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The Role of Host (Endogenous) T Cells in Chronic Graft-Versus-Host Autoimmune Disease

Fangqi Chen,* Michael A. Maldonado,* Michael Madaio,† and Robert A. Eisenberg‡*

Chronic graft-vs-host (cGVH) disease induced by the transfer of Ia-incompatible spleen cells from one normal mouse strain (such as B6.C-H2<sup>bm12</sup>/KhEg (bm12)) to another (such as C57BL/6) causes an autoimmune syndrome resembling systemic lupus erythematosus (SLE). The role of host-derived T cells in this response is not obvious. Previous reports suggested that host T cells might serve to down-regulate the autoimmune syndrome. To address this issue more definitively, we used CD4 knockout (KO) or CD8KO C57BL/6 (B6) mice as recipients in the bm12→C57B6 cGVH model. CD4KO B6 mice injected with allogeneic bm12 spleen cells (bm12→CD4KO group) showed no evidence of cGVH disease. They made no detectable autoantibodies, including anti-Chromatin, anti-dsDNA, anti-ssDNA, and rheumatoid factor. They survived at least 20 weeks after induction of cGVH disease; and they did not develop nephritis, based on the absence of detectable levels of proteinuria and normal renal histology at the time of sacrifice. By contrast, CD8KO B6 mice (bm12→CD8KO group) showed similar levels of mortality, nephritis, and autoantibodies, although the autoantibody titers declined somewhat after week 8 in the bm12→CD8KO group. Control groups of recipients injected with B6 spleen cells showed no induction of autoantibodies. A surprising finding, however, was that the B6→CD8KO group developed severe histologic glomerulonephritis in the absence of autoantibodies and with decreased immune deposits. These results indicate that endogenous (host) CD4<sup>+</sup> T cells play an essential role in the cGVH autoimmune syndrome. The Journal of Immunology, 1998, 161: 5880–5885.

S
ystemic lupus erythematosus (SLE<sup>3</sup>) is characterized by the production of autoantibodies against chromatin, Sm, DNA, and other nuclear antigens, as well as the deposition of Ig in various organs, including the kidneys and skin. These immunologic events are accompanied by the development of glomerulonephritis, although the underlying mechanisms responsible for the renal disease are not fully understood. An excellent experimental animal model of SLE can be induced by the transfer of spleen cells from nonautoimmune B6.C-H2<sup>bm12</sup>/KhEg (bm12) mice into coisogenic, nonirradiated C57BL/6 (B6) recipient mice (1–4). The bm12 strain has a mutant form of I-A that differs by three amino acids in the β-chain from the I-A<sup>B</sup> of B6 (5). Since murine T cells do not express MHC class II molecules, the donor T cells are not recognized as foreign by the recipient and are thus not rejected. Therefore, the chronic graft-vs-host (cGVH) disease induced by interparental transfers between B6 and bm12 is entirely comparable to what is usually seen in the DBA/2→C57BL/6F<sub>1</sub> and other parent→F<sub>1</sub> strain combinations used by other investigators (reviewed in Refs. 6 and 7). In the DBA/2→C57BL/6F<sub>1</sub> model, both MHC class I and class II differences are present between donor and recipient, and the development of cGVH disease depends on a relative inactivity of the donor CD8<sup>+</sup> T cells (6). Accordingly, this system can be driven toward an acute GVH by the in vivo administration of rIL-12 (8) or anti-OX40 Ab (9), or by increasing the percentage of donor CD8<sup>+</sup> T cells (10).

Our working hypothesis for the cellular mechanism of this autoimmune syndrome has been that the allogeneic donor T cells recognize the recipient B cells’ MHC class II molecules together with some peptides that may or may not be derived from the autoantigens against which the B cells react. This allogeneic effect then delivers an abnormal T cell help signal to the B cells and drives them to produce inappropriate specificities, i.e., autoantibodies. Such a model would not postulate any essential role for the host’s endogenous T cells. Nevertheless, several lines of evidence have suggested that recipient T cells might be involved in the cGVH autoimmune responses. Rolink et al. (11) reported that adult-thymectomized, irradiated, bone marrow-reconstituted recipients of allogeneic T cells had a more severe cGVH syndrome than intact recipients. On the other hand, repeated attempts in our laboratory to induce cGVH in athymic (nu/nu) B6 recipients by the transfer of varying numbers of bm12 spleen cells have not led to the production of autoantibodies (our unpublished data). Finally, Gonzalez et al. (12) observed that the ability of B cells from F<sub>1</sub> hybrid mice to respond to allogeneic help from parental T cells in vitro depended on the presence of CD4<sup>+</sup> T cells in the B cell donor. Collectively, these studies suggest that the cellular interactions that induce the production of autoantibodies in the cGVH may require recipient T cells.

