FUNCTIONAL HETEROGENEITY OF L3T4+ T CELLS
IN MRL-lpr/lpr MICE

L3T4+ T Cells Suppress Major Histocompatibility
Complex-self-restricted L3T4+ T Helper Cell Function
in Association with Autoimmunity

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MRL/MpJ-lpr/lpr (MRL/l) mice develop a disease similar to human SLE, consisting of massive lymphoproliferation, autoantibody production, and an immune complex glomerulonephritis that results in a 50% mortality by ~20 wk of age (1-3). Congenic MRL/MpJ +/+ (MRL/+ ) mice share >98% of their genome with MRL/l mice but lack the gene for lymphoproliferation (lpr) (1, 3). As a result, MRL/+ mice do not develop the massive lymphadenopathy characteristic of MRL/l mice. Autoimmunity in MRL/+ mice is milder and occurs later in life, making them a useful control strain for MRL/l mice.

The lupus-like disease of MRL/l mice is thought to be primarily due to a disorder of T cells based on the following observations: (a) the massive lymphadenopathy induced by the lpr gene consists primarily of T cells with an unusual phenotype (Thy 1.2+, L3T4-, Lyt2-, Ly-5+, B220+) (4, 5); (b) neonatal thymectomy retards all aspects of the disease (6, 7); (c) in vivo depletion of Thy-1.2+ or L3T4+ T cells improves disease (8, 9); and (d) MRL/l T cells spontaneously produce factors that induce B cell differentiation and Ig secretion (10, 11). A paradoxical aspect of this model is that, despite the evidence for increased Th activity for B cells (10, 11), Th activity for T cell responses (e.g., IL-2 production, IL-2 responsiveness, and in vitro CTL generation) has been reported to be reduced (12-14).

In the present study, we demonstrate that MRL/l mice, and not MRL/+ mice, have an age-dependent loss of Th function that involves MHC-self-restricted L3T4+ Th responses and not Lyt-2+ Th responses. Associated with this defect is the appearance of suppressor cell activity that selectively inhibits L3T4+ MHC-self-restricted responses. We have previously reported similar suppressor cell activity in two murine graft-vs.-host (GVH) models of autoimmunity (also characterized by excessive Th activity for B cells) (15-17), suggesting that these suppressor cells represent a common immunoregulatory mechanism in the setting of autoimmunity.

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1 Abbreviations used in this paper: CAS, Con A-stimulated spleen cell supernatant; MRL/l, MRL/MpJ-lpr/lpr; MRL/+ , MRL/MpJ-++.
Materials and Methods

Mice. Young adult male MRL/+ and MRL/l mice and adult B10.D2 mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

In Vitro Generation and Assay for Cell-mediated Lympholysis. Spleen cells were tested for in vitro sensitization against TNP-modified syngeneic cells (TNP-self) or unmodified allogeneic (B10.D2 or C57Bl/10) cells as previously described (18), with the following modifications. Unless otherwise noted in the figure legends, 3 x 10^6 MRL responder cells were cultured with 2 x 10^6 irradiated stimulator cells and TNP modification (2 mM) was performed on MRL/+ spleen cells. In coculture experiments, 3 x 10^6 MRL/+ cells were cocultured with 3 x 10^6 MRL/+ or MRL/l cells, and stimulator cells consisted of 10^6 TNP-modified MRL/+ cells or 10^6 unmodified allogeneic stimulator cells. Results are expressed as mean percent lysis of ^51Cr-labeled targets tested in triplicate for a given E/T ratio. SEs were consistently <5% and have been omitted.

Exogenous Helper Factors. 24-h culture supernatant from Con A-stimulated BALB/c spleen cells (CAS) or rIL-2 (Cetus Corp., Emeryville, CA) was used as a source of soluble Th factors. The CAS was supplemented with 0.2 M a-methyl-D-mannoside to neutralize excess Con A. Final concentration of CAS during the culture period was 10% (vol/vol).

In Vitro Generation and Measurement of IL-2 Production. Responder and stimulator cell numbers and culture conditions for the stimulation of IL-2 production were identical to those described above for the cell-mediated lympholysis assay. In addition, 0.1% final concentration of ascites fluid containing the anti-murine IL-2-R mAb 7D4 (19) was added in vitro to inhibit IL-2 consumption during the culture period. After 4 d, supernatants were harvested and frozen. IL-2 activity was assessed as the ability of the supernatants to stimulate the proliferation of the IL-2-dependent cell line, CTLL, as measured by [3H]thymidine incorporation into DNA (16). Supernatants were tested in serial, twofold dilutions and varying dilutions of rIL-2 were tested in parallel. Results are expressed as mean cpm for four replicate wells of a given supernatant dilution. SEs were always <10% (in most instances, <5%) and have been omitted. The concentration of 7D4 used in these experiments does not inhibit CTLL proliferation.

