# CD4+ T CELLS ARE REQUIRED FOR DEVELOPMENT OF A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY SYNDROME (MAIDS)

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Adult C57BL/6 (B6) or C57BL/10 mice inoculated with LP-BM5 murine leukemia viruses (MuLV)[1] develop a syndrome characterized by progressive lymphadenopathy, splenomegaly, and increasingly profound immunodeficiency with death occurring at 16–26 wk after infection (1–3). The advanced stages of this syndrome, termed murine acquired immunodeficiency syndrome (MAIDS), are associated with enhanced susceptibility to infection (4) and the development of B cell lineage lymphomas (5, 6).

The present studies were based on the observations that congenitally-athymic C57BL/10-nu/nu mice are resistant to the immunodeficiency disease induced by LP-BM5 MuLV, but that susceptibility is reconstituted in these animals by injection of purified, mature T lymphocytes (7). These findings clearly demonstrate that development of B cell dysfunction in MAIDS is dependent on the presence of functional T cells. Almost all peripheral T cells in the mouse belong to one of two subpopulations that are mutually exclusive in their expression of the cell surface antigens CD8 and CD4 (8). A number of functional attributes also serve to distinguish between $CD8^+,CD4^-$, and $CD8^-,CD4^+$ T cells, henceforth referred to as $CD8^+$ and $CD4^+$ cells, respectively. $CD8^+$ T cells usually respond to antigens associated with class I molecules of the MHC, whereas $CD4^+$ cells generally recognize antigens in association with class II MHC determinants. In addition, activated $CD8^+$ T cells can exhibit CTL or suppressor activities, whereas $CD4^+$ T cells usually help or induce other T cells or B cells to proliferate, differentiate, or perform a variety of effector functions (8).

[1] Abbreviations used in this paper: CU, cytotoxic units; eco, ecotropic MuLV; FFU, focus-forming units; MAIDS, murine acquired immunodeficiency syndrome; MCF, mink cell focus-forming; MuLV, murine leukemia virus; PFC, plaque-forming cells; PFU, plaque-forming units.

The observations of polyclonal B cell activation and differentiation during the course of MAIDS, together with the understanding that normal B cell activation is usually dependent on lymphokines produced by $CD4^+$ rather than $CD8^+$ T cells (9–12), suggested to us that hyperactivity of $CD4^+$ cells might contribute to the pathogenesis of this syndrome, and that elimination of these cells could inhibit the development of disease. The opportunity to evaluate this possibility was provided by the demonstration that $CD4^+$ cells can be depleted in vivo by treatment with the anti-CD4-specific mAb GK1.5 (13, 14). We report here that depletion of $CD4^+$ T cells in mice before infection with LP-BM5 MuLV inhibited the development of many of the immunologic abnormalities characteristic of MAIDS, and that established disease was reversed by modifications of this depletion regimen.

## Materials and Methods

*Mice and Viruses.*    C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were inoculated with 0.1 ml LP-BM5 MuLV at 4 wk of age. LP-BM5 MuLV consist of a mixture of B-tropic ecotropic (eco) and B-tropic mink cell focus-inducing (MCF) MuLV (1–6, 15). Adult B6 mice inoculated with this virus mixture (LP-BM5 eco + MCF) exhibit replication of both virus classes in spleen and lymph node, develop lymphadenopathy, splenomegaly, and immune defects within 4 wk of infection, and die between 12 and 26 wk after injection (1–6). Biologically cloned isolates of LP-BM5 eco, obtained by limiting dilution titrations in tissue culture, replicate efficiently in lymphoid tissues of B6 mice infected as adults, but the animals do not develop prominent lymphoproliferation or immunodeficiency and live for at least 1 yr (Lee, J. S., R. A. Yetter, K. L. Elkins, K. L. Holmes, J. W. Hartley, H. C. Morse, III, manuscript in preparation). Uncloned stocks of LP-BM5 MCF MuLV, free of ecotropic virus, replicate poorly in tissues of mice infected as adults, and inoculated mice live for more than 6 mo without signs of disease (our unpublished observations). Development of disease thus appears to be related to unique features of the LP-BM5 MCF virus that are dependent on LP-BM5 eco for efficient spread in vivo, or to undefined interactions of the eco and MCF components of the virus mixture.

Virus stocks of LP-BM5 eco and LP-BM5 eco + MCF were prepared from chronically infected SC-1 cells. Titers of ecotropic MuLV were determined by the XC plaque test (16), and mink-infectious (MCF) viruses were titered using a fluorescent antibody focus-forming assay (17). The LP-BM5 eco pool contained $10^{3.5}$ plaque forming units (PFU)/0.2 ml. The LP-BM5 eco + MCF pool contained $10^{4.3}$ PFU/0.2 ml ecotropic virus and $10^{3.3}$ focus-forming units (FFU)/0.2 ml of MCF virus.

