Altered Differentiation, Diminished Pathogenicity, and Regulatory Activity of Myelin-Specific T Cells Expressing an Enhanced Affinity TCR

Rajshkekar Alli, Phuong Nguyen and Terrence L. Geiger

*J Immunol* 2011; 187:5521-5531; Prepublished online 24 October 2011; doi: 10.4049/jimmunol.1102202

http://www.jimmunol.org/content/187/11/5521

Supplementary Material

http://www.jimmunol.org/content/suppl/2011/10/24/jimmunol.1102202.DC1

References

This article cites 60 articles, 30 of which you can access for free at:

http://www.jimmunol.org/content/187/11/5521.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Altered Differentiation, Diminished Pathogenicity, and Regulatory Activity of Myelin-Specific T CellsExpressing an Enhanced Affinity TCR

Rajeshkhar Alli, Phuong Nguyen, and Terrence L. Geiger

Whereas increased affinity enhances T cell competitiveness after immunization, the role of affinity in modulating the pathogenicity of self-reactive T cells is less established. To assess this, we generated two myelin-specific, class II MHC-restricted TCR that differ only in a buried hydroxymethyl that forms a common TCR β-chain V region variant. The variation, predicted to increase TCR stability, resulted in a ∼3log10 difference in TCR sensitivity with preserved fine specificity. The high-affinity TCR markedly diminished T cell pathogenicity; T cells were not deleted, did not upregulate Foxp3, and barring disease induction were predominantly naive. However, high-affinity CD4+ T cells showed an altered cytokine profile characterized by the production of protective cytokines prior to experimental allergic encephalomyelitis induction and decreased effector cytokines after. Further, the high-affinity TCR promoted the development of CD4+CD8+ and CD8+ T cells that possessed low intrinsic pathogenicity, were protective even in small numbers when transferred into wild-type mice and in mixed chimeras, and outcompete CD4+ T cells during disease development. Therefore, TCR affinities exceeding an upper affinity threshold may impede the development of autoimmunity through altered development and functional maturation of T cells, including diminished intrinsic CD4+ T cell pathogenicity and the development of CD4−Foxp3− regulatory populations. The Journal of Immunology, 2011, 187: 5521–5531.

Postinfection or immunization, T cells with higher Ag affinity outcompete lower affinity cells and come to dominate the immune response (1–4). The role of TCR affinity in determining pathogenicity among autoreactive T cells is less clear. This has been indirectly probed by examining T cell responses to altered peptide ligands (APL) (5). Antagonists or weak agonists can block or ameliorate autoimmune disease, implying that low-affinity cross-reactive interactions are fundamentally non-pathologic (6, 7). Indeed, such APL have shown mixed results in clinical trials (8, 9). Implicitly, higher affinity recognition is a prerequisite for pathogenicity. However, high-affinity APL may also generate tolerance. Immunosuppression with a proteolipid-protein (PLP)-derived APL stimulated T cells with a higher affinity for the APL than heteroclitic self-antigen. This led to the development of a Th0/Th2 response to self-antigen that inhibited disease development (10, 11). Indeed, hyperstimulatory APL can also promote T cell apoptosis and tune down TCR responsiveness to cognate Ag and thereby confer protection (12, 13). This suggests that T cells with either too low or high an affinity for stimulating Ag may be diverted into nonpathologic or even protective developmental pathways.

Whereas studies of APL have indicated that affinity thresholds may guide autoreactive T cell responses, few studies have directly assessed the role of affinity among T cells responding to cognate autoantigen. NRP-A7–specific CD98+ T cells in NOD mice showed avidity-based competition during diabetes development, with higher affinity cells accumulating during disease progression (14). Likewise, 4E3 TCR transgenic (Tg) mice, which develop spontaneous fulminant experimental autoimmune encephalomyelitis (EAE), bear a higher affinity TCR than 5B6 TCR Tg mice, which have a lower incidence of spontaneous disease (15). In contrast, in a direct assessment of the affinity of T cells responding in myelin oligodendrocyte glycoprotein-induced EAE (MOG-EAE) using two-dimensional binding assays, the affinity range of responding cells varied by >100-fold (16). The majority of cells were low affinity, suggesting that high- or moderate-affinity cells were excluded from the response or otherwise tolerized. These low-affinity cells demonstrated in vitro effector responses equivalent to those with higher affinity. In a separate study, higher affinity pancreatic Ag-specific T cells were irreversibly tolerized, whereas lower affinity cells persisted and were capable of initiating autoimmunity under stimulatory conditions (17). Two EAE studies also failed to correlate avidity with disease progression, one in which functional measurements of PLP-specific T cell avidity was longitudinally followed (18) and a second exploring spontaneous EAE in retroviral transgenic (retrogenic) mice expressing a series of different TCR (19). These results imply that increased affinity is not necessarily associated with pathogenicity.

In prior analyses of the role of T cell affinity in autoimmunity, T cells expressed wholly distinct TCRs. How differences in fine specificity, degenerate recognition of additional Ags, or other recognition parameters influenced pathogenicity could not be dissected. To better characterize the role of affinity in T cell
pathogenicity, we generated a mutant self-specific TCR with minimal structural differences compared with its parental receptor, but dramatically increased sensitivity for cognate autoantigen. In a prior report, we identified a variation in the use of G and S residues at position 107 in the TCR β-chain V region (TRBV); TRBV13-2 possesses a G107, whereas most other TRBV have an S. This amino acid lies within the buried N-terminal core of the CDR3, with the side chain intercalating into the loop structure of the CDR3. We predicted based on structural modeling and molecular dynamics that substitution of the TRBV13-2 G with a S residue more typical of other TCR would position the S hydroxymethyl side chain to fill an internal gap within the CDR3 loop and through increased van der Waals interactions and H-bonding stabilize it without substantially altering its structure (20). Indeed, a G107S substitution in the TRBV13-2*1-Aβ-MOG35–55-specific 1MOG9 TCR yielded a ∼3log10 increase in cognate Ag sensitivity while preserving fine specificity.

