responses. Furthermore, injection of these T cells into immunized mice significantly inhibited clinical EAE (14). Taken together, these data suggest that 3G11+ T cells may have a regulatory function, while 3G11+ T cells act as Teff.

Here we report that targeting 3G11 epitope during the priming phase of EAE induction suppresses disease and that anti-3G11 mAb injected during the first attack in the relapsing-remitting EAE (RR-EAE) model suppresses relapse. Mechanistically, anti-3G11 mAb reduces the number of CD4+ T cells and increases the proportion of Treg cells in the spleen. In agreement with this, targeting 3G11 suppresses antigen-specific immune responses and promotes IL-10 production. The suppressive effect of anti-3G11 mAb in EAE is mediated by IL-10. These findings show that targeting specific glycolipids on the surface of T cells is a potential therapeutic approach for treatment of autoimmune disorders.

Materials and methods

Mice and reagents

Female C57BL/6, B6129SF2/J and IL-10−/− mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Experimental procedures were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University. Monoclonal 3G11 IgM antibody was prepared from supernatants of hybridoma SM3G11 (gift of Dr M. Greene, University of Pennsylvania) as described (15). SM3G11 hybridoma cells were grown in protein-free hybridoma medium (Invitrogen). Supernatant was centrifuged and filtered through a 0.22-μm filter to remove cells and debris. 3G11 mAb was purified by chromatography over Sephadex G200 HPLC column (Pharmacia Biotech) and then concentrated by centrifugation over 100 kD cutoff membrane (Millipore, Billerica, MA, USA). The final mAb concentration was measured by capture ELISA using anti-IgM antibodies purchased from Jackson ImmunoResearch. IgM used for treatment of control groups of mice was purchased from Jackson ImmunoResearch.

Induction of chronic-progressive EAE and RR-EAE

To induce chronic-progressive EAE, female C57BL/6 mice, 8–10 weeks of age, were immunized with 100 μg MOG35–55 emulsified in CFA. Pertussis toxin (200 ng per mouse per injection) (List Biological, Campbell, CA, USA) was given intra-peritoneally at the time of immunization and 48 h later. To induce RR-EAE, female B6129SF2/J mice were immunized with MOG35–55 + CFA in the same way as C57BL/6 mice (16). A relapse was defined as an increase in at least one clinical grade sustained for at least two consecutive days after animals had previously improved at least a full clinical grade and stabilized (17).

Flow cytometry

PE-labeled anti-CD44 mAb, PerCP-cy5.5-labeled anti-CD4 mAb, PerCP-cy5.5-labeled anti-CD25 mAb, APC-labeled anti-CD4 mAb, APC-labeled anti-CD62L mAb and APC-cy7-labeled anti-CD8 mAb were purchased from BD Bioscience. FITC-labeled rat anti-mouse-IgM mAb and PE-labeled anti-Foxp3 mAb were purchased from eBioscience. For immunostaining, mononuclear cells (MNCs) were re-suspended in the staining buffer (PBS, 1% FCS and 0.02% NaN3) and incubated with antibody for 30 min at 4°C. For 3G11 staining, splenocytes were incubated with purified anti-3G11 antibody (IgM), washed and then incubated with rat anti-mouse-IgM antibody. Intracellular staining was performed for detection of cytokines and Foxp3. Cells that had been stained for surface markers were fixed and permeabilized using the Cytofix/Cytoperm system (BD Bioscience) for cytokine or with a Foxp3 staining kit (eBioscience) for Foxp3. After permeabilization, cells were stained with mAb for 30 min at 4°C.

Histopathology

Mice were sacrificed and spinal cords were harvested at day 22 after immunization. Five-micron sections were stained with H&E or Luxol fast blue (myelin stain). Slides were assessed in a blinded fashion for inflammation and demyelination (18).

Cytokine production

Supernatants from the cultures were harvested and levels of IL-2, IL-10, IL-17, IFN-γ and tumor necrosis factor (TNF)-α were determined by ELISA assays according to the manufacturer’s instructions (BD PharMingen).