In Vitro Manipulation of Spleen Cells. Spleen cell populations were depleted of APC by passage over Sephadex G-10 columns as previously described (20). T cell depletions were carried out using either monoclonal anti-Lyt-2 antibody 116-13.1 (American Type Culture Collection, Rockville, MD) or monoclonal anti-L3T4 antibody RL172 (21). Cells were incubated with antibody for 40 min at 4°C, washed, then incubated with rabbit complement (Low Tox; Cedarlane Lab, Hornby, Ontario, Canada) for 30 min at 37°C. Cells were then washed twice before culture. In Fig. 1, after anti-L3T4 antibody plus complement treatment of spleen cells, in vitro L3T4+ function was further blocked by the addition of 20% (vol/vol) culture supernatant containing monoclonal anti-L3T4a antibody GK1.5 (22) (kindly provided by Dr. F. Fitch, Univ. of Chicago, Chicago, IL). All Lyt-2+ cell-depleted populations were unable to generate an in vitro CTL response to alloantigen despite the addition of either rIL-2 or CAS, yet demonstrated normal IL-2 production to the L3T4+ T cell-dependent antigen TNP-self. All cell populations depleted of L3T4+ cells demonstrated a loss of in vitro IL-2 production to either TNP-self or to unmodified syngeneic cells (autologous MLR) with no concomitant loss of CTL effector function when exogenous helper factors were added.

Statistics. Statistical differences between groups was determined by the student's t test.

Results

MRL/l Mice Have a Selective Defect in Th Function. A comparison of in vitro generated CTL responses by spleen cells from 4-mo-old MRL/+ and MRL/l mice, to TNP-self and H-2 alloantigens is shown in Fig. 1. The data of Fig. 1 A and B demonstrate that depletion of L3T4+ Th before sensitization resulted in complete abrogation of the MRL/+ TNP-self CTL response, whereas the allogeneic CTL response remained intact in these mice. The addition of Th factors contained in CAS restored the TNP-self CTL response to above normal levels and moderately enhanced allogeneic CTL responses. These results are in agreement with previously published
studies, which have shown in other normal murine strains that the TNP-self CTL response is mediated exclusively by an L3T4+ Th-dependent pathway requiring self APC (i.e., MHC-self restricted), whereas allogeneic CTL responses are mediated by both an L3T4+ Th-dependent and L3T4+ Th-independent (i.e., Lyt-2+) pathway (23-25).

Untreated MRL/l spleen cells exhibited a loss of the TNP-self CTL response (Fig. 1 C), and depletion of L3T4+ Th had no additional detectable effect. The in vitro addition of CAS restored the defective TNP-self CTL response in MRL/l mice to levels similar to that observed for L3T4+-depleted MRL/+ spleen cells cultured with CAS. The allogeneic CTL response for untreated MRL/l spleen cells appeared to be intact and was not abolished by L3T4+ Th depletion (Fig. 1 D). The defective TNP-self CTL response of MRL/l mice can also be restored by the in vitro addition of rIL-2 (Table I). These results are suggestive of an L3T4+ Th defect in MRL/l autoimmune mice because: (a) the naturally occurring selective loss of the TNP-self CTL response in MRL/l mice can be mimicked in MRL/+ mice by L3T4+ Th depletion; and (b) the addition of Th factors in the form of CAS or rIL-2 can restore the abrogated TNP-self CTL response in MRL/l mice.

The Selective Loss of Th Function in MRL/l Mice Is Age Related. The data of Fig. 2 (upper panel) demonstrate that the loss of the TNP-self CTL response in MRL/l mice occurs as a function of age. MRL/+ mice (2-6 mo of age) exhibited strong CTL responses to TNP-self with no significant age-related differences throughout this time period. TNP-self CTL responses were initially present in young (<2 mo) MRL/l mice, although, in some instances, they were reduced compared with the responses of MRL/+ mice. By 3-4 mo of age, however, MRL/l mice consistently exhibited defective TNP-self CTL responses compared with MRL/+ mice (p < 0.0001). Specifically, the anti-TNP-self CTL response of MRL/l mice was always reduced by more than eightfold and, in most instances, was reduced by 16-32-fold.
Table 1
The Defective TNP-self CTL Response in MRL/l Mice
Is Corrected by rIL-2

| Group | Responder* | rIL-2 | Percent lysis of MRL/+ TNP |
|-------|------------|-------|--------------------------|
|       |            |       | 40 | 20 | 10 | 5  |
| 1     | MRL/+      | –     | 40 | 29 | 20 | 16 |
| 2     | MRL/11     | –     | 9  | 4  | 0  | 0  |
| 3     | MRL/12     | +     | 62 | 47 | 41 | 34 |
| 4     | MRL/11     | –     | 1  | 2  | 1  | 0  |
| 5     | MRL/12     | +     | 53 | 40 | 37 | 26 |

* 3 x 10^6 responder cells from individual MRL/+ (6 mo old) or MRL/l mice were cultured with 10^6 MRL/+ TNP-modified stimulator cells (2,000 rad). MRL/11 and MRL/12 responder groups represent two individual 5-mo-old and 7-mo-old MRL/l mice, respectively.