We compare in this study development and pathogenicity of T cells expressing these TCR. Either the high- or low-affinity MOG-specific T cells were well selected and predominantly naïve in the absence of immunization. However, the high-affinity TCR markedly diminished disease susceptibility. This resulted from a diminished pathogenicity of autoreactive CD4+ T cells. More prominently, the high-affinity TCR led to the development of coreceptor independent, CD4+CD8− (double-negative [DN]), and CD8+ T cells that showed low intrinsic pathogenicity, out-compete CD4+ T cells, and inhibited disease after transfer to wild-type (wt) mice. Therefore, high-affinity autoantigen-specific T cells can suppress autoimmune responses through both altered lineage differentiation and functional maturation. Implicitly, the pathologic potential of an autoantigen will depend on the affinity distribution of specific TCR; higher affinity clones may be protective.

Materials and Methods

Animals

C57BL/6 (B6), B6.129S7-Rag1<sup>+/−</sup>/MjmxJ (Rag1−/−), and B6.SJL-Ptprc<sup>a</sup> Pep8<sup>b</sup>/B10J (CD45.1) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were bred and maintained under specific pathogen-free conditions, including detectable strains of helicobacter. Experiments were performed under a protocol approved by and in accordance with Institutional Animal Care and Use Committee guidelines.

Peptides, Abs, and flow cytometry

MOG<sub>35–55</sub> peptide (MEVGWYRSPFSRVVHLYRNGK) was synthesized and HPLC purified by the St. Jude Hartwell Center for Biotechnology. mAbs specific for CD4 (clone H129.19), CD8 (clone 53-6.7), CD25 (clone MR5-2), CD69 (clone H1.2F3), CD45RB (clone 16A), CD44 (clone IM7), and mAbs specific for CD4 (clone H129.19), CD8 (clone 53-6.7), CD25 (clone MR5-2), CD69 (clone H1.2F3), CD45RB (clone 16A), CD44 (clone IM7), and TCR Vβ8.1, 8.2 (clone MR5-2) were from BD Biosciences (Franklin Lakes, NJ). CD62L (MEL-14) and the Foxp3 staining kit were from eBioscience (San Diego, CA). Flow cytometry was performed on an FACSCalibur (BD Biosciences), and flow cytometric sorting was performed on a MoFlo high-speed cell sorter (DakoCytomation, Fort Collins, CO).

TCR constructs and generation of retrogeneic and chimeric mice

1MOG9 and G107S TCR α- and β-chains were isolated by PCR and cloned into the murine stem cell virus (MSCV)-I–GFP MSCV-based retroviral vector and retroviral mice generated as described (19, 20). Briefly, bone marrow (BM) cells were harvested from the femurs of Rag1−/− mice 48 h after the administration of 0.15 mg 5-fluorouracil/g body weight. The pooled cells were cultured in complete Click’s medium (Invitrogen, Carlsbad, CA) containing 20% FCS, IL-3 (20 ng/ml), IL-6 (50 ng/ml), and stem cell factor (50 ng/ml) for 48 h at 37˚C/5% CO<sub>2</sub>. The cells were then cocultured for an additional 48 h with 1200 rad-irradiated retroviral producer cells. The hematopoietic progenitor cells (HPC) were harvested, washed with PBS, transduction confirmed by flow cytometry for GFP, and

![FIGURE 1. Immunophenotype of G107S and 1MOG9 retrogeneic T cells. Thymocytes, LN cells, and splenocytes were isolated from retrogeneic mice ∼8 wk following stem cell transfer or B6 controls. A, CD4 and CD8 labeling of thymocytes is shown. B, CD4 and CD8 expression on TCRB-gated LN cells or splenocytes. C, CD44, CD45RB, and CD62L labeling was used to indicate the memory or naive status of CD4+ TCRB+ T cells. Data are from representative animals.](http://www.jimmunol.org/)
injected i.v. into sublethally irradiated (450 rad) Rag1−/− mice at a ratio of two recipient mice per BM donor. For the generation of chimeric mice, transduced HPC were admixed with B6 BM cells and transferred i.v. into 900 rad-irradiated B6 recipients. Mice were assessed at the indicated times posttransplant.

EAE induction and clinical evaluation
EAE was induced by s.c. immunization with 100 μg MOG35–55 peptide emulsified in CFA containing 0.4 mg (4 mg/ml) Mycobacterium tuberculosis H37RA (Difco, Franklin Lakes, NJ). Two hundred nanograms pertussis toxin (List Biological Laboratories, Campbell, CA) was administered i.v. on days 0 and 2. In some experiments, CD4+, CD8+, and DN TCRβ+ cells were flow cytometrically isolated and transferred into recipient mice 1 d prior to immunization. Clinical scoring was as follows: 1, limp tail; 2, hind limb paresis or partial paralysis; 3, total hind limb paralysis; 4, hind limb paralysis and body/front limb paresis/paralysis; and 5, moribund.