Apoptosis assay

3G11 mAb (1.0 mg per mice per day) i.v. was injected into C57BL/6 mice for three consecutive days. The same volumes of IgM were injected into mice in parallel as control (n = 5 in each group). Mice were sacrificed 3 days after the last injection. Splenocytes and thymocytes were harvested. Cells were labeled for CD4, CD8 and Annexin-V and analyzed by flow cytometry.

Statistics

The Mann–Whitney U-test was used for comparison of average clinical scores and the Student’s t-test for other parameters among different groups. All tests were two sided.

Results

The majority of Treg cells lack 3G11 expression

(3G11 Foxp3+CD4+)

To determine whether absence of the 3G11 epitope on the surface of CD4+ T cells identifies a regulatory phenotype,
we correlated expression of 3G11 and Foxp3 (19). In spleen of naive mice, among 3G11+CD4+ T cells, only 2.5 ± 0.7% cells expressed Foxp3, while 34.3 ± 1.6% of 3G11+CD4+ T cells were Foxp3+ (Fig. 1A and B). When analyzed from a different angle, only 21.7 ± 0.8% of all Foxp3+CD4+ T cells were 3G11+, while 80.2 ± 4.3% of Foxp3+CD4+ T cells were 3G11+ (Fig. 1C). This result demonstrates that 3G11+ T cells are highly enriched in Treg cells and that the lack of 3G11 expression characterizes the majority (~80%) of Treg cells. At the same time, the vast majority of 3G11+ cells (>95%) do not express Foxp3 and can be considered to be a Teff population.

3G11 mAb suppresses EAE when given early but not after disease onset

To test in vivo effects of 3G11 targeting, we injected anti-3G11 mAb into C57BL/6 mice immunized with MOG35–55 peptide for EAE induction. Early mAb injection delayed EAE onset and significantly reduced clinical disease (Fig. 2A); however, injection at the peak of EAE did not change its severity (Fig. 2D). As shown in Fig. 2(B), clinical disease was significantly suppressed when anti-3G11 mAb was administered every 3 days during the experiment (mean clinical score 0.5 in anti-3G11 group versus 3.0 in control groups). When anti-3G11 mAb was injected at EAE onset [days 11–15 post-immunization (p.i.)], a more pronounced recovery after the peak of disease was noted compared with control mice (Fig. 2C, P < 0.05). Consistent with clinical findings, histopathological studies showed significantly less inflammatory infiltration and demyelination in spinal cords of anti-3G11-treated animals than in the control group (Fig. 3). These data demonstrate that targeting 3G11 early in immune response suppresses its full development, while later in the response anti-3G11 mAb has no effect.

We then determined the effect of anti-3G11 treatment on RR-EAE in B6129SF2/J mice [F1(SJLxC57BL/6)] immunized with MOG35–55. Anti-3G11 mAb injected following the first peak of clinical disease significantly suppressed relapses immediately after the injection and the suppressive effect lasted until mice were sacrificed 3 weeks later (Fig. 2E).

Anti-3G11 mAb increases proportion of Treg cells

To determine whether anti-3G11 mAb perturbed a normal composition of immune cells, C57BL/6 mice were immunized for EAE induction and anti-3G11 mAb was injected into mice every third day starting from day 3 before immunization. Mice were sacrificed on day 17 p.i. when clinical EAE reached its peak. Splenocytes were isolated and percentage of CD4+ and CD8+ T cells was determined by flow cytometry. Treatment of EAE mice with anti-3G11 mAb resulted in a reduction of ~33% in the number of CD4+ cells, while the percentage of CD8+ cells remained relatively unaffected by the mAb treatment (Fig. 4A). Mice treated with anti-3G11 mAb had an increased proportion of 3G11+CD44highCD4+ and

![Fig. 1. Correlation between 3G11 and Foxp3 expression in CD4+ T cells. To determine the correlation between 3G11 and Foxp3 expression, splenocytes of naive C57BL/6 mice were stained for 3G11, Foxp3 and CD4. (A) CD4+ T cells were gated and the percentage of Foxp3+ versus 3G11+ cells was determined. (B) Percentage of Foxp3+ cells in 3G11+CD4+ and 3G11+CD4+ T cells. (C) Percentage of 3G11+ cells in Foxp3+CD4+ and Foxp3+CD4+ T cells. Columns refer to the mean values and bars to standard deviation (n = 5 each group). ***P < 0.001. Data are representative of three experiments.](image-url)
Anti-3G11 antibody suppresses EAE

