# THE CONTRIBUTION OF L3T4$^+$ T CELLS TO LYMPHOPROLIFERATION AND AUTOANTIBODY PRODUCTION IN MRL-*lpr/lpr* MICE

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The in vivo administration of mAbs against T cells and T cell subsets has provided insight into the cellular mechanisms that operate in the development of autoimmunity. Repeated injections of mAb to the L3T4 antigen has a salutary effect on disease expression in type II collagen–induced arthritis (1), experimental allergic encephalomyelitis (2), diabetes-prone NOD mice (3), and the spontaneous systemic lupus erythematosus (SLE)–like syndromes manifested by (NZB × NZW)F$_1$ (4) and BXSB (5) mice. These studies have demonstrated that the L3T4$^+$ (CD4$^+$) T cell subset plays a pivotal role in a variety of distinct autoimmune diseases and suggest further that a T cell response to antigen in the context of self class II MHC molecules, or possibly to self class II MHC molecules alone, may be required for the induction of autoimmunity.

MRL-*lpr/lpr* mice also develop a lupus-like illness (6). The expression of autoimmune features in mice that bear the *lpr* gene coincides with the massive accumulation of an unusual subset of T cells, which are phenotypically Thy-1$^+$, Ly-1$^+$, Lyt-2$^-$, L3T4$^-$, and aberrantly express the B220 antigen (7). Neonatal thymectomy, which abrogates *lpr*-dependent lymphoproliferation, prevents autoantibody production and the development of immune-complex glomerulonephritis (8, 9). Similarly, treatment of MRL-*lpr/lpr* mice with anti-Thy-1.2 mAb retards both lymphoproliferation and the progression of autoimmune disease (10). These observations indicate that autoimmunity in MRL-*lpr/lpr* mice is T cell dependent, and have been used to implicate Thy 1$^+$, B220$^+$ cells in the etiopathogenesis of disease. Recently, we reported the presence of functional abnormalities in splenic L3T4$^+$ cells from MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice (11). The current study demonstrates an important role for the L3T4 T cell subset in the pathogenesis of IgG autoantibody production and lymphoproliferation in *lpr*-bearing mice.

## Materials and Methods

*Mice.* MRL mice were purchased from the Jackson Laboratory, Bar Harbor, ME. The mice were bred and maintained under conventional conditions in the animal research

facility at the Denver Veterans Administration Medical Center, Denver, CO. Only females were used in these studies.

*Antibodies.* The rat hybridomas GK1.5 and RA3 were obtained from the American Type Culture Collection (Rockville, MD), and D4.69 was obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). The hybridomas were grown as ascites in sublethally irradiated, pristane-primed [C57BL/6 × DBA/2]$F_1$ mice. GK1.5 secretes an IgG mAb that recognizes a nonpolymorphic determinant present on $L3T4^+$ T cells (12). The hybridoma D4.69 (control rat Ig) produces an IgG mAb with specificity for the RT1-N.A. antigen and shows no activity against murine cells (13). RA3 secretes an IgM mAb that reacts with an antigen (B220) present on B cells and pre-B cells that is expressed on the mutant T cells of *lpr* mice (7). Fluoresceinated anti-Thy-1.2, anti-Lyt-2, anti-IgM, and MAR 18.5, a murine antibody to rat K chains, were purchased from Becton Dickinson & Co. (Mountain View, CA).

*Treatment Regimens.* In the first study, nine mice (4 wk old) were treated with 1 mg of anti-L3T4 antibody given by intraperitoneal injection twice weekly. The control groups consisted of untreated mice ($n = 6$) and mice treated with 1 mg of control rat Ig twice weekly ($n = 8$). The study was terminated 10 wk later when two mice from the anti-L3T4-treated group became moribund coincident with administration of antibody. The latter mice were not included in the overall analysis of results.