T cell proliferation
CD4+, CD8+, and DN TCRβ+ T cells were isolated by flow cytometry or Ab-based magnetic bead selection (MACS; Miltenyi Biotec, Auburn, CA) per the manufacturer’s instructions. These were from mice either unimmunized or 7 d postimmunization with MOG35–55 peptide. Cells were cultured at 5 × 10^4/well in 96-well plates with 3 × 10^5 irradiated B6 APCs and the indicated stimulus, pulsed with 1 μCi [3H]thymidine after 72 h of culture, and then harvested for scintillation counting. Samples were analyzed in triplicate.

Cytokine analysis
CD4+, CD8+, and DN TCRβ+ T cells were isolated and cultured as described above. Culture supernatants were collected at 48 h and analyzed for IL-2, IL-4, IL-10, IL-17, and IFN-γ using the Milliplex Map kit assay (Millipore, Billerica, MA).

Cytotoxicity assay and quantitative flow cytometry
Flow cytometrically purified CD4+, CD8+, and DN TCRβ+ cells from CD45.1+CD45.2+ G107S mice were stimulated for 4 d with MOG35–55, then incubated at a 1:1 ratio with freshly purified congenic CD45.1+CD45.2+ B cells or BM-derived dendritic cell (DC) targets for 6 or 18 h with or without 100 μg/ml MOG35–55. BM-derived DCs were matured with GM-CSF and IL-4 (R&D Systems, Minneapolis, MN) as described (21). Samples were stained for the indicated target B cell or DC population and then analyzed by quantitative flow cytometry as described (22). Viable cell counts are plotted for individual samples assayed in triplicate.

Results

Retrogenic mouse production and characterization
The parental MOG35–55-specific, I-A^b–restricted 1MOG9, and G107S TCR are identical except for the added hydroxymethyl group in the TRBV 107 side chain in the G107S receptor. This G/S variant residue is at the immediate junction of the TRBV and TCR β-chain J region, does not directly engage peptide–MHC, and is predicted to fill a buried gap in the 1MOG9 TRBV. The G107S TCR was previously observed in transduced hybridoma cells to possess an ∼3log10 increase in Ag sensitivity (20).

The TCRA and B chains of the 1MOG9 and G107S TCR were subcloned into the MSCV-I–GFP retroviral vector separated by a viral 2A sequence to facilitate their stoichiometric expression. Retrovirus was transduced into Rag1−/− HPC, which were trans-
planted into Rag1−/− recipients, thereby ensuring that only the retrogenic TCR was expressed.

**G107S lineage infidelity**

Skewed thymic development of CD4+ single-positive T cells was seen in mice receiving HPC transduced either with the 1MOG9 or G107S TCR (Fig. 1A). Both 1MOG9 and G107S retrogenic mice also showed peripheral engraftment with the self-specific TCR (Fig. 1B). In each case, the CD4+ T cells were predominantly naive, bearing a CD44lo, CD45Rbhi, CD62Lhi phenotype (Fig. 1C). Little expression of the activation and regulatory marker CD25 was apparent on either cell type, and despite the enhanced affinity for a self-antigen in the G107S mice, few Foxp3+ regulatory T cells were seen. In contrast with the virtually exclusively CD4+ T cell engraftment in mice expressing the wt 1MOG9 TCR, substantial numbers of peripheral CD8+ T cells were observed in G107S mice (Fig. 1B). These CD8+ cells, as the CD4+, were predominantly naive (Fig. 1D). A smaller population of DN T cells was also seen in the G107S mice, whereas few were present in the 1MOG9 animals. These also displayed a largely naive phenotype (Fig. 1E). Therefore, the high-affinity G107S TCR promotes lineage infidelity and T cell subset heterogeneity.

Increased affinity for self is associated with the deletion of autoantigen-specific T cells (23). However, this was not apparent with the G107S TCR. To the contrary, 7–12 wk after stem cell transfer, the G107S mice showed significantly more splenic CD4+ TCR+ T cells than matched wt 1MOG9 TCR retrogenic mice ($p < 0.0001$; Fig. 2). Therefore, the G107S substitution does not adversely affect the development and engraftment of self-reactive T cells. Rather, it supports increased engraftment as well as production of CD8+ and smaller numbers of DN T cells. Notably, in a prior study of retrogenic mice expressing different MOG-specific TCR (19), we were unable to associate affinity with engraftment level, and the recognition parameters that are promoting this increased engraftment remain to be determined.

**Heightened responsiveness of primary G107S T cells**

Developmental tuning can dampen the responsiveness of high-affinity T cells (24, 25). To determine if the increased Ag sensitivity previously observed in G107S-TCR-transduced cell lines was retained in primary T lymphocytes, we compared the proliferative response of isolated CD4+ T cells from 1MOG9 and G107S retrogenic mice. Similar to transduced cell lines, a $\sim 3\log_{10}$ increase in MOG sensitivity was seen in primary CD4+ T cells expressing the G107S TCR (Fig. 3A). Isolated CD8+ and DN T cells present in the G107S mice also proliferated strongly in response to cognate Ag with response profiles similar to that of CD4+ T cells (Fig. 4A), verifying our prior observation in hybrids that the G107S TCR is coreceptor independent. Therefore, a G107S substitution in a TRBV13-2 TCR is fully compatible with the development of functional T lymphocytes bearing markedly enhanced sensitivity for cognate Ag.