1 Approximately 32 U/well of rIL-2 were used, where 1 U is defined as the amount of rIL-2 required to induce half-maximal proliferation of the IL-2-dependent cell line CTLL.

(as estimated by LU comparison of CTL activity at four E/T ratios). This pattern of unresponsiveness in MRL/l mice was also present in 5–6-mo-old MRL/l mice.

Allogeneic CTL responses for 2–6-mo-old MRL/+ mice were also strong with no significant age related differences. Allogeneic CTL responses of MRL/l mice were reduced for both 3–4-mo-old mice (p < 0.005) and for 4–6-mo-old mice (p < 0.001) when compared with MRL/+ mice. However, the reductions in the MRL/l anti-allogeneic CTL response were neither as pronounced (generally 0–8-fold by LU comparison) nor as consistently observed as were the reductions in the TNP-self CTL response. Furthermore, the anti-allogeneic CTL response of older MRL/l mice was never completely abrogated as was the anti-TNP-self response for these mice. Thus, although both allogeneic and TNP-self CTL responses are defective in MRL/l mice, the magnitude of the defect is greater for TNP-self and occurs earlier in life than does the defect in the allogeneic response.

MHC-self-restricted Th Function Is Defective in MRL/l Mice. It has been previously demonstrated by the laboratories of Singer (23, 24) and Schmitt-Verhulst (25) that TNP-self responses are exclusively dependent on L3T4+ Th that recognize TNP in the context of self Ia on APC (i.e., an MHC-self-restricted response). In contrast, allogeneic CTL responses can proceed by both an MHC-self-restricted as well as a non-self-restricted pathway. In this latter pathway, allogeneic APC directly stimulate either L3T4+ or Lyt-2+ responder Th. It is possible that this age-related abrogation of the TNP-self CTL response in MRL/l mice reflects an inability of L3T4+ Th to function in an MHC-self-restricted manner, and that the apparently intact allogeneic CTL response observed in these mice is mediated by non-self-restricted Th function. Depletion of stimulator APC before culture eliminates the non-self-restricted component of the allogeneic response and permits assessment of only the MHC-self-restricted component. Using this approach, we have tested the self-restricted L3T4+ Th component of the allogeneic CTL response for MRL/+ and MRL/l mice. As previously shown in Figs. 1 and 2, both MRL/+ and MRL/l mice have strong allogeneic CTL responses when unmanipulated stimulator cells are used (Fig. 3, left panel) although, as noted for Fig. 2, the response of MRL/l mice is often weaker...
than that of MRL/+ mice. However, when allogeneic stimulator cells are depleted of APC (making the response solely dependent on MHC-self-restricted L3T4+ Th function), the CTL response of MRL/l, but not MRL/+, spleen cells is lost (Fig. 3, right panel). Thus, MHC-self-restricted L3T4+ Th function in MRL/l mice is defective for alloantigen as well as for TNP-self. Additionally, the CTL response of MRL/l
mice using unmanipulated allogeneic stimulator cells (Fig. 2, lower panel and Fig. 3, left panel) can be explained by non-self-restricted Th function.

Selective Defect in IL-2 Production by MRL/l Mice. The above data strongly suggest that MRL/l mice have defective L3T4+ Th function as measured by their ability to provide help for in vitro CTL responses. It has been previously demonstrated that MRL/l mice have defective Th function as measured by Con A-stimulated IL-2 production of unfractionated spleen cells (12, 13). It would not be surprising, then, for MRL/l mice to exhibit reduced IL-2 production to weaker antigens such as TNP-self. Based on the data shown above, which demonstrate that allogeneic CTL responses are not as compromised in MRL/l mice as are TNP-self CTL responses, it might be expected that alloantigen-stimulated IL-2 production by MRL/l mice would be less compromised than TNP-self-stimulated IL-2 production. In Fig. 4A, IL-2 production by MRL/l mice is reduced in response to TNP-self by approximately >16-fold compared with MRL/+ mice. Although alloantigen-stimulated IL-2 production by MRL/l mice (Fig. 4B) is also reduced by ~8-16-fold compared with that of MRL/+ mice, the response is nevertheless 8-16-fold higher than TNP-self-stimulated IL-2 by MRL/l mice. Taking Figs. 3 and 4 together, the increased alloantigen-stimulated IL-2 production (compared with TNP-self-stimulated IL-2 production) by MRL/l mice is due to non-MHC-self-restricted Th function and can account for the relative preservation of allogeneic CTL responses (as opposed to the complete abrogation of TNP-self CTL responses) in these mice. These data are consistent with a selective defect in self-restricted L3T4+ Th function by MRL/l mice that can be obscured by the alternate Th pathways that are operative in the allogeneic response but not in the TNP-self response.