3G11+CD62L-CD4+ T cells, which correspond to Teff/memory T cells (Supplementary Figure 1, available at International Immunology Online). To analyze whether anti-3G11 treatment impacts cytokine secretion by CD4+ T cells, intracellular staining was performed. Significantly, fewer IL-2- and TNF-α-producing cells were observed in both 3G11+CD4+ and

Fig. 2. Anti-3G11 mAb suppresses both chronic EAE and ongoing RR-EAE. Chronic EAE was induced by MOG35–55 + CFA and pertussis toxin immunization of C57BL/6 mice. 3G11 mAb (1.0 mg per mouse per day) was injected i.v. into mice on days −3, −2 and −1 before immunization (A); every 3 days starting from day −3 before immunization (B); daily on days 11–15 p.i. (C) and daily on days 16–21 p.i. (D). The same volumes of PBS and IgM (1.0 mg per mouse per day) were injected into control mice in parallel (n = 5 in each group). Data were expressed as the mean clinical score on each day. (E) RR-EAE was induced in B6129SF2/J mice by MOG35–55 immunization. Anti-3G11 mAb (1.0 mg per mouse per day) was injected i.v. into mice at days 22–26 p.i. The same doses of IgM were injected into control mice (n = 10 in each group). Data were expressed as the mean clinical score on each day. *P < 0.05, **P < 0.01. Data are representative of three experiments.

Fig. 3. Central nervous system pathology of EAE mice treated with anti-3G11 mAb. Mice described in Fig. 2(B) were sacrificed and spinal cords were collected on day 21 when clinical EAE was at its peak. (A) Sections of spinal cords, 5 μm thick, were stained with H&E or Luxol fast blue (myelin stain). (B) Mean scores of inflammation and demyelination ± SD in IgM i.v. and anti-3G11 i.v. groups. **P < 0.01. One representative experiment of three is shown.
Fig. 4. Correlation of 3G11, Foxp3 and CD25 expression by CD4+ T cells. (A) EAE in C57BL/6 mice was induced with MOG35–55 + CFA and pertussis toxin. Anti-3G11 mAb (1.0 mg per mouse per day) was injected i.v. into five mice every third day starting from day −3 before immunization. The same amount of control IgM was injected into mice in parallel as control (n = 5 in each group). Mice were sacrificed on day 17 p.i. when clinical EAE was at its peak. Splenocytes were isolated and percentage of CD4+ and CD8+ T cells was determined by flow cytometry. Columns refer to mean values and bars to standard deviation (SD) (n = 5 each group). *P < 0.05, **P < 0.01. Data are representative of two experiments. (B) The effect of anti-3G11 mAb on IL-2 and TNF-α production was assayed by intracellular staining of splenic CD4+ T cells derived from mice represented in Fig. 2(B). Columns refer to the mean values and bars to SD (n = 5 each group). *P < 0.05, **P < 0.01. Data are representative of three experiments. (C) Mice from experiments represented in Fig. 2(B) were sacrificed on day 21 when clinical EAE was at its peak. Splenocytes were isolated and expression of 3G11, Foxp3 and CD25 on CD4+ cells was determined by flow cytometry. Columns refer to the mean values and bars to SD (n = 5 each group). **P < 0.01. Data are representative of three experiments. (D) Splenocytes were collected from naive C57BL/6 mice and CD4+ T cells were purified with magnetic microbeads. CD4+ T cells were cultured with or without anti-3G11 mAb or anti-IL-2R (anti-yc) antibody at different doses of IL-2 (0, 10, 20 and 40 ng/ml). [3H]thymidine incorporation was measured by a scintillation counter. ***P < 0.01. Data are representative of three experiments.
3G11+CD4 populations of anti-3G11-treated mice compared with IgM-treated control mice (Fig. 4B).