**Distinct cytokine profiles of 1MOG9 and G107S T cells**

To assess the differentiation potentials of 1MOG9 and G107S T cells, CD4+ T cells were bead isolated from 8 wk retrogenic mice, and equal numbers stimulated in vitro with MOG35–55, IL-2, IL-4, IL-10, IFN-γ, and IL-17 were measured as representative cytokines. Either 1MOG9 or G107S CD4+ T cells showed good IL-2 production, though levels were greater with the G107S T cells (Fig. 3A). Both cell types also produced IFN-γ, associated with differentiation into Th1 cells that are pathologic in EAE (26, 27), though in this study more was produced by the lower affinity 1MOG9 T cells. G107S T cells produced modest amounts of IL-17, also associated with EAE pathogenicity (28), whereas none was seen with the 1MOG9 T cells. IL-4 is associated with disease protection (29). Little or no IL-4 was produced by the 1MOG9 T cells, whereas substantial quantities were produced by the G107S cells. The G107S T cells also exclusively demonstrated significant production of IL-10, a cytokine with pronounced regulatory activity in EAE (30–34). Therefore, CD4+ T cells from 1MOG9 mice showed a restricted cytokine production pattern characterized by IL-2 and IFN-γ production and little IL-4 and IL-10 synthesis. G107S T cells showed a more degenerate and pronounced cytokine profile that included cytokines characteristic of Th1, Th2, and Th17 cells. IL-10 and IL-4 production, protective in EAE, was limited to the G107S cells.

CD8+ and DN T cells were similarly isolated, though from older 16-wk retrogenic mice, necessary to obtain adequate DN...
numbers by cell sorting. These demonstrated less IL-2 and more IFN-γ than similarly isolated CD4+ G107S T cells (Fig. 4B). Neither DN nor CD8+ T cells produced substantial quantities of IL-10. However, production of other cytokines diverged between the cell types. DN cells produced more IL-17, whereas CD8+ T cells produced little. CD8+ T cells also produced little IL-4 when compared with DN and CD4+ T cells. Therefore, G107S T cell subsets possess distinct functional profiles when stimulated with Ag, with variability in the production of both inflammatory and protective cytokines.

Cytolytic activity of DN and CD8+ T cells
To determine if the T cells were able to kill MOG-expressing APCs, primary B cells or BM-derived DCs were pulsed with MOG35–55 and cocultured for either 6 or 18 h with G107S T cells at a 1:1 E:T ratio. The DN and CD8+ T cells lysed the B cell populations at either time point. G107S (Fig. 5A) or 1MOG9 (not shown) CD4+ T cells were unable to kill these cells. At the 1:1 E:T ratio, DCs proved insensitive to CTL killing (Fig. 5B); such DC resistance to lysis has been previously reported (35). However, at a 4:1 ratio, modest DC killing by the DN and CD8+ populations was evident (data not shown). Therefore, the CD8+ and DN but not CD4+ effector cells are capable of killing cells presenting Ag, though the extent of this is dependent on lineage sensitivity to lysis.

Diminished EAE severity in G107S mice
We would predict that the altered differentiation profile of the higher affinity G107S T cells would influence EAE susceptibility. To test this, 1MOG9 and G107S mice were immunized with MOG35–55. The 1MOG9 mice developed fulminant disease, which proved nearly uniformly fatal by 2 wk after immunization (Fig. 6A, Table I). In contrast, despite the increased T cell affinity for Ag, the G107S mice manifested milder disease, which, though not

![FIGURE 5. Ag-specific cytolysis by G107S CD8+ and DN T cells.](http://www.jimmunol.org/) Purified CD4+, CD8+, and DN G107S T cells were stimulated for 4 d with MOG35–55 Ag, isolated, and then cocultured with purified B6 B lymphocytes (A) or BM-derived DCs (B) at a 1:1 E:T ratio in triplicate. Cultures were either pulsed with 100 μg/ml MOG35–55 or left without Ag. At 6 or 18 h, numbers of residual viable target cells were quantitatively assessed by flow cytometry. The ratio of residual cells in Ag-pulsed/nonpulsed cultures is plotted. Mean ± 1 SD is shown. Representative of two experiments.

![FIGURE 6. Diminished EAE severity and altered subset expansion in G107S retrogenic mice.](http://www.jimmunol.org/) A, EAE was induced by immunization with MOG35–55. Clinical disease score was monitored longitudinally. Mean ± SEM is plotted. Representative of two experiments. Additional data parameters are provided in Table I. B–E, T lymphocytes were isolated from the spleen and CNS of mice 6 d after induction of EAE or spleen of control mice in which disease was not induced. The immunized 1MOG9 mice had disease scores ranging from 3 to 4 and the G107S mice all scores of 1. B, Sample plots showing CD4 and CD8 labeling profiles of splenocytes (immunized and unimmunized) or CNS (immunized only) TCRβ+ T cells. Absolute numbers of splenic T cell populations in 1MOG9 (C) or G107S (D) mice are plotted. Circles indicate individual animals and lines represent means. E, Absolute numbers of isolated TCRβ+ CNS T cell subsets are similarly plotted.
delayed in onset relative to the 1MOG9 mice, failed to clinically progress. Mortality was absent among the G107S mice. Therefore, a high-affinity TCR in G107S retrogenic mice, despite promoting significantly enhanced CD4+ T cell engraftment, led to markedly diminished disease severity.

**Distinct T lineage responses in G107S and 1MOG9 mice**

To better characterize the responding cells in the different mice, we examined splenic and CNS cell numbers prior to and after immunization. As described above, CD4+ T cells were the predominant lymphoid constituent in preimmune 1MOG9 mice, with only small numbers of CD8+ or DN T cells detected. After EAE induction, a dramatic expansion of CD4+ T cells was seen. These remained the dominant cell type in the spleen, and little expansion of CD8+ or DN T cells was apparent (Fig. 6). The CD4+ population expanded less than in the 1MOG9 mice. CD8+ and DN T cells expanded to a much greater degree than CD4+ T cells, reversing the CD4+/CD8+ and CD4+/DN T cell ratios. Analysis of the CNS lymphoid infiltrate showed a predominance of CD4+ T cells in 1MOG9 mice, but CD8+ and DN significantly outnumbered CD4+ cells in G107S mice (Fig. 6E). Therefore, the autoimmune response in 1MOG9 and G107S mice is characterized by the differential expansion and CNS infiltration by CD4+, CD8+, and DN T cell subsets.