Defective L3T4+ Th Cell Function in MRL/l Mice Is Due to Suppression. It is possible that the observed loss of self-restricted L3T4+ Th function is due to a reduction of L3T4+ T cells in the spleen of MRL/l mice. Using flow cytometry, we have observed that the relative frequency of L3T4+ T cells in spleens from 5-mo-old MRL/l mice is ~20-24% and is slightly less than that of 4-6-mo-old MRL/+ spleens (26-28%). Because older MRL/l spleens have a greater cell yield, absolute numbers of L3T4+ T cells are not less than that of MRL/+ spleens. Because numbers of

![Figure 4](image-url)

**Figure 4.** IL-2 production by 5-mo-old MRL/+ (O) or MRL/l (●, ▲) spleen cells to either TNP-modified MRL/+ spleen cells (A) or to allogeneic B10.D2 spleen cells (B). MRL/l responder cells were pooled from two mice. MRL/+ responder cells are from two mice tested individually. Results are expressed as 3H incorporation by CTLL cells as a function of supernatant dilution.
splenic L3T4+ T cells in MRL/l mice appear adequate, the defect in L3T4+ Th function observed in these mice is likely due to another mechanism, possibly suppressor cells. Therefore, we tested the ability of MRL/l spleen cells to suppress MRL/+ splenic T cell responses. In Fig. 5, the results of nine independent experiments are shown in which $3 \times 10^6$ MRL/+ spleen cells were cocultured with an equal number of either MRL/+ or MRL/l spleen cells, and TNP-self or allogeneic CTL responses were measured. Coculture of MRL/+ spleen cells with an equal number of MRL/+ or young (2–3-mo-old) MRL/l spleen cells resulted in strong CTL responses to TNP-self and to alloantigen. Coculture of MRL/+ cells with an equal number of old (4–6-mo-old) MRL/l spleen cells resulted in a significant reduction in CTL responses to TNP-self ($p < 0.0001$), but not to alloantigen. Suppression of the MRL/+ anti-TNP-self CTL response was observed for all but one of the 18 older MRL/l mice tested in coculture. In contrast, suppression of MRL/+ allogeneic CTL responses by older MRL/l spleen cells was observed in only 4 of 16 mice. It is noteworthy that

**Figure 5.** MRL/l spleen cells suppress the TNP-self CTL response of MRL/+ mice as a function of age. CTL responses to either TNP-modified MRL/+ spleen cells (upper panel) or to allogeneic B10.D2 spleen cells (lower panel). Pooled MRL/+ responder spleen cells were cocultured with an equal number of MRL/+ (○) or MRL/l (●) spleen cells from individual mice. Percent lysis is expressed at an E/T ratio of 40:1, although four E/T ratios were tested.
SUPPRESSION OF L3T4+ T HELPER CELLS IN MRL/lpr MICE

for all experiments, the TNP-self and allogeneic CTL response of \(3 \times 10^6\) MRL/+ spleen cells alone was comparable (≤ twofold) with that of \(6 \times 10^6\) MRL/+ spleen cells (data not shown). Thus, the reductions observed represent suppression below the response level for \(3 \times 10^6\) cells MRL/+ cells and do not reflect merely the addition of nonfunctional cells. Further underscoring this point, is the ability of radiation to remove, in large part, the suppressive effect of MRL/l spleen cells. The data shown in Table II demonstrate that: (a) the TNP-self response of \(4 \times 10^6\) or \(8 \times 10^6\) MRL/+ responder cells is comparable; (b) coculture of \(4 \times 10^6\) MRL/+ spleen cells with an equal number of MRL/l spleen cells results in a suppression of the TNP-self CTL response by approximately >8-16-fold; and (c) irradiation of MRL/l cells before coculture removes much of this suppressive effect. Thus, if MRL/l cells were merely nonfunctional rather than suppressive in nature, the expected effect on the TNP-self CTL response would be that of either \(4 \times 10^6\) MRL/+ cells alone or \(4 \times 10^6\) MRL/+ cells cultured with irradiated MRL/l cells.