Mice with EAE represented in Fig. 2(B) were sacrificed on day 21 when clinical EAE was at its peak. Analysis of splenic cells showed that the percentage of 3G11+ cells among CD4+ T cells was significantly lower after anti-3G11 mAb injection compared with the IgM control group (43.8 versus 70.8%), with significantly higher percentages of Foxp3+CD4+ and CD25+CD4+ T cells (Fig. 4C). The main reduction of 3G11+CD4+ T cells occurred in the 3G11+CD25− population, which was presumably Teff cells, while the main increase in CD25 expression occurred in the 3G11−CD4+ population. Furthermore, the highest percentage of Foxp3 expression was seen in the 3G11−CD4+ population (8.7% in IgM versus 20.3% in anti-3G11 group), while the 3G11+CD4+ population had the lowest level of Foxp3+ cells (Fig. 1C).

We directly compared the effect of anti-3G11 and anti-IL-2R antibodies as a control. Splenic CD4+ T cells of naive C57BL/6 mice were purified and cultured with anti-3G11 mAb or anti-IL-2R mAb, and their proliferative responses to increasing concentrations of IL-2 were determined. Both antibodies significantly blocked proliferative responses of CD4+ T cells to IL-2 (Fig. 4D).

Anti-3G11 mAb suppresses immune responses, promotes IL-10 production and inhibits EAE in an IL-10-dependent manner

To examine the effect of targeting the 3G11 epitope on autoantigen-induced immune responses, splenocytes derived from mice with EAE at its peak were stimulated with MOG35–55. Antigen-specific proliferative responses were significantly suppressed in anti-3G11-treated mice (Fig. 5).

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**Fig. 5.** Autoantigen recall responses after anti-3G11 mAb treatment. Mice from the experiment represented in Fig. 2(B) were sacrificed and splenocytes were isolated on day 21 when clinical EAE was at its peak. Cells were cultured for 72 h with or without added MOG35–55 (20 μg ml−1). After 60 h of incubation, cells were pulsed for 12 h with 1 μCi of [3H]thymidine. Thymidine incorporation was measured by a scintillation counter. Supernatants were collected and cytokines levels were determined in triplicate by ELISA. *P < 0.05, **P < 0.01. NS: non-stimulated. Data are representative of two experiments.
Splenocytes of 3G11-treated mice produced lower levels of T1 cytokines IL-2, TNF-α and in particular IFN-γ, as well as lower levels of T17 cytokines IL-17 and IL-21 (20, 21). In contrast, a significantly higher expression of IL-10 was observed in anti-3G11-treated mice (Fig. 5). In RR-EAE, anti-3G11 treatment suppressed responses not only against immunizing peptide MOG35-55 but also against other myelin antigens (MBP1-1 and PLP139-151) known to be involved in epitope spreading (22) (data not shown).

To examine the role of IL-10 in anti-3G11-induced EAE suppression, we injected anti-3G11 mAb into IL-10-/- mice immunized for EAE induction. In contrast to wild-type mice, anti-3G11 mAb had no effect on EAE in IL-10-/- mice (Fig. 6), despite a slight reduction in IL-17 production (data not shown).

Anti-3G11 mAb induces apoptosis of CD4+ T cells

Suppression of EAE can be induced via different mechanisms including clonal deletion, anergy and active suppression of T cells (23, 24). We have previously shown that 3G11+ cells exhibit anergic properties (14). Anti-3G11 mAb was injected into mice for three consecutive days, and 3 days later, Annexin-V expression was determined on splenocytes and thymocytes. Anti-3G11 treatment resulted in more apoptotic cells among CD4+ T cells of the spleen, but not the thymus, than treatment with control antibody (21.0 versus 9.7%; Fig. 7). Anti-3G11 mAb enhanced apoptosis of CD4+, but not CD8+ T cells, most likely because 3G11 is not expressed on CD8+ T cells (2). These data suggest that apoptosis contributes to reduction of CD4+ T cells among splenocytes of anti-3G11 mAb-treated mice.