**Functional potential of immune T cells**

To functionally assess the responding cells, we analyzed the proliferative potential of purified T cell populations 7 d after immunization. In contrast to the distinct responses of preimmune T cells, comparison of the T cell populations from mice with EAE demonstrated nearly equivalent in vitro proliferation of the 1MOG9 CD4+ T cells and G107S T cell subsets (Fig. 7A). This differed from results with preimmune populations (Fig. 3). Indeed, direct comparison of the proliferative responses of purified CD4+ T cells isolated from immunized and unimmunized mice showed a corresponding enhanced responsiveness to Ag among immunized 1MOG9 but not G107S T cells (data not shown) compared with unimmunized controls. Therefore, prior activation differentially influences subsequent Ag sensitivity by G107S and 1MOG9 T cells, with only the lower affinity cells developing increased reactivity.

Cytokine profiles of the 1MOG9 and G107S T cells were further assessed at day 7, which, though early after disease induction, only shortly preceded the demise of most of the 1MOG9 mice (Fig. 7B–F). 1MOG9 CD4+, CD8+, and DN T cells showed significantly decreased IL-2, IFN-γ, and IL-17 compared with 1MOG9 cells. This indicates an overall diminished production of Th1 and Th17 cytokines relevant to EAE. Only modest IL-4 and IL-10 was produced by immune cells from either mouse type.

Therefore, an increased TCR affinity in G107S mice is associated with both attenuated disease course and diminished effector cytokine production. Notably, similar to the preimmune mice (Fig. 1), few Foxp3+ regulatory T cells were detectable in either G107S or 1MOG9 mice after induction of EAE (data not shown).

**Pathogenicity and protection by isolated G107S cellular subsets**

Our results indicated that multiple T cell subsets responded in G107S EAE; the dominant T cell response was CD4+, and G107S T cells had diminished effector cytokine production. To examine the pathogenicity of isolated high-affinity subsets, CD4+, CD8+, or DN T cells were purified from G107S mice and CD4+ T cells from 1MOG9 mice. These were transferred into Rag1-/- recipients and disease induced. CD4+ T cells from G107S mice manifested diminished disease relative to lower affinity 1MOG9 CD4+ T cells, indicating that increased TCR affinity decreases T cell pathogenicity in this population (Fig. 8, Table I). In contrast to the G107S CD4+ T cells, mild or no disease was seen in recipients of CD8+ or DN G107S T cells. Therefore, pathogenicity is a function of both TCR affinity and cell lineage. DN and CD8+ T cells, which show the greatest expansion in immunized G107S mice, have limited capacity to independently promote disease.

To determine whether G107S T cells could also suppress disease development and progression in a competitive environment, we analyzed their impact in B6 mice. Small numbers (1 × 10^5) of flow cytometrically purified CD4+, CD8+, or DN T cells were transferred into Rag1-/- recipients, and disease induced. CD4+ T cells, with only the lower affinity cells developing increased reactivity.

Cytokine profiles of the 1MOG9 and G107S T cells were further assessed at day 7, which, though early after disease induction, only shortly preceded the demise of most of the 1MOG9 mice (Fig. 7B–F). 1MOG9 CD4+, CD8+, and DN T cells showed significantly decreased IL-2, IFN-γ, and IL-17 compared with 1MOG9 cells. This indicates an overall diminished production of Th1 and Th17 cytokines relevant to EAE. Only modest IL-4 and IL-10 was produced by immune cells from either mouse type.

Therefore, an increased TCR affinity in G107S mice is associated with both attenuated disease course and diminished effector cytokine production. Notably, similar to the preimmune mice (Fig. 1), few Foxp3+ regulatory T cells were detectable in either G107S or 1MOG9 mice after induction of EAE (data not shown).

**Pathogenicity and protection by isolated G107S cellular subsets**

Our results indicated that multiple T cell subsets responded in G107S EAE; the dominant T cell response was CD4+, and G107S T cells had diminished effector cytokine production. To examine the pathogenicity of isolated high-affinity subsets, CD4+, CD8+, or DN T cells were purified from G107S mice and CD4+ T cells from 1MOG9 mice. These were transferred into Rag1-/- recipients and disease induced. CD4+ T cells from G107S mice manifested diminished disease relative to lower affinity 1MOG9 CD4+ T cells, indicating that increased TCR affinity decreases T cell pathogenicity in this population (Fig. 8, Table I). In contrast to the G107S CD4+ T cells, mild or no disease was seen in recipients of CD8+ or DN G107S T cells. Therefore, pathogenicity is a function of both TCR affinity and cell lineage. DN and CD8+ T cells, which show the greatest expansion in immunized G107S mice, have limited capacity to independently promote disease.

To determine whether G107S T cells could also suppress disease development and progression in a competitive environment, we analyzed their impact in B6 mice. Small numbers (1 × 10^5) of flow cytometrically purified CD4+, CD8+, or DN T cells were transferred into Rag1-/- recipients and disease induced. CD4+ T cells, with only the lower affinity cells developing increased reactivity.