If this suppressor activity of MRL/l spleen cells is causally related to the defective L3T4+ Th function observed in these same mice, then it should affect only self-restricted L3T4+ Th function such as the TNP-self response. The allogeneic response should be less susceptible to suppression than the TNP-self response by virtue of the alternate Th pathways described above. Removal of stimulator APC before culture would be expected to render the allogeneic response equally susceptible to suppression, because the response would then be solely due to self-restricted L3T4+ Th function. In Fig. 6, IL-2 production (A–C) and CTL generation (D–F) were measured for MRL/+ spleen cells cocultured with an equal number of MRL/+ spleen cells or MRL/l spleen cells. Coculture of MRL/+ spleen cells with an equal number of MRL/l spleen cells resulted in suppressed CTL and IL-2 responses to TNP-self (Fig. 6, A and D). Secondly, coculture of MRL/+ spleen cells with MRL/l cells resulted in only a mild (~2–4-fold) reduction in IL-2 and CTL responses to untreated allogeneic stimulator cells (Fig. 6, B and E) when compared with the responses of MRL/+ spleen cells cocultured with an equal number of MRL/+ spleen cells. This degree of mild suppression can also be seen in Fig. 5 (lower panel, MRL/l

| Table II |
|-----------------|-----------------|
| **Suppressor Cells in MRL/l Spleens Are Radiosensitive** | **Percent lysis of MRL/+ TNP** |
| Group | Responder+ | Cocultured with: | 40\% | 20 | 10 | 5 |
|-------|------------|-----------------|-----|---|---|---|
| 1     | MRL/+     | –               | 61  | 56 | 50 | 46 |
| 2     | MRL/+     | MRL/+           | 62  | 56 | 53 | 42 |
| 3     | MRL/+     | MRL/l1         | 32  | 19 | 17 | 5  |
| 4     | MRL/+     | MRL/l2         | 15  | 10 | 2  | 2  |
| 5     | MRL/+     | MRL/l1 (2,000 rad) | 53 | 43 | 32 | 23 |
| 6     | MRL/+     | MRL/l2 (2,000 rad) | 54 | 48 | 34 | 18 |

* \(4 \times 10^6\) MRL/+ pooled spleen cells were cultured alone or with an equal number of pooled MRL/+ spleen cells or MRL/l spleen cells from individual mice. MRL/l1 represents the mean results of two 3-mo-old MRL/l mice tested individually. MRL/l2 represents mean results obtained from two 5-mo-old MRL/l mice tested individually.

1 E/T ratio.
MRL/l spleen cells can suppress IL-2 or CTL responses of MRL/+ spleen cells. IL-2 production (upper panels) or CTL responses (lower panels) are shown after stimulation with either TNP-modified MRL/+ stimulator cells (A and D), allogeneic C57BL/10 stimulator cells (B and E), or APC-depleted C57BL/10 stimulator cells (C and F). Responder cells consist of $3 \times 10^6$ pooled MRL/+ spleen cells (4–6-mo-old) cocultured with either: (a) $3 \times 10^6$ MRL/+ spleen cells from two individual mice (○); or (b) $3 \times 10^6$ MRL/l spleen cells from two individual mice aged 6 mo (●, △). Results from (a) are shown as the mean CTL activity for the two mice tested; in (b), results are shown for each individual mouse.

VIA AND SHEARER

>4 mo) and is consistent with the loss of only the self-restricted Th pathway, leaving non-self-restricted Th function to account for the observed response. However, when allogeneic APC are depleted and the allogeneic response is solely dependent on self-restricted L3T4+ Th function, MRL/l spleen cells completely suppress the allogeneic CTL response of MRL/+ mice (Fig. 6 F) and suppress IL-2 production to levels similar to that seen in response to TNP-self (compare Fig. 6 C with 6 A). Thus, MRL/l spleen cells can induce a defect in the CTL and IL-2 responses of MRL/+ mice that mimics the naturally occurring Th defect in MRL/l mice. Therefore, reduced or abrogated MHC-self-restricted Th function observed in MRL/l mice appears to be due to active suppression.

The Suppresser Cell Is an L3T4+ Cell. Using coculture experiments, cell depletion studies were performed to determine the phenotype of the suppressor cell. Preliminary experiments involving depletion of surface Ig+ MRL/l spleen cells before coculture with an equal number of unfractionated MRL/+ spleen cells resulted in increased suppression of TNP-self CTL response of MRL/+ mice, implying that the suppressor cell was a non-B cell (data not shown). Experiments were then performed to determine if the suppressor cell was a T cell (Fig. 7). MRL/+ or MRL/l
spleen cells were either: untreated (Fig. 7 A); Lyt-2+ T cell depleted (Fig. 7 B); or L3T4+ T cell depleted (Fig. 7 C) before coculture with an equal number of unfractionated MRL/+ spleen cells, and the affect on the TNP-self CTL response was measured. Both unfractionated and Lyt-2+ T cell-depleted MRL/+ spleen cells suppressed the TNP-self CTL response of MRL/+ spleen cells by ~4-8-fold as determined by LU comparison (Figs. 7, A and B). Conversely, depletion of MRL/+ spleen cells of L3T4+ cells removed, almost entirely, the suppressive ability of MRL/+ spleen cells (Fig. 7 C). As in other coculture experiments, the CTL response of 3 × 10^6 MRL/+ spleen cells was comparable with that of 6 × 10^6 MRL/+ spleen cells. The reduced TNP-self CTL responses observed when MRL/+ cells are cocultured with unfractionated (Fig. 7 A) or Lyt-2+ -depleted (Fig. 7 B) MRL/+ cells represent suppression to levels below that observed for 3 × 10^6 MRL/+ cells alone.