To address this issue, we used CD4 knockout (KO) and CD8KO B6 mice as recipients in our cGVH model (13, 14). In these strains, the CD4<sup>+</sup> or CD8α genes have been inactivated by homologous recombination. We found that allogeneic splenocyte transfer did not induce a cGVH disease in CD4KO mice, as determined by mortality, autoantibody production, and renal disease. CD8KO recipients, on the other hand, produced somewhat lower levels of autoantibodies in the late stage of cGVH disease than normal B6 mice.
recipient mice, while they showed comparable severity of renal disease. These results indicate that host (endogenous) T cells, especially host CD4+ T cells, play very important roles in the cGVH autoimmune responses.

Materials and Methods

Mice

C57BL/6-Cd8a+tm1Mak (CD4KO), C57BL/6d (B6), and B6.C-H2+tm1/KhlEg (bm12) mice, originally obtained from The Jackson Laboratory (Bar Harbor, ME), and C57BL/6-Cd8a+tm1Mak (CD8KO) mice, originally obtained from Tak W. Mak (University of Toronto, Canada), were bred and maintained in our mouse colony at the University of Pennsylvania Medical Center. Strains B6 and bm12 differ only by three amino acids in the β-chain of the I-A molecule. Recipient and donor mice were sex- and age-matched within each independent experiment.

Experimental cGVH disease protocol

cGVH disease was induced as previously described (1). Briefly, recipient mice between 2–5 mo of age were injected (i.p.) with single cell suspensions of 1 × 10^6 donor splenocytes, prepared by pressing donor spleens through a wire mesh screen in HBSS. Blood samples were obtained from experimental mice at the induction of cGVH disease and at 2- to 4-wk intervals thereafter. Sera were stored at −20°C for later analysis.

Detection of autoantibodies

The expression of autoantibodies was assessed by ELISA, as previously described (4). Autoantigens (see below) were diluted in borate-buffered saline (BBS), added to polyvinyl microtiter plates (Dynatech Laboratories, Alexandria, VA), and incubated 4 h at room temperature or overnight at 4°C. The plates were washed with BBS and blocked with BBS supplemented with Tween and BSA (BBT:BBS, 0.01 M diethanolamine, pH 9.8, was added. plates were washed and incubated fo r1ha t room temperature with avidin-biotinylated rat anti-mouse IgM, (clone HB63) and mouse IgM (clone CBPC 112) were used as standards in

Experimental design

Table I. cGVH disease experimental design

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mice</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<td>B6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>CD8KO experiments</td>
<td>bm12→B6</td>
<td>B6</td>
<td>10</td>
<td>9</td>
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"Data are from one of three comparable experiments. 
"Data are pooled from two of three comparable experiments.

Results

Induction of cGVH autoimmune disease

cGVH disease was established in unirradiated recipient mice (Table I) by i.p. injections of a single dose of 1 × 10^8 age-sex-matched donor cells. Mice were followed for survival and for periodic determination of urinary protein and collection of serum. As shown in Fig. 1, bm12→CD8KO group and bm12→B6 groups showed similar mortality rates, reaching 50–60% at week 20 after the induction of cGVH disease. By contrast, 10/11 mice in the B6→CD8KO groups and all mice in B6→CD4KO and bm12→CD4KO groups survived to week 24. Additional control groups of B6→B6 (n = 10), CD4KO→CD4KO (n = 6), and CD8KO→CD8KO (n = 8) also showed no mortality by week 20 (data not shown). The difference in mortality rates between bm12→B6 and bm12→CD4KO groups was highly significant (p < 0.01).