Discussion

Targeting disialoganglioside GD1c, present on the cell surface of naive CD4+ T cells, with anti-3G11 mAb reduces the number of these cells at the periphery and increases the proportion of Treg cells. As a consequence, CD4+ T cell immune responses are suppressed, as evidenced by ameliorated EAE clinical course and milder pathological changes in the central nervous system, reduced magnitude of antigen-specific responses and lower production of pro-inflammatory cytokines. This is accompanied by greater IL-10 production, which is necessary for the suppression of EAE by anti-3G11 mAb.

Strong suppressive effects of IL-10 on EAE are well documented (25-28). IL-10 is broadly expressed by many cells of the adaptive immune system, including T1, T12, T9, T17 and Treg cells (29-32) as well as by cells of the innate immune system, including DCs, and macrophages. By acting on DCs and macrophages, IL-10 limits their innate effector functions and inhibits the development of T1 responses (29). However, IL-10 enhances the differentiation of IL-10-secreting Treg cells, thus providing a positive regulatory loop for its induction (33, 34). Our data indicate that anti-3G11 mAb amplifies this regulatory loop since treatment with the mAb increases both IL-10 production and proportion of Treg cells and suppresses EAE in an IL-10-dependent manner. The exact mechanisms that lead to promotion of IL-10 production by anti-3G11 mAb remain to be elucidated, but apoptotic CD4+ T cells might play a role. Since anti-3G11 mAb increases apoptosis of CD4+ cells, there is a possibility that APCs acquire tolerogenic phenotype due to phagocytosis of a large number of apoptotic cells as this process results in immunoregulation of APCs to promote self-tolerance (35-39). In the non-obese diabetic mouse model of autoimmune diabetes, induction of limited apoptosis of pancreatic cells prevents progression of the diabetogenic response, which correlates with the establishment of tolerogenic DCs and subsequent induction of Treg cells (40). The presence of apoptotic cells during monocyte activation increases their secretion of IL-10 and decreases secretion of pro-inflammatory cytokines TNF-α, IL-1 and IL-12 (39).

These findings support the possibility that targeting the 3G11 epitope promotes apoptosis on CD4+ T cells, which in turn induces tolerogenic APCs leading to diminished T1, but increased Treg responses and IL-10 production.

Injection of anti-3G11 mAb in mice immunized for EAE induction resulted in a reduction of ~33% in the total number of CD4+ cells among splenocytes. It is likely that apoptosis induced by anti-3G11 mAb is an important underlying reason for the reduced numbers of CD4+ T cells. However, the possibility that mechanisms other than apoptosis contribute to the reduction of CD4+ T cell numbers, such as direct inhibition of cell proliferation by anti-3G11 mAb and anergy induction, cannot be excluded. An intriguing possibility is that the observed blockade of proliferation by anti-3G11 mAb, when CD4+ T cells are stimulated with IL-2, indicates a molecular mechanism by which cells are rendered either apoptotic or anergic.

We have demonstrated previously that the lack of 3G11 expression (3G11-CD4+ phenotype) is strongly associated with the regulatory function of CD4+ T cells (14). However, the 3G11- cells that we have characterized, which were generated by i.v. tolerance and also naturally occurring Foxp3+ Treg cells, have not been analyzed separately in this
model. Here we show that the Foxp3+ Treg cell population is predominantly 3G11+/C0, while Foxp3−3G11+ cells represent a minority of Treg cells. This is consistent with our previous finding that 3G11+/C0 cells exhibit strong suppressive function both in vitro and in vivo (14), which is not surprising given that the 3G11− fraction of CD4+ cells contains a vast majority of Foxp3+ Treg cells.

In addition to reducing the total number of CD4+ T cells, targeting the 3G11 epitope disturbs the normal composition of this cell population. There was a pronounced loss of 3G11− cells (>40% reduction), while, as a consequence, the proportion of 3G11+/C0 cells increased. It is likely that other changes in composition of the CD4+ population, such as increase in percentages of Foxp3+ Treg, CD44high and CD62L− cells, are largely a consequence of diminished size of the 3G11+ sub-population.