**APC Function Is Normal in MRL/+ Mice.** It is possible that the loss of MHC-self-restricted L3T4+ T cell function in MRL/+ mice results from a defect in APC function rather than a primary defect in L3T4+ Th. Therefore, we tested the ability of APC from MRL/+ or MRL/+ mice to present the antigen TNP to MRL/+ responder spleen cells that had been depleted of APC (Table III). Groups 1–3 confirm that the MRL/+ spleen cells to be used as a source of APC in this experiment exhibited defective CTL responses when compared with age matched (5 mo) MRL/+ mice. The intact MRL/+ CTL response to TNP-modified MRL/+ stimulator cells (group 1) could be abrogated if APC were depleted from both the stimulator and the responder population (group 4). The abrogated TNP-self response could be then be restored by: (a) the in vitro addition of CAS (group 5); (b) the use of TNP-modified APC from MRL/+ mice (group 6); or (c) the use of TNP-modified APC from MRL/+ mice (groups 7 and 8). Thus, although MRL/+ mice exhibit defective Th responses to TNP-modified MRL/+ cells (groups 2 and 3), these same MRL/+ spleen cells were able to provide APC function for the antigen TNP when APC-depleted MRL/+ responder cells were used (groups 7 and 8).

**Discussion**

The results of the present study demonstrate a selective defect in Th function in MRL/+ mice that is age related and progresses in parallel with the development of
autoimmunity. Specifically, L3T4+ Th from MRL/l spleens exhibit defective MHC-self-restricted responses to two different antigens, TNP-self and an alloantigen. In contrast, MRL/l Lyt-2+ T cells exhibit no defect for either effector function or helper function. Thus, 4-6-mo-old MRL/l mice have profound reductions in L3T4+ T cell-dependent IL-2 and CTL responses to TNP-self compared with age-matched MRL/+ mice, yet Lyt-2+ anti-TNP-self CTL effector function in MRL/l mice appears normal, since the defective TNP-self CTL response can be restored by the in vitro addition of Th factors. Alloantigen-stimulated IL-2 production in MRL/l mice is eightfold greater than TNP-stimulated IL-2 production by MRL/l mice. The greater IL-2 response to alloantigen compared with TNP-self for MRL/l mice can be explained by the contribution of Lyt-2+ Th (and possibly L3T4+ Th) functioning in a non MHC-self-restricted fashion. These alternate pathways have been previously shown to exist for allogeneic responses but not for TNP-self responses (23-25). When the non-self-restricted Th contribution is eliminated (by depletion of the stimulator population of APC) and the anti-allogeneic CTL response of MRL/l mice is made solely dependent on self-restricted L3T4+ Th function, the antiallogeneic CTL response, like the TNP-self CTL response, is abrogated. Thus, the Th defect in MRL/l mice is not antigen- (i.e., TNP-self) specific, but rather, Th pathway selective, involving MHC-self-restricted L3T4+ Th function. Studies are in progress to determine whether non-self-restricted L3T4+ Th function is also defective in MRL/l mice.

Defective T cell function in MRL/l mice has been previously demonstrated for a wide variety of stimuli. For example, decreased IL-2 production and IL-2 responsiveness to Con A (12, 13) occurring in an age-related fashion (26) have been reported. Additionally, Scott et. al (14) have shown decreased in vitro CTL generation to either virus or TNP-self for MRL/l mice. In contrast, allogeneic CTL responses by MRL/l mice have been reported to be preserved at an age when other parameters

| Group | Responder* | TNP-modified stimulator cells | CAS (vol/vol) | CTL |
|-------|------------|------------------------------|--------------|-----|
| 1     | MRL/+      | MRL/+                        | -            | 58  |
| 2     | MRL/l      | MRL/+                        | -            | 10  |
| 3     | MRL/l      | MRL/+                        | -            | 5   |
| 4     | MRL/+ (G-10)| MRL/+ (G-10)                | -            | 0   |
| 5     | MRL/+ (G-10)| MRL/+ (G-10)                | 10%          | 34  |
| 6     | MRL/+ (G-10)| MRL/+                        | -            | 24  |
| 7     | MRL/+ (G-10)| MRL/+                        | -            | 34  |
| 8     | MRL/+ (G-10)| MRL/+                        | -            | 25  |

* Responder spleen cells (3 x 10^6) were cultured with 2 x 10^6 TNP-modified stimulator spleen cells (2,000 rad). In group 1, two MRL/+ responder spleens were pooled. In groups 2 and 3, two MRL/l responder spleens were tested individually. Age-matched 5-mo-old mice were used. Spleen cells from responder groups 1, 2, and 3 served as TNP-modified stimulator cells for groups 6, 7, and 8, respectively.