Cytokine profiles of the 1MOG9 and G107S T cells were further assessed at day 7, which, though early after disease induction, only shortly preceded the demise of most of the 1MOG9 mice (Fig. 7B–F). 1MOG9 CD4+, CD8+, and DN T cells showed significantly decreased IL-2, IFN-γ, and IL-17 compared with 1MOG9 cells. This indicates an overall diminished production of Th1 and Th17 cytokines relevant to EAE. Only modest IL-4 and IL-10 was produced by immune cells from either mouse type.

Therefore, an increased TCR affinity in G107S mice is associated with both attenuated disease course and diminished effector cytokine production. Notably, similar to the preimmune mice (Fig. 1), few Foxp3+ regulatory T cells were detectable in either G107S or 1MOG9 mice after induction of EAE (data not shown).

**Pathogenicity and protection by isolated G107S cellular subsets**

Our results indicated that multiple T cell subsets responded in G107S EAE; the dominant T cell response was CD4+, and G107S T cells had diminished effector cytokine production. To examine the pathogenicity of isolated high-affinity subsets, CD4+, CD8+, or DN T cells were purified from G107S mice and CD4+ T cells from 1MOG9 mice. These were transferred into Rag1-/- recipients and disease induced. CD4+ T cells from G107S mice manifested diminished disease relative to lower affinity 1MOG9 CD4+ T cells, indicating that increased TCR affinity decreases T cell pathogenicity in this population (Fig. 8, Table I). In contrast to the G107S CD4+ T cells, mild or no disease was seen in recipients of CD8+ or DN G107S T cells. Therefore, pathogenicity is a function of both TCR affinity and cell lineage. DN and CD8+ T cells, which show the greatest expansion in immunized G107S mice, have limited capacity to independently promote disease.
ferred into B6 recipients, and 1 d later, EAE was induced (Fig. 9A).

Transfer of G107S CD4+ T cells diminished disease severity when compared with control treated mice. However, CD8+ T cells blocked G107S disease development and DN T cells markedly delayed time to disease onset as well as disease severity, indicating that these subsets are protective in wt mice.

To determine if the MOG-specific CD4+ T cells would develop not only in Rag2−/− mice but in the context of a wt thymus, we diluted G107S, 1MOG9, or retroviral vector-transduced Rag2−/− HPC with an excess of untransduced B6 BM cells and transplanted these into B6 recipients. Only small numbers of retrogenic T cells, identifiable through their coexpression of GFP, were identified. They averaged fewer than 2% of TCRαβ+ T cells in mice receiving 1MOG9-transduced progenitors and were comprised virtually exclusively of CD4+ T cells (Fig. 9B). Approximately 7% of TCRαβ+ cells in the G107S chimeras expressed the retrogenic TCR. Quantities of CD4+ retrogenic T cells were not significantly different from those in the 1MOG9 mice. However, CD8+ and DN G107S T cells were also observed, making up the balance of cells. Therefore, the high-affinity MOG-specific TCR promotes the cross-differentiation of T cells into coreceptor independent CD8 and DN T cell subsets when developing as a minority population in wt mice.

To determine if the MOG-specific CD4+ T cells would protect against EAE development, disease was induced in these chimeric mice (Fig. 9C). Whereas mice receiving control MSCV vector- or 1MOG9 TCR-transduced HPCs demonstrated similar levels of disease, mice receiving G107S-transduced cells demonstrated a milder disease course. Therefore, as with T cell transfer into wt mice, G107S T cells developing in mixed stem cell chimeras diminish EAE severity.

**FIGURE 7.** Functional profiles of 1MOG9 and G107S T cells in MOG-immunized mice. T cell subsets were isolated by flow cytometry from 1MOG9 (CD4+) or G107S (CD4+, CD8+, DN) mice 7 d after MOG immunization. A, Proliferation to the indicated concentration of MOG35–55 was measured by [3H]thy midine incorporation 72 h after stimulation. B–F, Production of the indicated cytokines was measured by multiplex assay 48 h after stimulation. Responses of purified cells cultured without or with 100 μg/ml MOG35–55 is shown. Mean ± 1 SD is plotted. Representative of two experiments.

**FIGURE 8.** Pathologic potential of isolated T cell subsets. Flow cytometrically isolated TCRαβ subsets from 1MOG9 (CD4+) or G107S (CD4+, CD8+, DN) were transferred i.v. into Rag1−/− recipients (5 × 104/mouse), and EAE was induced 1 d later. Clinical score was followed longitudinally. Mean ± 1 SEM is plotted. Representative of two experiments with flow cytometrically sorted T cells and two with bead-selected cells. Additional data are provided in Table I.
The cross-reactive NF-M18–30 epitope are the only Ags identified from two similar experiments. Metrically purified T cell subset or saline was transferred i.v. into B6 mice. Maturation is avidity dependent. Thus, the response to pigeon mental signals in the functional maturation of T cells. Th subset susceptibility by altering differentiation potential. Study, increased affinity can also lead to diminished disease susceptibility to autoimmunity. Thymic and peripheral T cell deletion may determine the quality of the response to Ag and hence susceptibility to induce EAE (36, 37). The available T cell repertoire will reflect the natural differentiation potential of this TCR.