Infectious center assays in tissue culture were performed by cocultivation of mitomycin C–treated spleen cells with SC-1 cells for assays of ecotropic MuLV by the XC plaque test and with mink lung cells for assays of MCF viruses by the fluorescent antibody focus-forming tests (16, 17). Titers of ecotropic MuLV are expressed as $\log_{10}$ PFU/$10^7$ cells and titers of mink-infectious virus as $\log_{10}$ FFU/$10^7$ cells.

*Treatment of Mice with Anti-CD4 Antibody.*    Anti-CD4 mAb GK1.5 was grown in ascites and prepared for inoculation of mice as described (14). Inoculation protocols are detailed in the text.

*Flow Microfluorometry (FMF).*    Single cell suspensions of peripheral lymph nodes were prepared and stained for FMF assays as described (18, 19). The origins, characteristics, and nominal specificities of mAbs to Thy-1.2, Ia, CD8, CD4, Mac-1, and xenoantibodies to κ light chain used in this study were detailed previously (18, 19). All antibodies used were directly conjugated with FITC. FMF assays were performed on $3 \times 10^3$ viable cells, determined by light scatter and exclusion of propidium iodide, using a flow cytometer (FACS 440; Becton Dickinson & Co., Mountain View, CA) equipped with an argon and an argon/dye laser (18, 19).

*Serum IgM Assays.*    Levels of IgM in sera were determined by an ELISA assay, to be described elsewhere (Lee, J. S., et al., manuscript in preparation) using TEPC 183 IgM as a standard.

*Immunization.*    Mice were inoculated with an optimally immunogenic dose (10 μg i.p.) of

TNP-Ficoll (20). 4 d later, TNP-specific plaque forming cell (PFC) responses of spleen cells were determined by established techniques using TNP-coupled SRBC (20).

*Generation of CTL.* Cytotoxic effector cells were generated in 24-well trays by coculturing 4 × 10⁶ irradiated (2,000 rad) BALB/c ($H$-$2^d$) stimulator spleen cells with 3 × 10⁶ B6 ($H$-$2^b$) responder spleen cells in a final volume of 2 ml for 5 d (21). The activity of effector cells was tested in a 4-h ⁵¹Cr-release assay with Con A-stimulated BALB/c spleen cells as targets. The relative CTL activity generated in individual mixed lymphocyte cultures was compared using a standardized measure of cytotoxic units (CU) per culture (21).

*Histopathology.* Portions of spleen and lymph node obtained at autopsy were sectioned, stained with hematoxylin and eosin, and examined microscopically.

## Results and Discussion

*Depletion of CD4⁺ T Cells from Virus-infected Mice Inhibits Lymphoproliferation and Phenotypic Changes in Lymph Node Cell Subpopulations.* Adult B6 mice were depleted of CD4⁺ T cells by intraperitoneal inoculations with 1 mg of mAb GK1.5 at days -4 and -1 before infection with LP-BM5 eco+MCF and every 3–5 d thereafter for the duration of the experiments. Mice treated with the mAb were compared with untreated mice infected with the virus mixture, and with untreated mice injected with the relatively nonpathogenic LP-BM5 eco virus alone. Untreated animals infected with LP-BM5 eco+MCF exhibited splenomegaly (Table I) and lymphadenopathy (by gross observation) at 4 wk after infection that was progressive through 12 wk. In contrast, spleen weights of mice infected with LP-BM5 eco+MCF but treated with mAb GK1.5 before infection were similar to those of mice infected with LP-BM5 eco or uninfected controls at each time point (Table I). Lymph nodes of mice infected with LP-BM5 eco+MCF and treated with mAb GK1.5 were also noted to be of similar size to mice infected with LP-BM5 eco.

To evaluate the effects of infection with LP-BM5 eco+MCF and treatment with mAb GK1.5 on lymph node cells, single cell suspensions prepared from peripheral nodes were examined by FMF for the proportions of cells expressing Thy-1, CD8, CD4 (Fig. 1), κ Ig light chain, Ia, and Mac-1 (Fig. 2) cell surface antigens. In contrast to normal mice or mice infected with LP-BM5 eco, mice infected with LP-BM5 eco+MCF demonstrated a significant depletion of Thy-1⁺ cells in lymph node (Fig. 1 *a*) due to losses of both CD8⁺ (Fig. 1 *c*) and CD4⁺ T cells (Fig. 1 *e*). The propor-

