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CD4⁺ T Cells from Lupus-Prone Mice Avoid Antigen-Specific Tolerance Induction In Vivo

Farida Bouzahzah,* Sungsoo Jung,2* and Joseph Craft3*†

Activated T cells in spontaneous lupus presumably bypass normal tolerance mechanisms in the periphery, since thymic tolerance appears intact. To determine whether such T cells indeed avoid in vivo peripheral tolerance mechanisms, we assessed their activation and recall responses after in vivo Ag stimulation in the absence of exogenously supplied costimulatory signals. Naïve CD4⁺ AND (transgenic mice bearing rearranged TCR specific for pigeon cytochrome c, peptides 88–104) TCR-transgenic T cells, specific for pigeon cytochrome c, from lupus-prone Fas-intact MRL/Mp⁺Fas-/+ and from II-2⁻⁻ mice were adoptively transferred into MRL × CBA/F₁ or (MRL × B10)F₁ recipients transgenically expressing membrane-bound pigeon cytochrome c as a self-Ag. MRL.AND and control B10.AND and B10.AND-transgenic T cells were activated and divided after transfer, indicating encounter with their cognate Ag; however, T cells from B10.AND mice were impaired in their ability to proliferate and produce IL-2 after challenge with pigeon cytochrome c in ex vivo recall assays, a typical phenotype of anergized cells. By contrast, MRL.AND T cells proliferated more, and a significantly higher percentage of such cells produced IL-2, compared with control T cells. This observation that MRL T cells avoided anergy induction in vivo was confirmed in an in vitro system where the cells were stimulated with an anti-CD3 in the absence of a costimulatory signal. These experiments provide direct evidence that CD4⁺ T cells from Fas-intact lupus-prone MRL mice are more resistant than non-autoimmune control cells to anergy induction. Anergy avoidance in the periphery might contribute to the characteristic finding in lupus of inappropriate T cell activation in response to ubiquitous self-Ags. The Journal of Immunology, 2003, 170: 741–748.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by high titer IgG autoantibodies to certain intracellular components, including chromatin and ribonucleoproteins (1). Several inbred mouse strains also develop spontaneous lupus, with the same spectrum of autoantibodies. Certain of these pathologies are pathogenic, including those against chromatin that can induce immune-complex glomerulonephritis. Such autoantibodies in lupus appear to arise as a consequence of autoantigen-specific CD8 T cell help, a notion originally derived from the demonstration that neonatal thymectomy of lupus-prone mice led to abrogation of anti-dsDNA IgG synthesis and glomerulonephritis and to increased survival (2). Similar results were obtained after Ab depletion of either Thy.1⁺ (3) or CD4⁺ cells (4), or genetic deletion of αB T cells in lupus-prone mice (5). Autoreactive T cells that provide pathogenic B cell help in lupus appear to be specific for ubiquitous self-peptides, including those derived from chromatin and ribonucleoproteins (6–8).

The mechanisms of tolerance escape for T cells responsive to ubiquitous self-peptides in lupus are unknown, although central tolerance appears intact, as determined by studies of thymocyte deletion using superantigens (9–11) as well as conventional peptide Ags (12–14), including in Fas (CD95)-intact mice (15). Thus, it appears that activation of autoreactive T cells in lupus is a consequence of peripheral tolerance abrogation, a concept that finds support in vitro studies of human T cells (16). By contrast, in vivo work has suggested that peripheral T cell tolerance is intact in murine lupus (17).

We have hypothesized that lupus T cells have intrinsic (genetic) defects that render them more susceptible to activation through their TCR-CD3 complex after contact with self-peptides, leading to genetically abnormal lupus B cells and subsequent pathogenic autoantibody production (18). This hypothesis stems in part from the observations that T cells from humans with SLE appear to have abnormalities in TCR signaling (19–25) and apoptosis (26–28), as well as in expression of effector molecules, including CD40 ligand (CD154) (29–33), and from genetic studies that suggest a locus on chromosome 7 from lupus-prone New Zealand mixed mice contributes to a lower threshold of T cell activation and a higher threshold for apoptotic death (34, 35). More recent data from our laboratory has supported the concept that lupus T cells are intrinsically abnormal, demonstrating that naïve, mature CD4⁺ T cells from lupus-prone Fas-intact MRL/Mp⁺Fas-/+ (MRL/Fas-/+ mice are hyperproliferative after engagement of MHC-self-peptide complexes in vitro (36).