† CTL assays were run at four E/T ratios. The data are shown for E/T, 40:1.

§ Stimulator cells were depleted of APC by passage over Sephadex G-10 columns.
of T cell function are depressed (12). This puzzling exception in defective T cell function can be explained by the data of the present study, which demonstrate that the preserved allogeneic CTL response in older MRL/l mice is also defective when it is solely dependent on MHC-self-restricted L3T4+ Th function. Although a Th defect in MRL/l mice has been postulated previously (14), our study demonstrates the specific and selective nature of this Th defect to involve only L3T4+ Th.

Because MHC-self-restricted responses depend on L3T4+ Th as well as self APC, it was possible that the observed defects in L3T4+ Th function reflect defective APC function. Previous studies of APC function in MRL/l mice have shown it to be normal by a variety of parameters (27, 28), including presentation of TNP (14). In this latter study, however, the results were inconclusive because APC were not removed from the MRL/+ responder population. Our data demonstrate that when CTL responses by MRL/+ spleen cells are solely dependent on MRL/l APC, the TNP-self response is normal. Thus, the defect in L3T4+ Th in MRL/l mice is not due to defective function of MRL/l APC. Additionally, the defect in MRL/l Th function could be mimicked in MRL/+ mice by in vitro depletion of L3T4+ Th, further supporting our contention that L3T4+ Th, and not Lyt-2+ Th or APC, are defective in MRL/l mice.

The mechanism(s) responsible for defective Th function in MRL/l mice has not been previously defined. Some investigators have ascribed MRL/l T cell defects to a dilution of normal responding T cells by a massively proliferating population of abnormal T cells (Thy-1.2+, L3T4-, Ly-5+, B220+) (4, 5). This explanation has not been widely accepted, because it has been shown that the defect in MRL/l Th occurs early in life, before the massive influx of abnormal T cells (4, 12, 28). Additionally, extensive dose-response studies using MRL/l T cells have failed to show any evidence of a “dilution” effect by the abnormal T cells on in vitro CTL function (14).

Suppressor cells are not generally thought to play a role in mediating the defective Th responses in MRL/l mice (3), since coculture experiments have failed to show evidence of suppression (10, 11). However, these studies used Con A as a stimulus, which could have obscured suppressor cell effects of the type reported herein by virtue of its ability to elicit Th activity through non MHC-self-restricted pathways (29, 30). The presence of suppressor cell activity in MRL/l mice has been reported by Naides (31), who noted that MRL/l lymph node cells could suppress IL-2 production by TNP-primed MRL/+ lymph node cells restimulated in vitro with TNP-self. However, similar suppressor cell activity was also detected in age-matched MRL/+ mice. Also, it is not clear whether the observed decrease in supernatant IL-2 concentration was due to active suppression of IL-2 production or to increased IL-2 consumption, since IL-2 consumption was not blocked. In the present study, we have used an mAb to the IL-2-R to block IL-2 consumption. Using TNP-self and alloantigen as stimuli, we have demonstrated the presence of suppressor cells in MRL/l spleens, but not in age-matched MRL/+ spleens, which are capable of inducing a Th defect in MRL/+ spleen cells that is remarkably similar to the naturally occurring Th defect in MRL/l mice. That is, MRL/l cells can reduce by 8-16-fold IL-2 production by MRL/+ spleen cells for L3T4+ T cell-dependent MHC-self-restricted responses, i.e., to TNP-self or to APC-depleted alloantigen. Consequently, CTL responses to these antigens are also abrogated. Thus, MRL/l spleen cells suppress the MHC-self-restricted function of MRL/+ L3T4+ Th. MRL/l cells do not
appear to suppress the non-self-restricted Th contribution to the anti-allogeneic response by Lyt-2+ (and possibly L3T4+) Th, because IL-2 and CTL responses by MRL/+ cells to non-APC-depleted alloantigen are only slightly reduced by coculture with MRL/I spleen cells. This mild reduction (as opposed to a complete abrogation) in Th function is consistent with the loss of only one of several possible Th pathways shown to be operative in allogeneic responses. These data strongly argue that the observed defect in MRL/I L3T4+ Th function in vitro is due to the action of suppressor cells, because they are capable of inducing a similar selective Th defect in normal MRL/+ L3T4+ Th.