In a second trial, five mice (4 wk old) were treated with 1 mg of anti-L3T4 antibody intraperitoneally per week. Control mice ($n = 5$) received PBS. mAb injections were discontinued after 6 wk of therapy and the study was terminated 2 wk later.

*Quantification of Antinuclear Antibodies and Total IgG in Sera.* Sera were assayed for antibodies to total histones and single-stranded DNA by an ELISA as reported earlier (14). Antibody activity is expressed as units related to OD at 405 nm. IgG levels in sera were also measured by ELISA, and OD values were converted to mg/ml as described (14).

*Immunofluorescence and Cytofluorographic Analyses.* For direct staining, cells were incubated with a previously determined optimal concentration of fluorescein-labeled mAb (anti-Thy-1.2, anti-Lyt-2, anti-IgM). Indirect immunofluorescence was performed by incubating cells with mAb (anti-L3T4 or anti-B220), and then staining them with excess purified fluorescein-conjugated MAR 18.5. Controls included cells stained with the second-step reagent only. Fluorescence intensity was determined using an Epics C cell sorter (Coulter Electronics Inc., Hialeah, FL) as previously reported (11).

*Expression of Data.* Data are expressed as the mean ± SE. Significance was determined using a nonpaired Student's *t* test.

## Results

After 10 wk of twice weekly treatment with 1 mg of anti-L3T4, MRL-*lpr/lpr* mice exhibited a marked reduction in spleen and lymph node mass (Fig. 1). The lymph node and spleen weights in anti-L3T4-treated mice were reduced greater than 90 and 80%, respectively, compared with control groups, and approximated those of MRL-+/+ mice. Mice treated with control rat Ig showed no difference in the weights of lymphoid tissue compared with untreated mice.

Cells bearing the L3T4 antigen were undetectable in the spleen and lymph nodes of anti-L3T4-treated mice (Fig. 2). Compared with the control rat Ig-treated mice, the percentage of $B220^+$ T cells was also significantly decreased after anti-L3T4 treatment. However, the residual level of these abnormal cells remained much greater than that seen in MRL-+/+ or normal mouse strains in which $B220^+$, Thy $1^+$ cells were undetectable (data not shown). An increase in the percentage of $Lyt-2^+$ cells was apparent in the anti-L3T4 group, while no change was observed in the percentage of $sIg^+$ or total $Thy-1.2^+$ cells (Fig. 2).
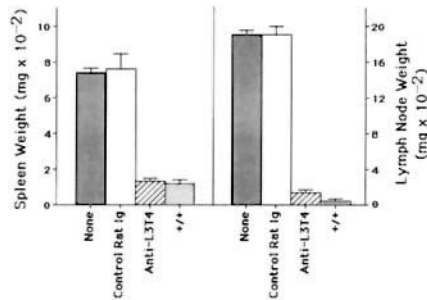
FIGURE 1.   Reduced lymphoid mass in anti-L3T4-treated mice. Individual spleens (left panel) and peripheral (submandibular, axillary, inguinal) lymph nodes (right panel) were isolated from MRL-lpr/lpr mice in the different study groups. The weights of spleens and lymph nodes from 14-wk-old MRL-+/+ mice are shown for comparison. Spleen and lymph node weights were significantly different ($p < 0.01$) in the group that received anti-L3T4 treatment relative to those of untreated mice and mice given control Ig.
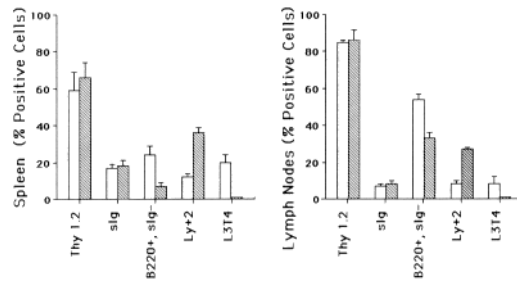


FIGURE 2.   Changes in lymphocyte subsets after anti-L3T4 treatment. Individual spleens and lymph nodes from mice receiving control rat Ig (open bars) or anti-L3T4 (hatched bars) were isolated and analyzed for phenotypic composition by fluorescence staining and cytofluorographic analyses. The percentage of $B220^+sIg^-$ cells was determined by subtracting the percentage of $sIg^+$ cells from that of $B220^+$ cells. Less than 1% of cells stained positive with the second-step reagent alone. The percentages of $L3T4^+$ cells, $B220^+sIg^-$ cells, and $Lyt-2^+$ cells were significantly different ($p < 0.05$) in the anti-L3T4 group compared with controls.