Injection of anti-3G11 mAb into mice immunized for chronic EAE induction suppressed the disease when treatment was initiated close to the time of immunization. Injection of the mAb at disease onset or peak had no, or only a modest, effect on its progression. This is consistent with the lack of 3G11 expression on activated T cells (5) that are prevalent in the ongoing immune response. Thus, in order for anti-3G11 mAb to suppress development of the immune response, it seems necessary that the pool of antigen-specific cells be exposed to the mAb before encountering an antigen. When naive CD4+ T cells are activated by their cognate antigen, they down-regulate expression of the 3G11 epitope and become resistant to mAb action. Therefore, the ensuing immune response develops in a largely unaffected manner if anti-3G11 mAb is injected after the priming phase of an immune response has already begun.

Interestingly, treatment with anti-3G11 mAb that preceded immunization has a lasting effect throughout the disease. One explanation for extended suppressive effect of the mAb is that repopulation of the CD4+ T cell pool, after being anergized/depleted by the mAb, happens at too slow a pace to provide sufficient naive antigen-specific precursors for development of a normal immune response. Another possibility is that anti-3G11 treatment induces Treg cells, which continue to suppress development of the immune response long after the mAb has been catabolized. This possibility is strengthened by elevated IL-10 production by splenocytes from anti-3G11 mAb-treated mice. In addition to a greater percentage of Foxp3+ Treg cells in mice treated with anti-3G11 mAb, there is the possibility that antibody treatment generated Foxp3+ adaptive (inducible) Treg cells, also known as T regulatory type 1 (Tr1) cells. Although Foxp3+ Treg cells have a dominant function in active immune suppression and the maintenance of immune homeostasis (41), other Tregs such as Tr1 cells also contribute substantially to active suppression in the periphery (42, 43). Tr1 cells produce mainly IL-10 and are efficient regulators of inflammation and autoimmunity (44). The inability of anti-3G11 mAb to completely abrogate new immune responses can be either due to its limited efficacy in suppressing activation of naive T cells or, potentially, because, even at the beginning of a new immune response, a portion of T cells is recruited from the pool of pre-activated 3G11− T cells, which are thus not a target of the mAb.

Fig. 7. Anti-3G11 antibody induces apoptosis of CD4+ T cells in the periphery. 3G11 mAb (1.0 mg per mouse per day) was injected into mice for three consecutive days. The same quantities of IgM were injected into control mice in parallel (n = 5 in each group). Mice were sacrificed 3 days after the last injection. Splenocytes and thymocytes were harvested. Cells were labeled for CD4 and CD8. (A) Live CD4+ and CD8+ T cells from spleens were gated and percentages of Annexin-V are shown. (B) Percentage of Annexin-V+ cells in CD4+ and CD8+ T cells from spleen. (C) Percentage of Annexin-V+ cells in CD4+, CD8+ and CD4+CD8+ T cells from thymus. Columns refer to the mean values and bars to standard deviation (n = 5 each group). **P < 0.01. Data are representative of three experiments.
In contrast to the model of chronic EAE, in the RR-EAE model, late treatment, during remission, prevented relapses and gradually led to almost complete recovery from disease, indicating a difference in mechanisms that underlie chronicity and relapse of EAE. Anti-3G11 mAb suppresses chronic EAE most effectively when administered early during the priming phase of encephalitogenic response. A similar suppressive effect seen in the case of relapse suggests that newly recruited myelinspecific T cells cause relapses, which is consistent with the epitope spreading view of EAE relapse (45).

Although there have not yet been reports that anti-3G11 mAb recognizes targets on human CD4+ T cells, structural studies of the 3G11 epitope showed its close similarity to GD1c(NeuGc,NeuGc), an important component of ganglioside on the surface of human CD4+ T cells. Dittrich et al. (2) reported that antigen recognized by anti-3G11 mAb was isolated from mouse thymoma and characterized as GD1c, which may act as a functional molecule critical to the differentiation of thymocytes to a particular subset of T1 cells (46). GD1c cooperates with CD3 and CD4 in T cell activation (47, 48). These results, combined with our current observation, provide a unique opportunity to explore 3G11-like molecules as a new target on human CD4+ T cells for therapy in autoimmune diseases.

Supplementary data
Supplementary data are available at International Immunology Online.

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