Autoantibodies in cGVH CD4KO and CD8KO recipient mice

The levels and specificities of autoantibody production during cGVH disease were assessed by ELISA. Surprisingly, the bm12→CD4KO group showed no autoantibody titers different from the negative control group (B6→CD4KO), except for a modest, transient elevation of anti-chromatin and RF at week 2, and anti-dsDNA at week 4 (Fig. 2A). As expected, the concomitant bm12→B6 positive control group showed substantial elevations of autoantibody titers of anti-chromatin, RF, anti-ssDNA, anti-dsDNA at all time points tested after week 0. The bm12→CD8KO group also had significant levels of anti-chromatin, RF, anti-ssDNA, anti-dsDNA compared with the negative control (B6→CD8KO group) at all time points except week 0 (p < 0.01). However, the concomitant bm12→B6 groups in these experiments showed even higher levels of anti-chromatin, RF, anti-ssDNA, and anti-dsDNA at later time points (Fig. 2B). The pattern of a gradual falloff in titer over time was unchanged if the analysis included data only from mice that survived the entire experiment (not

basement membrane and vascular immune deposits were judged independently. Multiple sections at a minimum of two different levels were observed. Each section typically involved evaluation of over 50 glomeruli, more than 25 blood vessels, and the interstitium contained within two to three longitudinal sections of kidney.

Statistical and data analyses

The autoantibodies and proteinuria data presented are from a single CD4KO experiment or from two pooled CD8KO experiments, which individually showed similar results. Two subsequent experiments with the CD4KO recipients and one with the CD8KO recipients again gave comparable results. Statistical analysis of data was performed according to the Student t test.

Evaluation of nephritis

Urine protein concentrations were detected at 2- to 4-wk intervals using Uristix reagent strips (Miles Laboratories, Elkhart, IN). The presence and severity of nephritis was determined by hematoxylin and eosin light and immunofluorescence microscopy, as previously described (16). Briefly, for light microscopy, the severity of glomerular, interstitial, and vascular lesions was determined independently by blinded grading by one of us (M.M.) on a scale of 0 to 4+. Similarly, the presence of glomerular, tubular

Table I. cGVH disease experimental design

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shown). In addition, anti-cathepsin C and anti-collagen IV autoantibodies also showed the same pattern as anti-ssDNA in both the CD4KO and CD8KO experiments (data not shown). Striking increases in total serum IgM and IgG were seen in the cGVH groups (bm12→B6 and bm12→CD8KO). The bm12→CD4KO group showed a modest, transient (but significant) elevation of total IgM and IgG at weeks 2, 4, and 8 (Fig. 3).

Nephritis in cGVH CD4KO and CD8KO recipient mice

Proteinuria was measured at 2- to 4-wk intervals in female recipients to assess renal involvement during cGVH disease. As shown in Fig. 4, bm12→CD4KO and B6→CD4KO mice failed to show any significant elevation of renal protein excretion, while concomitant bm12→B6 mice showed substantial levels. In contrast, bm12→CD8KO mice showed elevated proteinuria levels comparable to the concomitant positive control (bm12→B6 group).

To examine the degree of renal involvement directly, surviving recipient mice were sacrificed at 20 wk (CD4KO experiment) or 24 wk (CD8KO experiments) after the induction of cGVH disease. Hematoxylin and eosin (H&E)-stained kidney sections were scored by light microscopy for severity of glomerular lesions, interstitial lesions, and vascular lesions. The quantity and location of IgG and C3 deposits were determined by immunofluorescence microscopy. Individual kidney scores are shown in Fig. 5. The mean values of all kidney scores of bm12→B6 mice in the CD4KO experiments were significantly higher than those of bm12→CD4KO or those of B6→CD4KO mice (p < 0.005), while the latter two groups did not differ (p > 0.05). The light microscopy scores of the
mice showed significant renal abnormalities (data not shown). Neither unmanipulated CD8KO mice nor control CD8KO B6 mice showed pathologic character of the glomerular involvement of the scores for IgG and C3 deposition, while bm12 B6 was surprisingly, so were those of B6 CD4KO and B6 CD8KO at the same time point (p < 0.002); +, either bm12→B6 or bm12→CD8KO is significantly different from B6→CD8KO at the same time point (p < 0.002). The SE for all data points was <5% of the mean.