An intrinsic T cell abnormality, perhaps contributing to a hyperproliferative phenotype after encounter with self-Ag, would seem logical in a disease such as lupus characterized by autoreactive T cell activation to ubiquitous self-Ags. However, these studies asking whether lupus T cells are intrinsically abnormal have not determined that such cells avoid peripheral tolerance induction in vivo, as a potential contributing pathway for autoreactive T cell

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4 Abbreviations used in this paper: SLE, systemic lupus erythematosus; PCC, pigeon cytochrome c; DC, dendritic cell; mPCC, membrane PCC; L, ligand.
activation. To address this question, we asked whether CD4+ T cells from lupus-prone mice could be anergized by contact with ubiquitous self-Ags in the periphery in comparison to control T cells from nonautoimmune mice. To this end, CD4+ AND (transgenic mice bearing TCR specific for pigeon cytochrome c, peptides 88–104) TCR-transgenic T cells, specific for pigeon cytochrome c (PCC), from lupus-prone Fas-intact MRL/Mp+/Fas–/– and from H-2d–matched control CBA/Ca and B10.BR mice (MRL.AND, CBA.AND, and B10.AND, respectively) were adoptively transferred into (MRL × CBA)F1 or (MRL × B10)F1 recipients transgenically expressing membrane-bound PCC (mPCC) as a self-Ag. Ag encounter in this protocol, in the absence of exogenously supplied costimulatory triggers, normally induces anergy in the adoptively transferred cells, even if such Ags are presented by bone marrow-derived or dendritic cells (37, 38). As expected, MRL.AND and control CBA.AND- and B10.AND TCR-transgenic CD4+ T cells were activated and divided after transfer, indicating encounter with their cognate Ag; however, T cells recovered from CBA.AND and B10.AND mice were impaired in their ability to proliferate and produce IL-2 after challenge with PCC in ex vivo recall assays, a typical phenotype of anergized cells. In comparison, MRL.AND T cells avoided anergy induction, with significantly more proliferation and IL-2 production than control T cells, a phenotype that was confirmed with an in vitro system where the cells were stimulated with anti-CD3 in the absence of costimulation. In addition to providing evidence that CD4+ T cells from lupus-prone Fas-intact MRL mice avoid anergy induction in periphery after contact with ubiquitous self-Ags, these experiments suggest that such cells are intrinsically abnormal.

Materials and Methods

Mice

AND-transgenic mice, expressing an aβ TCR (Vα11+, Vβ3+) recognizing PCC, were originally provided on the B10.BR background by S. Hedrick (University of California, San Diego, CA) (39). The transgenic locus was serially backcrossed to the Fas-intact MRL/Fas–/– (MRL.AND) and to the H-2d–matched background B10.BR (B10.AND) for >20 generations, and to the control H-2d CBA/Caj strain (CBA.AND) for >10 generations. The three test strains lack endogenous (viral) superantigens, products of murine mammary tumor viruses that bind the transgenic Vβ3 chain, with resultant central deletion in the context of I-E–E (40). Levels of expression of the transgenic Vα and Vβ chains and the CD4 coreceptor were equivalent among the three strains, as determined by flow cytometry. TCR-transgenic mice were maintained as heterozygotes, with screening performed for expression of the transgenic Vα (H-2k) and Vβ (H-2d) chains and the CD4 coreceptor by gating on the CFSE fluorescence on TCR-transgenic T cells, specifically CD4+ T cells from pooled lymph nodes and spleens extracted from nontransgenic F1, respectively. The comparison of responses between CD4+ T cells from pooled lymph nodes and spleens extracted from nontransgenic MRL, B10.BR and CBA/CAJ mice were incubated at 1 × 106 cells/well in 1 ml in 24-well plates previously coated with purified anti-CD3 mAb at 10 μg/ml. Two days later, the cells were washed and incubated in fresh medium for 2 days (resting). The cells were labeled with CFSE as described above and finally restimulated in vitro with anti-CD3 mAb (10 μg/ml) and anti-CD28 mAb (1 μg/ml), with cell divisions measured by CFSE dilution using flow cytometry. As a control, CD4+ T cells from these mice were also initially stimulated with anti-CD3 in the presence of anti-CD28, resteed, and then restimulated with anti-CD3 and anti-CD28 as above.