Interestingly, the percentage of $Thy-1^+$ cells that apparently lacked B220, Lyt-2, and L3T4 antigens was increased after anti-L3T4 treatment relative to that of control mice in both the spleen (23 vs. 3%) and lymph node (26 vs. 15%).

Sera from mice treated with anti-L3T4 or control rat Ig were tested for IgG and IgM antibodies to ssDNA and total histones (Fig. 3). A marked reduction in IgG antinuclear antibody levels was seen in the anti-L3T4 group. Serum IgM anti-DNA and antihistone antibodies were, however, comparable in both groups (Fig. 3) and significantly increased over levels in the sera of nonautoimmune
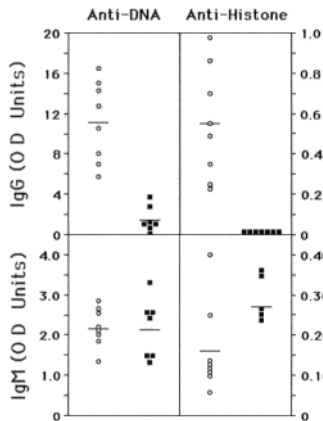


FIGURE 3.   Quantification of anti-DNA and antihistone antibody levels in anti-L3T4-treated mice. Sera from individual mice given control rat Ig (open circles) or anti-L3T4 (closed squares) were assayed for IgG autoantibodies (upper panels) or IgM autoantibodies (lower panels). The levels of IgG anti-DNA and IgG antihistone antibodies were significantly reduced ($p < 0.001$) in the anti-L3T4-treated group relative to controls.

mice (data not shown). Total serum IgG levels in the anti-L3T4-treated mice (10.1 ± 3.4 mg/ml) were similar to those in immunologically normal sex- and age-matched (C57BL/6 × DBA/2)$F_1$ mice (7.4 ± 1.2 mg/ml), but significantly decreased compared with MRL-*lpr/lpr* mice administered control rat Ig (25.0 ± 3.7 mg/ml).

Although the expression of autoimmune features was significantly reduced in MRL-*lpr/lpr* mice given 2 mg of anti-L3T4 weekly, the treatment itself was accompanied by morbidity with two of nine mice becoming moribund during week 10 of the trial coincident with antibody administration. Although repeated high doses of anti-L3T4 have not been reported to induce a host-immune response to rat Ig, a reaction to foreign protein appeared to be the most likely cause of these acute deaths. Autopsy of both mice revealed minimal lymphadenopathy, normal spleen weight and architecture, normal renal histology, and no evidence of viral or bacterial infection (data not shown).

Another study using a reduced dose and shortened course of anti-L3T4 antibody (1 mg/wk for 6 wk) was undertaken, with control mice receiving PBS alone. Immediately after treatment, lymphadenopathy was not clinically detectable in four of five antibody-treated mice. In contrast, all five untreated mice displayed evidence of lymphoproliferation. After 6 wk of anti-L3T4 treatment, mean levels of IgG antihistone antibodies were 0.68 ± 0.20 U in control mice and 0.09 ± 0.04 U in the four anti-L3T4-treated mice without lymphadenopathy. Mean IgG anti-DNA levels were 2.5 ± 0.24 U in the untreated group and 0.85 ± 0.18 U in the four mice that received anti-L3T4 therapy and were without lymphadenopathy. The single treated mouse with lymphadenopathy had both elevated antihistone (0.45 U) and anti-DNA (3.8 U) antibody levels. Within 2 wk of discontinuation of anti-L3T4 treatment, all mice exhibited generalized lymphadenopathy. Furthermore, IgG antihistone and anti-DNA antibodies rapidly increased to 0.98 ± 0.29 and 2.55 ± 0.58 U, respectively.