FIGURE 3. Total IgG and total IgM during cGVH disease. Total IgG and total IgM were assayed by quantitative ELISA in the sera from cGVH disease mice. *, bm12→CD4KO is significantly different from B6→CD4KO at the same time point (p < 0.01); **, bm12→B6 is significantly different from both bm12→CD4KO and B6→CD4KO at the same time point (p < 0.002); +, either bm12→B6 or bm12→CD8KO is significantly different from B6→CD8KO at the same time point (p < 0.002). The SE for all data points was <5%.

FIGURE 4. Proteinuria during cGVH disease. Proteinuria was detected at 2- to 4-wk intervals using Uristix reagent strips. The strips were coated with fresh urine and immediately scored according to color change with a scale of 0–4. **, bm12→B6 is significantly different from both bm12→CD4KO and B6→CD4KO at the same time point (p < 0.02); +, either bm12→B6 or bm12→CD8KO is significantly different from B6→CD8KO at the same time point (p < 0.02). The SE for all data points was <5%.

bm12→B6 and bm12→CD8KO were comparably abnormal, but surprisingly, so were those of B6→CD8KO mice. However, B6→CD8KO mice showed normal kidney immunofluorescence scores for IgG and C3 deposition, while bm12→CD8KO and bm12→B6 mice showed comparably elevated scores. The histopathologic character of the glomerular involvement of the bm12→CD8KO mice was similar to that of the bm12→CD8KO group. Neither unmanipulated CD8KO mice nor control CD8KO→CD8KO mice showed significant renal abnormalities (data not shown).

Discussion
The cGVH induced by the transfer of spleen cells between mouse strains that differ by MHC class II provides a very useful model of human SLE (1–4, 17–18). In this autoimmune syndrome, the alloreactive donor T cells recognize the recipients’ B cells’ MHC Class II molecules, together with peptide that may or may not be derived from the autoantigen against which the B cells react (19). This allogeneic effect then delivers a T cell help signal to the B cells and drives them to produce inappropriate specificities, i.e., autoantibodies. Such a model does not postulate any essential role for the recipients’ endogenous T cells. It is therefore striking that in the current study of CD4 “knockout” and CD8 “knockout” mice as recipients in the cGVH model, we found that autoimmune cGVH, including early mortality, autoantibody production, and renal disease, could not be induced in CD4KO mice by allogeneic splenocyte transfer. CD8KO mice recipient of bm12 spleen cells produced somewhat lower levels of autoantibodies in later stages of cGVH disease than did normal B6 recipients, while they showed comparable severity of renal disease. These results indicate that host (endogenous) T cells, especially CD4+ T cells, play very important roles in the cGVH autoimmune responses. The findings are consistent with our own experience with athymic (nu/nu) B6 recipients of bm12 cells, where we never saw autoantibody induction (L. Davignon et al., unpublished observations). On the other hand, our data seem to contradict Gleichmann’s finding that adult-thymectomized, irradiated, bone marrow-reconstituted recipients of alloreactive T cells actually had a more severe cGVH syndrome than intact recipients (11). Gleichmann’s experiments utilized a different strain combination, (DBA/2×DBA/2×C57BL/6)F1, which has the potential complication of Class I reactivities. In addition, adult-thymectomized, irradiated, bone marrow-reconstituted mice do not have T cell deficits that are entirely comparable to those of the knockout animals in the present study or the congenitally athymic mice in our previous unpublished work.

Thus, the cellular interactions that induce the production of autoantibodies may be much more complicated that we had thought. What role could the endogenous T cells play in this syndrome? They could supply T cell factors in a way that does not require any particular specificity of interaction between T cells and B cells. Alternatively, they could be essential for development of the B cell repertoire that eventually becomes autoreactive. This may entail the selection of particular autoantibody-specific B cells, or it could involve a more general influence on B cell ontogeny that might influence the ability of B cells to respond to allogeneic help. Another possibility is that the endogenous T cells are themselves induced to become autoreactive as a result of interaction with autoreactive B cells that have in turn been activated by interaction with the donor alloreactive T cells. Such a mechanism would parallel the stepwise T-B interactions proposed by Lin, Mamula, and Janeway, in which immunization with foreign and self cytochrome c induces foreign reactive T cells that can provide help for B cells that see both the self and the foreign protein and which, in turn, then can induce the activation of T cells that are reactive with the
self protein (20). Such sequential breaking of T and B cell tolerance may be generally applicable to models of systemic autoimmunity in which a spectrum of autoantibodies is made that is largely reactive against nuclear components (21). These possibilities are currently being tested in our laboratory by cell-transfer experiments with purified T cells and B cells.