Proliferation assays

Briefly, 2 × 105 cells from pooled lymph nodes and spleens extracted from transfer recipients were cultured in 96-well flat-bottom tissue culture plates in the presence of different concentrations of synthetic PCC peptide targeted by the AND TCR transgenes (PCC 88–104; American Peptide Company, Sunnyvale, CA; peptide purity >90% by HPLC analysis). Seventy-two-hour cultures were pulsed with 1 μCi of [3H]thymidine and incubated for an additional 18 h. Cells were then harvested and proliferation was measured by determination of the amount of incorporated radioactivity.

Intracellular cytokine staining

CD4+ T cells from pooled lymph nodes and spleens taken from transfer recipients were stimulated with 1 μM PCC for 2 h and pulsed with a Golgi Plug (BD PharMingen) for an additional 4 h. Cells were then harvested, fixed, washed, and incubated in PBS with 2% formaldehyde for 20 min at room temperature, and permeabilized with PBS containing 0.5% saponin (Sigma-Aldrich, St. Louis, MO) for 10 min. Cells were incubated with PE-labeled anti-IL-2 mAb at room temperature. After 1 h, the cells were washed and then incubated with CyChrome-conjugated CD4, biotin-conjugated Vα11, and allophycocyanin-conjugated streptavidin, followed by flow cytometric analysis. The percentage of transgenic cells producing IL-2 was determined by gating on the CFSE–CD4+Vα11–transgenic population.

Anergy induction in vitro

Exposure to anti-CD3 mAb in the absence of a costimulatory signal induces anergy in resting murine and human T cells (45, 46). We adopted this principle to induce anergy in vitro, as previously described (47). Briefly, highly purified CD4+ T cells from nontransgenic MRL, B10.BR and CBA/CAJ mice were incubated at 1 × 105 cells/well in 1 ml in 24-well plates previously coated with purified anti-CD3 mAb at 10 μg/ml. Two days later, the cells were washed and incubated in fresh medium for 2 days (resting). The cells were labeled with CFSE as described above and finally restimulated in vitro with anti-CD3 mAb (10 μg/ml) and anti-CD28 mAb (1 μg/ml), with cell divisions measured by CFSE dilution using flow cytometry. As a control, CD4+ T cells from these mice were also initially stimulated with anti-CD3 in the presence of anti-CD28, resteed, and then restimulated with anti-CD3 and anti-CD28 as above.

Statistical analysis

Comparative data were analyzed using the unpaired Student’s t test. Data shown are representative for at least three separate experiments with two or three age- and sex-matched animals in each group.

Results

Characterization of transferred cells

PCC-specific TCR-transgenic CD4+ T cells were isolated from MRL.AND, CBA.AND, and B10.AND mice and transferred to F1 recipients ((MRL × CBA)F1) and (MRL × B10)F1 with or without expression of PCC as a membrane Ag (mPCC-transgenic F1 or nontransgenic F1, respectively). The comparison of responses between CD4+ CBA.AND and MRL.AND or between B10.AND and MRL.AND T cells was conducted in separate experiments...
using (CBA × MRL)F1 or (B10 × MRL)F1 recipients, respectively. The purity of transgenic CD4+ T cells before transfer was consistently >97%, with >98% expressing TCR Vα11, and >95% of these cells were naive as determined by CD44low and CD62Lhi expression (data not shown) (48). In vitro activation studies demonstrate that AND cells respond very efficiently to PCC (36).