## Discussion

The current study demonstrates for the first time that lymphoproliferation in *lpr*-bearing mice, which relates mainly to the accumulation of $B220^+$ T cells, is heavily dependent upon the presence of the L3T4 subset. After repeated administration of anti-L3T4 antibody beginning at 4 wk old in MRL-*lpr/lpr* mice, spleen and peripheral lymph node weights were reduced by greater than 80 and 90%, respectively. Moreover, a decrease in the percentage of $B220^+$ T cells in both spleen and lymph nodes suggests a greater than 90% total body reduction of these mutant T cells after anti-L3T4 treatment. It is noteworthy that $B220^+$ T cells remained easily detectable in the treated MRL *lpr/lpr* mice. However, massive expansion of this subset, which is characteristic of *lpr*-bearing mice (6–8), did not occur.

We also found that anti-L3T4 treatment of MRL-*lpr/lpr* mice resulted in a marked reduction in IgG anti-ssDNA and antihistone antibody production. After 10 wk of therapy, serum levels were reduced by 85 and greater than 95%, respectively, compared with control mice. In contrast, production of IgM antibodies to these nuclear antigens appeared to be unaffected by anti-L3T4 treatment. The selective dependence of IgG, but not IgM anti-DNA antibody pro-

duction, on the L3T4 subset was previously reported in (NZB × NZW)F$_1$ mice (4). The data suggest that L3T4$^+$ T cells in both MRL-*lpr/lpr* and New Zealand mice are independently involved in stimulation of autoantigen-specific B cells. An alternative, but not mutually exclusive, explanation is that anti-L3T4 treatment in MRL-*lpr/lpr* mice leads to decreased autoantibody production through a reduction in the number of B220$^+$ T cells. Whether anti-L3T4 treatment exerted a primary or indirect effect in our study is unclear.

The L3T4 (CD4) antigen is present on T cells that primarily recognize antigen in the context of class II MHC molecules (12). In immunologically normal mice, there exists a subset of L3T4$^+$ T cells that are specifically reactive to self class II MHC antigens alone (15). We have previously postulated a central role for class II MHC molecules and self-reactive T cells in the etiopathogenesis of spontaneous murine lupus-like diseases, including that which occurs in MRL-*lpr/lpr* mice (16). Hyperexpression of class II MHC antigens, which has been found on macrophages and B cells of MRL-*lpr/lpr* mice and New Zealand mice (17), might predispose to stimulation of self-reactive T cells and subsequent activation of other lymphoid cells. This hypothesis, which predicts that treatment with either anti-L3T4 or anti-Ia antibody will abrogate disease, is supported by earlier reports in (NZB × NZW)F$_1$ mice (4, 18) and by the results presented herein in MRL-*lpr/lpr* mice.

## Summary

The current study examines the role of the L3T4 T cell subset in the development of lupus-like autoimmunity and lymphoproliferation in *lpr*-bearing mice. Chronic treatment of MRL-*lpr/lpr* mice with anti-L3T4 antibody beginning at 4 wk old was found to markedly decrease the production of IgG anti-DNA and antihistone antibodies, while having no effect on IgM autoantibodies. A dramatic reduction in splenomegaly and lymphadenopathy was also observed coincident with a decrease in the percentage and total number of Thy-1$^+$, B220$^+$ cells. Together, the data suggest an important role for L3T4$^+$ T cells in the pathogenesis of disease in *lpr* mice and provide further evidence that a requirement for the L3T4 subset may be a common feature of murine autoimmunity.

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