Our results are consistent with a model in which T cell immunopathologic potential is bracketed by both low- and high-affinity thresholds and describe new mechanisms for this. Importantly, the G107S TCR differs only in the presence of a buried hydroxymethyl group in the TRBV (20). The S107 residue supplying this moieties is present in the majority of expressed TCR; 20 out of 23 mouse and 49 out of 54 human TRBV possess an S, whereas only 1 and 2, respectively, incorporate a G, and therefore, the substitution represents a natural variation at this site (20). The G leaves a gap at the base of the CDR3β, which is filled by the S side chain. This minor structural perturbation influences affinity for MOG35–55 without substantially altering fine specificity. Yet it has several functionally significant effects. Most prominently, whereas the wt 1MOG9 TCR drives T cells into the CD4 T cell lineage, the G107S TCR leads to lineage infidelity, with CD4+, CD8+, and DN T cells identifiable. These CD4+ populations also develop in mixed HPC chimeras in wt mice, indicating that they reflect the natural differentiation potential of this TCR.

Whether increased G107S TCR affinity for a self-ligand is itself responsible for the lineage infidelity is uncertain from this study. In one other model, a high-affinity self-specific TCR also guided T cell development into CD4–CD8– T cells (42), suggesting a role. Assessments of additional TCR series of varying affinity may help determine the recognition parameters controlling the fidelity of lineage assignments. Increased affinity is, however, essential for coreceptor-independent Ag recognition. Studies of MHC class I-restricted Tg cells, it has also been observed that Th2 cells require greater signal strength on rechallenge than Th1 cells for cytokine production (40).

Affinity likewise is essential in determining cell survival and proliferation. In the Listeria-specific response, early stimulation activates both low- and higher-affinity cells. The lower affinity cells, however, exit the lymph nodes (LNs) earlier and have a more abbreviated expansion phase, potentially explaining the affinity maturation that is observed (41). In other conventional responses, affinity maturation is also present, and higher affinity cells dominate the secondary response. Despite these findings, in autoimmune systems, strong agonists may be protective. Thus, a myelin APL superagonist did not lead to EAE, but promoted the desensitization and activation-induced cell death of autoantigen-specific T cells in a Fas-dependent manner (11, 12).

Our results are consistent with a model in which T cell immunopathologic potential is bracketed by both low- and high-affinity thresholds and describe new mechanisms for this. Importantly, the G107S TCR differs only in the presence of a buried hydroxymethyl group in the TRBV (20). The S107 residue supplying this moiety is present in the majority of expressed TCR; 20 out of 23 mouse and 49 out of 54 human TRBV possess an S, whereas only 1 and 2, respectively, incorporate a G, and therefore, the substitution represents a natural variation at this site (20). The G leaves a gap at the base of the CDR3β, which is filled by the S side chain. This minor structural perturbation influences affinity for MOG35–55 without substantially altering fine specificity. Yet it has several functionally significant effects. Most prominently, whereas the wt 1MOG9 TCR drives T cells into the CD4 T cell lineage, the G107S TCR leads to lineage infidelity, with CD4+, CD8+, and DN T cells identifiable. These CD4+ populations also develop in mixed HPC chimeras in wt mice, indicating that they reflect the natural differentiation potential of this TCR.

Whether increased G107S TCR affinity for a self-ligand is itself responsible for the lineage infidelity is uncertain from this study. In one other model, a high-affinity self-specific TCR also guided T cell development into CD4–CD8– T cells (42), suggesting a role. Assessments of additional TCR series of varying affinity may help determine the recognition parameters controlling the fidelity of lineage assignments. Increased affinity is, however, essential for coreceptor-independent Ag recognition. Studies of MHC class I-restricted Tg cells, it has also been observed that Th2 cells require greater signal strength on rechallenge than Th1 cells for cytokine production (40).

Discussion

The presence of self-specific T cells is inadequate for autoimmunity. These cells must also be stimulated in a manner that triggers their maturation into destructive effector forms, promotes their expansion, and sustains their pathogenicity over time. To achieve this balance experimentally, specific induction regimens are often needed. In most experimental systems, relatively few epitopes have been identified against which T cell responses can provoke autoimmunity. For example, in B6 mice, MOG35–55 and the cross-reactive NF-M18–30 epitope are the only Ags identified able to induce EAE (36, 37). The available T cell repertoire will determine the quality of the response to Ag and hence susceptibility to autoimmunity. Thymic and peripheral T cell deletion may lead to specificity holes and thereby tolerance. As described in this study, increased affinity can also lead to diminished disease susceptibility by altering differentiation potential.

TCR affinity plays a crucial role in conjunction with environmental signals in the functional maturation of T cells. Th subset maturation is avidity dependent. Thus, the response to pigeon cytochrome C is dominated by high-affinity cells that following in vitro priming generates a Th1-biased response. Purging the high-affinity cells leads to lower affinity repertoire that develop a Th2 bias (38). In a consistent manner, low Ag dose or weak APL signaling leads to a Th2 responses in TCR Tg cells, whereas high dose promotes Th1 maturation (10, 39). In contrast, in matured T cell lines, it has also been observed that Th2 cells require greater signal strength on rechallenge than Th1 cells for cytokine production (40).
to modulate autoimmune disease. Implicitly, in contrast to the competitive maturation to high-affinity responses typical after immunization or infection, the low or moderate affinities often observed in autoimmune may be necessary for the generation of pathologic responses.