AND TCR-transgenic CD4+ T cells divide after Ag encounter in vivo

Cell division was not observed when the CD4+ TCR-transgenic cells were transferred to nontransgenic F1 mice, as determined by the lack of CFSE dilution (Fig. 1, A and B, dashed histograms). By contrast, upon transfer to mPCC F1 animals, transgenic cells from all three strains (MRL.AND, CBA.AND, and B10.AND) underwent multiple cell divisions, suggesting that these cells encountered their cognate Ag in the PCC-expressing recipients (Fig. 1, A and B, solid histograms). In this study, adoptive transfers of MRL.AND and CBA.AND cells to nontransgenic and mPCC-expressing (MRL × CBA)F1 recipients were done side by side (see examples, Fig. 1A), as were transfers of MRL.AND and B10.AND cells to nontransgenic and mPCC-expressing (MRL × B10)F1 recipients (see examples, Fig. 1B). Even though the number of cell divisions was similar among the three groups, more MRL.AND T cells appeared to enter the cell cycle after transfer to mPCC-expressing hosts, as relatively fewer cells remained in the undivided pool (M0) compared with the two controls (compare the numbers of MRL.AND to CBA.AND cells in the pool labeled M0 in Fig. 1C, left panel, and to B10.AND cells in the pool labeled M0 in Fig. 1C, right panel).

We also asked whether the CFSE-labeled transferred cells acquired an activated phenotype. Spleen and lymph nodes were extracted from the recipients and the cells were stained with anti-CD44 and anti-CD62L mAbs. By using flow cytometry and gating on CD4+ Vα11+ CFSE+ cells, we could selectively analyze the AND-transgenic T cells. When transferred to mPCC F1 recipients, the transgenic AND T cells up-regulated CD44 and down-regulated CD62L compared with cells transferred to nontransgenic F1 animals (data not shown). These data, along with analysis of cell division (Fig. 1), provide evidence that the TCR-transgenic T cells encounter Ag in vivo.

Greater recovery of MRL AND T cells, compared with controls, after adoptive transfers

We next determined cell recovery after adoptive transfers. The same numbers of CFSE-labeled transgenic CD4+ T cells purified from MRL.AND, CBA.AND, and B10.AND mice were transferred to F1 recipients. Ten days later, the spleen and lymph nodes were extracted and the total number of transgenic cells and the number of transgenic cells determined as a percentage of the total lymphocytes were assessed by gating on the CD4+CFSE+Vα11+ cells. The total number of MRL.AND T cells recovered from nontransgenic mice was significantly greater than that of CBA.AND and B10.AND cells (Fig. 2), as was the percentage of transgenic MRL.AND cells compared with controls (data not shown). These findings suggest that MRL cells have a survival advantage over the nonautoimmune controls, even without cell division. Similarly, a greater number and a greater percentage of MRL.AND, compared with control, cells were recovered after transfer to mPCC F1 hosts.

FIGURE 1. Naive CD4+ AND TCR-transgenic T cells divide after Ag encounter in vivo. CD4+ T cells purified from MRL.AND, CBA.AND, and B10.AND mice were adoptively transferred to the F1 recipients with or without expression of PCC as membrane-bound Ag. Transfers of MRL.AND and CBA.AND cells to nontransgenic and mPCC-expressing (MRL × CBA)F1 recipients were done side by side (A), as were transfers of MRL.AND and B10.AND cells to nontransgenic and mPCC-expressing (MRL × B10)F1 recipients (B). Solid and dashed histograms represent transfers to mPCC transgenic and nontransgenic recipients, respectively. The percentages of MRL.AND and CBA.AND TCR-transgenic T cells in each division, and similarly MRL.AND and B10.AND cells, after transfer to mPCC-expressing (MRL × CBA)F1 and (MRL × B10)F1 recipients are shown (C: M0–M7, with M0 representing undivided cells and M7 representing cells having undergone seven divisions). Data shown are representative of three separate experiments with two or three age- and sex-matched animals in each group.
FIGURE 2. More MRL.AND T cells than control AND T cells were recovered after adoptive transfers to F1 recipients. The same number of PCC-specific CD4" T cells purified from MRL.AND, CBA.AND, and B10.AND mice was transferred to mPCC-transgenic and nontransgenic F1 recipients. Ten days after the adoptive transfer, spleens and lymph nodes were extracted and the total number of the transgenic cells was determined by gating on the CFSE"CD4"Vα11" cells. The total number of MRL.AND T cells vs CBA.AND T cells after transfer to nontransgenic and mPCC-expressing F1 mice were compared (A), as were the number of MRL.AND and B10.AND cells (B). Data shown are representative of at least three separate experiments with two or three age- and sex matched animals in each group. Non-Tg, Nontransgenic.