TABLE I

*Depletion of CD4⁺ T Cells Prevents Splenomegaly in Mice Infected with LP-BM5 eco + MCF*

| Weeks after infection | Spleen weight* | | |
|---|---|---|---|
| | LP-BM5 eco | LP-BM5 eco+MCF | LP-BM5 eco+MCF/CD4⁻ |
| | *mg* | | |
| 4 | 90 ± 3 | 210 ± 16 | 110 ± 10 |
| 8 | 100 ± 10 | 380 ± 40 | 90 ± 10 |
| 12 | 107 ± 10 | 450 ± 90 | 93 ± 12 |

Mice infected with LP-BM5 eco, LP-BM5 eco+MCF, or LP-BM5 eco+MCF after depletion of CD4⁺ cells (LP-BM5 eco+MCF/CD4⁻), were killed, and spleen weights were determined.
* Numbers indicate mean spleen weight ( ± 1 SE) for three to six mice in each group. Results at the 4- and 8-wk time points are representative of three and two experiments, respectively. Data at 12 wk after infection are from a single experiment. Mean spleen weight for eight uninfected controls is 80 ± 10 mg.
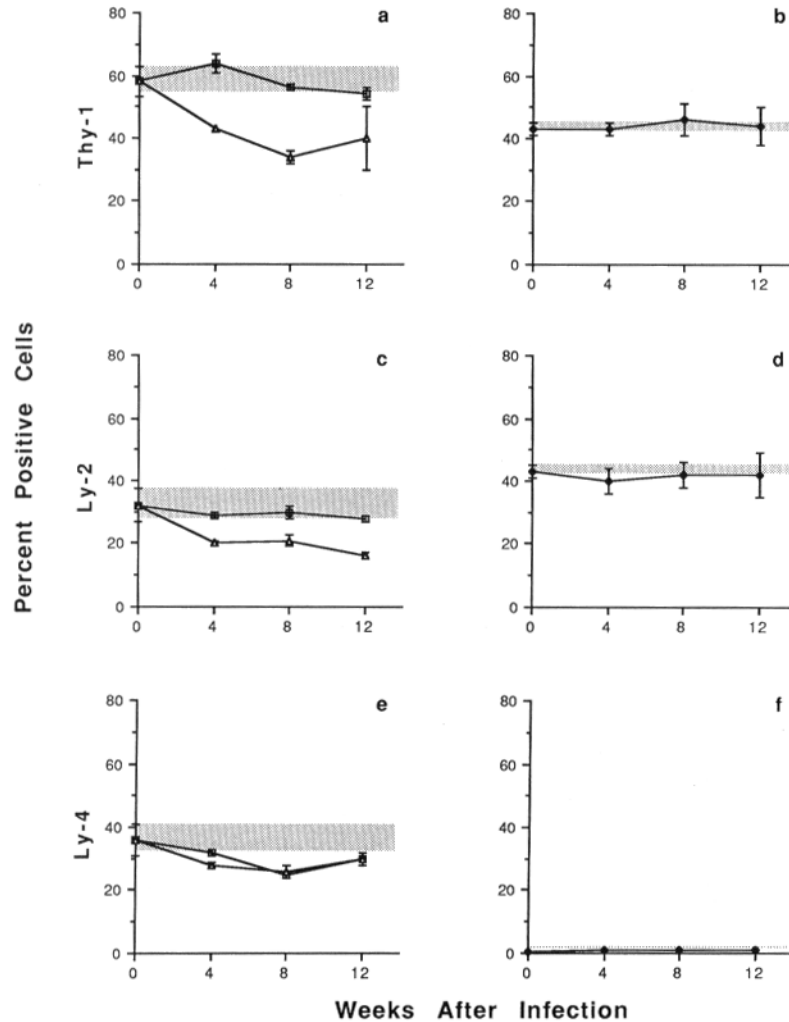
FIGURE 1.    FMF analyses of T cell populations in peripheral lymph nodes. Single cell suspensions from pooled peripheral lymph nodes were stained with FITC-labeled mAbs to Thy-1 (*a*, *b*), CD8 (*c*, *d*), and CD4 (*e*, *f*) and analyzed on a FACS. The shaded areas represent the mean (± 1 SE) percent antigen-positive cells in nodes of 10–21 uninfected normal mice (*a*, *c*, *e*) or 7 uninfected mice treated with mAb GK1.5 for 4 wk (*b*, *d*, *f*). Points indicate mean (± SE) percent positive cells for mice infected with LP-BM5 eco (-□-) LP-BM5 eco+MCF (-△-), or infected with LP-BM5 eco-MCF after treatment with mAb GK1.5 (-◆-), and assayed at the indicated times after infection (three mice/point). Mice treated with mAbs were judged to be numerically and functionally depleted of CD4^{+} cells by the following criteria. First, <1% of lymph node cells from mice injected with the mAb were reactive with FITC-labeled anti-CD4 antibody (*f*). Second, no more than 3% of cells from treated mice were reactive with FITC-labeled anti-rat Ig, indicating that few CD4^{+} cells coated with circulating mAb were present