A recent publication from Merino’s laboratory may help to elucidate our observation (12). These authors found that the ability of B cells to respond in vitro to allogeneic help by proliferation and production of IgG was dependent on the development of such B cells in an environment that had CD4+ T cells. B cells derived from athymic (nu/nu) mice or from mice treated with anti-CD4 mAb failed to be stimulated by MHC-disparate T cells, although they in turn could stimulate such T cells to produce normal amounts of IL-2, IL-4, and IL-10. The B cell defect was reversed by preincubation with IL-4. Whether IL-4 pretreatment or syngeneic B cell reconstitution of CD4KO mice can restore their autoimmune responsiveness to alloreactive T cells in our cGVH model is currently under investigation in our laboratory.

It is formally possible that the essential role of the host CD4+ T cells in the autoimmune cGVH is to react against the Ia+ donor cells in a host-vs-graft (HVG) reaction. Our previous published work with this model makes this highly unlikely. We showed that all of the autoantibodies in the cGVH were made by the host B cells and that we could detect only very transient evidence for the presence of the donor B cells in the recipient (4). Therefore, the requirement for CD4+ T cells in the recipient cannot be based on a direct role of donor B cells in autoantibody formation in this model. In addition, the experiments in which we compared the parent→parent with the parent→F1 protocols for cGVH, utilizing the cosisogenic B6 and bm12 strains, showed little difference between these two models (9). Since we would not expect any HVG recognition in the parent→F1 transfer, it is unlikely that HVG plays an important role in the parent→parent transfer.

We are currently investigating whether the B cells in CD4KO mice have functional abnormalities that may prevent them from responding to allogeneic help by the production of autoantibodies. Various molecules have been recently described as mediators of costimulatory signals necessary for the establishment of an efficient T-B cell collaboration. For instance, after the recognition of specific peptides by T cells, the interaction between CD28 and CTLA-4 with their ligands on B cells, B7-1 and B7-2, is essential to insure T cell activation instead of anergy or apoptosis (22, 23). Signals mediated through CD40 on B cells, which interacts with ligand for CD40 on activated T cells, seem to be required for the generation of germinal centers. Ig-class switch, and rescue from apoptosis of B cells expressing high affinity slg during the process of somatic mutation (24–26). Moreover, the interaction of some adhesion molecules, such as LFA-1, with their respective ligands can provide essential accessory signals in many immune reactions (27). In previous work, B cells from athymic CBF1 nu/nu mice expressed the above-mentioned molecules, as well as MHC class II, in a manner identical to that of euthymic CB6F1 mice (12, 28). On the other hand, it has also been reported that MHC class I-restricted Ag presentation by Langerhans cells is deficient in athymic nude mice, and that this defect can be restored after a thymus graft (29). It is therefore possible that Langerhans cells and B cells in CD4KO mice are deficient in autoreactive presentation to alloreactive T cells in our model.

The finding that the CD8KO recipients of B6 spleen cells had significant development of renal disease was unexpected. In three separate experiments, this group showed histologic glomerular lesions comparable to those seen in the bm12→B6 and bm12→CD8KO cGVH groups. On the other hand, B6→CD8KO mice never showed increased levels of autoantibodies (Fig. 2). In another experiment, involving sphen cell transfer (Fig. 5). In another experiment, involving sacrifice at 32 wk, some Ig and complement deposition was found, although not as much as in the positive cGVH controls (data not shown). We speculate that the glomerular disease in these animals represents a failure of normal immunoregulation that occurs as a result of the transfer of CD8 cells into the CD8KO recipients. It is also possible that persistent genetic contributions from the 129 genome in which the CD8KO mutation was first inserted might code for minor Ags that would be recognized by the donor B6 T cells in a cGVH reaction that could directly produce glomerular damage. Even though the B6CD8KO line is backcrossed nine generations to B6, 129 genes closely linked to CD8α would by definition cosegregate with the CD8KO locus. In any case, the B6→CD8KO group presents a potentially interesting model of pauciimmune glomerulonephritis which deserves further study.
Acknowledgments

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