(T2 and data not shown), a finding that might in part be attributable to greater survival of the former or perhaps more resistance to deletion after initial Ag encounter with greater numbers of cells after division. Although we recovered more MRL.AND cells than control cells after adoptive transfer, the comparison with CBA.AND cells after transfer to both nontransgenic and mPCC-expressing hosts was more variable, with one experiment (of three performed) demonstrating roughly equivalent survival (see Fig. 4 below).

T cells from MRL.AND mice avoid anergy induction in vivo

T cell anergy is a functionally defined state of hyporesponsiveness in which T cells neither proliferate nor produce IL-2 following subsequent TCR ligation (49). Exposure of TCR-transgenic T cells to their cognate Ag in vivo in the absence of costimulation, leads to an anergic phenotype (45, 46). To evaluate anergy induction, we next investigated the capacity of the adoptively transferred TCR-transgenic T cells to respond to PCC in vitro in proliferation assays. As expected, in one experiment, when retrieved from the mPCC-expressing hosts was more variable, with one experiment (of three performed) demonstrating roughly equivalent survival (see Fig. 4 below).

We also determined the capacity of adoptively transferred transgenic T cells to respond to PCC in vitro in proliferation assays. As expected, in one experiment, when retrieved from the mPCC-expressing hosts was more variable, with one experiment (of three performed) demonstrating roughly equivalent survival (see Fig. 4 below).

FIGURE 3. Control CD4" AND TCR-transgenic T cells retrieved from F1, mPCC-transgenic mice have impaired ability to produce IL-2 in ex vivo recall assays compared with MRL.AND cells. Spleen and lymph nodes were extracted from the recipients, stimulated ex vivo with PCC (1 μM), and stained for intracytoplasmic IL-2. AND-transgenic cells were analyzed using flow cytometry by gating on CFSE"CD4"Vα11" cells. Adoptive transfers of MRL.AND and CBA.AND cells and MRL.AND and B10.AND cells were done side by side (A and B, respectively). Non-Tg, Nontransgenic.
noted above (Fig. 2), cell survival after adoptive transfer to non-transgenic or to mPCC transgenic hosts was generally lower for control AND vs MRL.AND cells, a variable that might potentially confound proliferation assays using intact spleen and lymph nodes, as done here. To avoid this potentially confounding variable, we compared proliferation of CBA.AND and MRL.AND cells from the one experiment where the cell recovery was similar. In this experiment, CBA.AND T cells recovered in spleens and lymph nodes from mPCC F1 mice did not proliferate at any tested concentration of PCC (Fig. 4A). Under the same conditions, MRL.AND cells proliferated more and in a dose-dependent manner. These data further suggest that the control CBA.AND cells, after exposure to cognate Ag in vivo, were refractory to the secondary stimulation in vitro. Addition of IL-2 to the CBA.AND cultures led to a statistically significant recovery of proliferation in an Ag dose-dependent manner (Fig. 4B). This phenotype is consistent with anergy induction.

CD4+ cells from MRL lupus-prone mice are less susceptible to anergy induction in vitro

To corroborate our in vivo data, we used an in vitro system in which T cells are anergized by anti-CD3 in the absence of a costimulatory signal. In this study, we used both nontransgenic and AND TCR-transgenic CD4+ T cells, since these experiments were done with anti-CD3 triggering. In vitro anergized cells from autoimmune nontransgenic MRL mice proliferated significantly more in response to anti-CD3 and anti-CD28 stimulation than B10 and CBA controls, with the former undergoing more than five divisions compared with the latter that underwent more than two to three divisions (Fig. 5). Greater numbers of MRL cells also entered the cell cycle compared with control cells (Fig. 5B, compare columns labeled M0). Similar results were also obtained with AND TCR-transgenic T cells. These differences cannot be explained solely by any survival advantage of MRL T cells, since in this analysis we gated only on live cells. As a control, CD4+ T cells from the same nontransgenic mice were also stimulated with anti-CD3 in the presence of anti-CD28, followed by rest and then restimulation with anti-CD3 and anti-CD28. After such treatment, cells from all strains divided approximately equally at the tested concentration of anti-CD3, as shown by CFSE dilution (data not shown).