Phenotyping experiments reveal the suppressor cell in MRL/I spleens to also be an L3T4+ T cell. Although suppressor T cells were first associated with the Lyt-1-2+ phenotype (32), several laboratories have subsequently described cloned T suppressor cells that are Lyt-2- (33-35) and L3T4+ (36). Further studies using cloned L3T4+ T cells have shown them to be functionally heterogeneous (37-39). Although phenotypically distinct L3T4+ subsets have not been identified in murine systems, studies with cloned cells have allowed L3T4+ cells to be functionally subdivided with one population, termed Th2 cells, exhibiting predominantly "helper" activity in the form of help for B cell responses and the synthesis of IL-4, and a second population, termed Th1 cells, producing IL-2 and mediating delayed-type hypersensitivity. Since autoimmunity in the MRL/I model has been shown to be strongly related to the presence of circulating L3T4+ T cells (9), it is possible that autoantibody production and B cell hyperactivity is a result of excessive Th2 activity in vivo. The results of the present study suggest that such excessive Th2 activity is associated with activation of L3T4+ suppressor cells that can suppress IL-2 production by L3T4+ Th1 cells.

The reported defect in L3T4+ Th in MRL/I mice is remarkably similar to the observed defect in L3T4+ Th occurring in another model of autoimmunity, murine GVH disease (40). In this model, healthy unirradiated F1 mice can be induced to develop lupus-like disease after the injection of parental T cells that recognize, exclusively or predominantly, the class II MHC alloantigens of the host (15, 17). Although the etiology of autoimmunity is clearly different in these two models, excessive L3T4+ Th activity for B cells coupled with suppression of L3T4+ Th1 function are features common to both models (9, 11, 15-17). Additionally, such suppressor cell activity in both models is not detected until after the appearance of autoimmune phenomena, suggesting that the suppressor cells represent a common immunoregulatory event aimed at reducing autoimmunity, perhaps through a global reduction in class II-restricted, L3T4+ Th function.

Why, then, are these suppressor cells in either MRL/I or GVH mice not effective in preventing the development of autoimmunity? Several explanations are possible. First, the suppressor cells may actually be somewhat effective, and autoimmunity might be worse in their absence. Because suppressor cell activity appears to occur after the onset of autoimmunity in both models, the suppressor cells could be at a kinetic disadvantage. Second, the suppressor cell component may only effectively inhibit primary immune responses and not secondary responses. Because the immune system could be considered to be primed to the antigen(s) responsible for autoimmunity before the induction of suppressor cells, these cells may only efficiently suppress T cell responses to antigens that are unrelated to the development of au-
toimmunity. Third, these suppressor cells may inactivate only Th1 function and not Th2 function, thus allowing any excessive Th2 contribution to autoimmunity to continue unchecked. Finally, even if the suppressor cells can inhibit both Th1 and Th2 function, B cells, once activated, may be resistant to the effects of these suppressor cells and may continue producing autoantibodies.

Regardless of which, if any, of the above mechanisms is operative, the common occurrence of suppressor cells selective for L3T4+ Th in two very different murine models of lupus suggests that these suppressor cells are not related to the cause of autoimmunity, but rather are present as an unexpected immunoregulatory mechanism that occurs as a consequence of autoimmunity. Such a CD4+ selective regulatory mechanism may also be operative in human lupus, as well as in other autoimmune diseases, in which B cell hyperactivity occurs in the setting of reduced in vitro Th production of IL-2 (41).

Summary

The present study demonstrates in MRL-Ipr/Ipr autoimmune mice an age-dependent loss of MHC-self-restricted function by L3T4+ Th. This defect is not present in age-matched, congenic MRL-+/+ spleen cells and appears to be due to the presence of suppressor cells that are selective for L3T4+ Th and not for Lyt-2+ Th. Surprisingly, the suppressor cells are also L3T4+ T cells and can suppress the IL-2 production of congenic MRL+/+ L3T4+ Th to MHC-self-restricted antigens. These data support the idea of functional specialization within the L3T4+ population of T cells. Because L3T4+ suppressor cells are detected late in the course of autoimmunity, we interpret their presence not as a primary initiating event in the development of autoimmunity, but rather as a compensatory mechanism. Additionally, similar suppression of L3T4+ Th function has also been reported in a murine graft-vs.-host model of autoimmunity, suggesting that the suppressor cells represent an immunoregulatory mechanism that is a common feature of autoimmunity. Since excessive class II-restricted Th activity for B cells has been reported for both models of autoimmunity, L3T4+ suppressor cells may represent an attempt to down regulate such excessive Th activity. These findings may be relevant to human autoimmune diseases, such as systemic lupus erythematosus, in which B cell hyperactivity is also associated with reduced IL-2 production by Th.

The authors wish to express their thanks to Drs. H. C. Morse, III, National Institute of Allergy and Infectious Disease; H. Golding, W. Davidson, H. Dickler, and A. Singer, NCI; G. Tsokos, Uniformed Services University of Health Sciences; and B. Handwerger, University of Maryland Medical School, for reviewing this manuscript.

Received for publication 16 August 1988.

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