The mechanism of protection of these CD4+ T cells in this model remains uncertain, though some clues are present. DN T cells have been observed more broadly to possess regulatory properties, as evidenced in other models of allograft and autoimmune diseases (44, 45). It would seem likely in this study that the low pathologic potential of the DN cells coupled with their ability to outcompete more pathologic CD4+ T cells provides for their immunomodulatory activity. The source of these cells in natural circumstances has been debated. Small numbers of DN αβ T cells normally circulate, typically <2%, though these are increased in certain conditions such as autoimmune lymphoproliferative syndrome (46, 47). Defects in Fas signaling there suggest that alterations in this death-signaling pathway may allow the accumulation of DN cells. Indeed, DN T cells survive chronic stimulation better than their coreceptor-positive peers (48). In some situations, it is hypothesized that DN cells are generated through coreceptor downregulation with stimulation, which may occur genetically or epigenetically (49–52). However, other sources are possible (53), and the largely naïve state of the DN cells in unimmunized G107S mice and their distinct cytokine profile when compared with either CD4+ or CD8+ cells would indicate that they are a primary population. Indeed, diversion of a small proportion of autoreactive T cells to DN cells was observed when a high-affinity PLP-specific TCR was transgenically expressed on T cells in a disease-resistant genetic background. Whereas DN TCRαβ T cells form a small portion of most immune responses, this evidence supports their formation from high-affinity, self-specific cells through a common differentiation pathway (42). Further evaluation of these DN T cells and their role in EAE and other autoimmune diseases in unmanipulated mice is warranted.

CD8+ T cells recognizing epitopes internal to MOG35–55 can modify MOG-EAE, and both pathologic and protective properties have been attributed to them (54–56). EAE in β2-microglobulin (β2m)−/− mice shows increased disease severity, suggesting that, overall, the class I-restricted response is protective (57). In our analyses, the G107S CD8+ T cells recognized class II MHC-restricted Ag. Such lineage crossover by coreceptor-independent cells has been previously observed in immune responses (58), though the extent to which T cells are capable of crossing MHC restriction is unclear. Nevertheless, coreceptor independent signaling is adequate to activate T cells with high affinities (43). Further, the sheer magnitude of the degeneracy inherent in TCR recognition, in which it is anticipated that a single TCR may recognize >106 unique Ags, would suggest that some high-affinity, coreceptor-independent responses will exist, and these may cross MHC class barriers (59).

To our knowledge, it has not been previously shown that high-affinity cells specific for a class II-restricted Ag may actually develop in situ into CD8+ T cells. To test the MHC requirements for this development, we generated G107S retrogenic mice using transduced B2m−/−Rag1−/− HPCs transferred into β2m−/−Rag1−/− recipients. Although the relative numbers of CD8+ T cells was diminished compared with Rag1−/− retrogenic mice, a significant population was nevertheless present, and DN cells were increased (Supplemental Fig. 1). Therefore, both CD8+ and DN G107S T cells can develop in a class I MHC-independent manner.

It is of interest, though not surprising, that the CD8+ and DN G107S T cells can kill Ag-pulsed APCs, particularly B cells. Cytotoxicity is a characteristic of these populations, and it has been hypothesized that this can restrict autoreactive responses by reducing APC availability (54). The role of APC killing in the diminished pathogenicity and protection in this study will have to be established. However, DCs are a critical APC type in the EAE model system (60), and these possess mechanisms to resist lysis, including the expression of granzyme-inhibitory serpins (35). Indeed, at E:T ratios capable of easily killing B cells, DCs proved impervious to lytic attack.

The functional profile of the G107S and 1MOG9 T cells, defined in this study by their cytokine production patterns, differed significantly between the cells, and further explains the reduced pathogenicity of the higher affinity cells. Primary CD4+ G107S T cells produced increased IL-10 and IL-4, which are regulatory in EAE, compared with wt 1MOG9 T cells. In mice with active disease, substantive IL-10 and IL-4 responses were not detected. Nevertheless, the G107S T cells produced less IFN-γ and IL-17, indicating a disruption in Th1/Th17-mediated immunopathogenesis. Further, isolated CD4+ G107S T cells were less potent than CD4+ 1MOG9 T cells in transfer model. Therefore, consistent with data on the use of superagonistic APL to stimulate autoreactive T cells, and the observed diminished pathogenicity of high-affinity TCR transgenics, G107S CD4+ T cells possess an intrinsically reduced pathogenicity compared with paired 1MOG9 T cells. Further, lower affinity 1MOG9 but not G107S T cells demonstrated enhanced in vitro response to Ag after immunization, suggesting that prior stimulation differentially upregulates responsiveness and potentially supports expansion of the lower affinity cells upon Ag restimulation.

In summary, we demonstrate highly distinct T cell immunopathologic potential in two cohorts of mice expressing retrogenic TCR with minimal structural differences though more dramatically altered affinity for MOG35–55. Different sources are responsible, including cell-intrinsic effects on cytokine patterns by pathologic CD4+ T cells as well as altered T cell differentiation and the induction of protective DN and CD8+ T cell responses. The presence of high-affinity self-antigen–specific T cells may therefore provide a barrier, protecting the host against pathologic autoimmune responses. Further analyses of the effect of TCR affinity on autoimmune-specific T cell differentiation profiles are warranted to better define how T cell repertoire properties influence autoimmune susceptibility.

Acknowledgments

We thank Richard Cross, Greig Lennon, Stephanie Morgan, and the Immunology Core Flow Cytometry facility for assistance with flow cytometric sorting.

Disclosures

The authors have no financial conflicts of interest.

References


Downloaded from http://www.jimmunol.org/ by guest on April 12, 2017


Supplemental Figure S1. T cell engraftment in class I MHC deficient mice. To define the requirement for class I MHC in the development and engraftment of G107S CD8+ and DN T cells, transduced Rag1-/-β2m-/- or Rag1-/-β2m+/+ HPC were transplanted into mice of the same genotype. Relative CD4, CD8, and DN T cell engraftment among splenocytes is shown. The data indicates that in the absence of class I MHC, residual though diminished relative engraftment of CD8+ T cells and increased engraftment of DN cells is present (p<0.01 for each comparison).