Discussion

Activated T cells drive pathogenic autoantibody production in mice and presumably in humans with lupus (18). Such T cells presumably bypass normal tolerance mechanisms in the periphery, since thymic tolerance appears intact, at least in lupus-prone mice (9–15). In the current study, we asked whether T cells from such mice avoided in vivo peripheral tolerance mechanisms upon exposure to ubiquitous self-Ags in vivo. We used an experimental strategy of adoptive transfer of TCR-transgenic CD4+ T cells to mice expressing the cognate (self) Ag, an approach adapted from earlier studies that investigated tissue-specific T cell tolerance induction (37, 50) and one originally based on the studies of Jenkins and coworkers (44) dissecting Ag-specific responses in vivo. The former work demonstrated that encounter with the cognate Ag expressed on parenchymal or on bone-marrow derived cells, in the absence of exogenously supplied costimulatory signals, led to an initial phase of CD4+ T cell expansion, followed by deletion with the remaining cells becoming anergic (37, 50).

We found that MRL.AND CD4+ T cells, as well as CBA.AND and B10.AND control cells, retrieved from mPCC F1 mice had an activated phenotype consistent with Ag encounter; however, the
retrieved transgenic cells had impairments in their ability to proliferate and to produce IL-2 when challenged with PCC ex vivo. Nevertheless, under these conditions, significantly more MRL.AND T cells produced IL-2, with enhanced proliferation, suggesting that by comparison to H-2<sup>k</sup>-matched controls, MRL.AND cells were resistant to anergy induction in vivo. This notion was supported by in vitro experiments where MRL T cells, after initial stimulation with anti-CD3 in the absence of anti-CD28, proliferated more than control CBA and B10 T cells following restimulation with anti-CD3 and anti-CD28.

We believe that a principal mechanism of induced unresponsiveness in the cells adoptively transferred to recipients expressing the cognate Ag bound by the TCR-transgenic cells was anergy induction. In support of that idea, we were able to identify transferred cells isolated from mPCC-expressing F<sub>1</sub> recipients, with reduced IL-2 synthesis in recall assays ex vivo, compared with cells transferred to nontransgenic recipients (Fig. 3). Moreover, the addition of IL-2 to CBA.AND T cell cultures partially reversed the state of unresponsiveness (Fig. 4B). Such partial reversal is likely explained by the fact that in vivo anergized CD<sup>4+</sup> T cells are defective in their ability to synthesize IL-2 and in their responsiveness to signals through the IL-2R (51, 52). Finally, as noted above, other investigators have shown in similar systems that adoptively transferred TCR-transgenic CD<sup>4+</sup> T cells, upon exposure to cognate (self) Ag in vivo, undergo anergy induction (37, 50). It is now clear that the lack of exogenously supplied costimulation is important for the establishment of anergy in these systems. For example, treatment with Ab against CD40 prevents T cell unresponsiveness, converting tolerogenic signals into immunogenic ones (53), even when self-Ags are expressed by DC (38).

Cell deletion also likely contributed to tolerance induction following transfer of AND T cells to mPCC-expressing hosts in our experiments. As discussed above, TCR-transgenic CD<sup>4+</sup> T cells adoptively transferred in analogous experiments underwent deletion following an initial phase of expansion with surviving cells being anergized (37, 50). Likewise, presentation of a MHC class I-restricted self-Ag to TCR-transgenic CD<sup>4+</sup> T cells, using a similar adoptive transfer, induces tolerance in these cells, with clonal deletion as the mechanism (54). Nevertheless, we typically recovered more MRL.AND than control AND T cells following transfer to mPCC-expressing hosts, and MRL.AND cells on average divided more than controls (Figs. 1 and 2). In other words, MRL cells may be more resistant to deletion than control cells following Ag encounter in the absence of costimulation, with greater division and numbers of cells recovered at each division. MRL cells also appear to simply have a survival advantage in vivo, a notion supported by the finding that transfer of these cells to nontransgenic mice in these relatively short-term experiments generally led to a greater survival than for control cells (Fig. 2). The mechanisms for the presumed resistance to deletion and/or enhanced survival in vivo for MRL cells, compared with controls, are not apparent at present, but are under investigation in our laboratory, as are patterns of cell death. Thus, although our work suggests that T cells from lupus-prone mice are resistant to Ag-specific anergy induction in vivo and in vitro compared with control cells, other abnormalities in tolerance avoidance of MRL cells may also be operative in these experiments.

It would seem logical that anergy avoidance in the periphery might contribute to the characteristic finding in lupus of inappropriate T cell activation in response to ubiquitous self-Ags. The mechanism of anergy avoidance in MRL cells under these conditions, compared with control cells, remains undefined; however, recent work examining T cells from SLE patients perhaps provides some clues. Such T cells are resistant to anergy induction in vitro, with prolonged, high-level expression of CD40L (CD40L, CD154) even after being subjected to anergy-inducing conditions that suppress its expression on normal T cells. Prolonged expression of CD40L on lupus T cells in these experiments correlated with diminished phosphorylation of Cbl/Cbl-b (16), members of a family of adaptor molecules that play a role in the degradation of phosphorylated proteins (55, 56). Cbl-b has been shown to be a key regulator of activation thresholds in mature lymphocytes and immunological tolerance (57). Mice lacking the adaptor Cbl-b develop spontaneous autoimmune disease characterized by autoantibody production and infiltration of activated T and B cells in multiple organs (57).

Although we did not explore the cellular source of the self-Ag PCC and the nature of the APC that induce anergy, Adler et al. (37), in a very similar transgenic system, demonstrated an absolute requirement of bone marrow-derived APCs for cross-presentation of Ag in a tolerogenic manner. Although the conventional view is that DC are constitutively involved in T cell priming, accumulating data clearly show that such APC can also tolerize T cells under certain circumstances (58, 59). For example, in elegant work, Hawiger et al. (38) developed a system where they charged DC with Ag under steady-state conditions. Ag-specific transgenic T cells exposed to these DC in vivo either disappear or become anergic to Ag restimulation, a situation that was abrogated with appropriate costimulation.

There are data from human and murine studies suggesting that macrophages, DC, and/or B cells are defective in lupus-prone mice or in lupus patients (60–64), abnormalities that potentially could contribute to aberrant T cell phenotypes in MRL mice. However, in the current work transgenic T cells from the lupus-prone and control mice were transferred to the same F<sub>1</sub> recipients with a self-Ag that is broadly expressed. Therefore, anergy avoidance of MRL T cells compared with control cells is unlikely to be a consequence of selectively aberrant APC function.

In contrast to our data suggesting the CD4<sup>+</sup> T cells from lupus-prone mice are resistant to anergy induction in vivo, other work has indicated that T cell tolerance induction is normal in (NZB × NZW)<sub>F<sub>1</sub></sub> lupus-prone mice (17). Although these differences might be explained on the basis of analysis of different lupus models, this earlier work used a system where the lupus-prone mice transgenically expressed beef insulin, with tolerance investigated after self-Ag administration in CFA. Under these conditions, beef insulin-specific T cells could not be recalled ex vivo, suggesting that they were normally tolerized or perhaps deleted. However, insulin-specific T cells in these animals potentially could have been deleted centrally or perhaps existed in small numbers in the periphery, in contrast to our study where we transferred relatively large numbers of naive, Ag-specific T cells. B cell tolerance to beef insulin may have also contributed to the lack of T cell responsiveness, given the critical role of these cells as APC for autoreactive T cell activation in lupus (65).

The current data indicate that MRL T cells escape Ag-specific tolerance induction in vivo, with enhanced responsiveness after engagement with self-Ag in the absence of specific costimulation. We have recently demonstrated that MRL cells, compared with H-2<sup>k</sup>-matched controls, are hyperresponsive to MHC-peptide TCR interactions in vitro (36). Based on these two studies, we would argue that intrinsic T cell hyperresponsiveness after normal anergy signals in the periphery contribute to polyclonal T cell activation, a feature of lupus-prone individuals (66). Although it is possible that the abnormalities we have identified are the result of strain differences between the MRL and nonautoimmune backgrounds and are not related to the lupus phenotype, supportive data indicate that T cells from lupus-prone mice and humans are genetically
abnormal (19–27, 34, 35). We therefore would suggest the possibility that intrinsic T cell hyperresponsiveness with defective Ag-specific anergy induction contributes to T cell activation in lupus, leading to subsequent help for genetically abnormal B cells with resultant pathogenic autoantibody production.

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