in these tissues. Third, the frequencies of CD8^{+} cells in nodes (*d*) approximated, within 5%, the frequencies of Thy-1^{+} cells (*b*), indicating that few Thy-1^{+}, CD8^{−}, CD4^{−} T cells were present in these tissues. Finally, studies of similarly treated, uninfected mice demonstrated that they were unable to generate Th cell–dependent antibody responses after immunization with SRBC or infection with ectromelia virus (14).
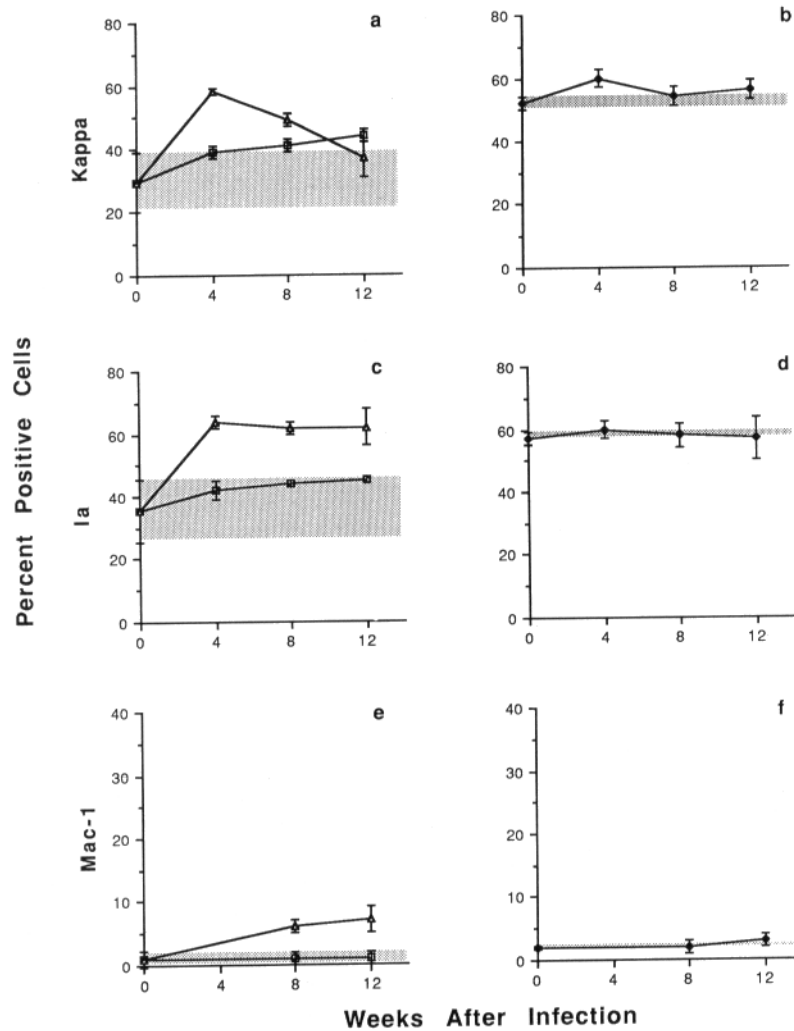
FIGURE 2.   FMF analyses of B cell and macrophage populations in peripheral lymph nodes. The same cell preparations described in the legend to Fig. 1 were stained with FITC-labeled goat anti–mouse κ antibodies (a, b) or FITC-labeled mAbs to Ia (c, d) or Mac-1 (e, f). Shaded areas and data points indicate means (± 1 SE) for normal and experimental mice as indicated in the legend to Fig. 1.

tional loss of CD4$^+$ T cells in mice infected with LP-BM5 eco + MCF was equivalent to that in mice infected with LP-BM5 eco, indicating that LP-BM5 MCF MuLV were not responsible for this change. By comparison, decreases in CD8$^+$ cells were observed only after infection with the mixed virus preparation.

These alterations in T cell frequencies in mice infected with LP-BM5 eco + MCF were associated with significant changes in the proportions of κ$^+$ B cells, Ia$^+$ cells (B cells and macrophages), and Mac-1$^+$ macrophages in relation to their representations in lymph nodes of normal mice or mice infected with LP-BM5 eco (Fig. 2). The frequency of κ$^+$ cells increased almost twofold at 4 wk after infection and

gradually decreased thereafter (Fig. 2 $a$). The reduced frequency of $\kappa^+$ cells at the 8- and 12-wk time points may be related to the progressive expansion of B cell immunoblasts that, like plasma cells, express very low levels of surface Ig (see below), and to clonal proliferations of B cells and pre-B cells that can first be detected around 12 wk after infection (5). The moderately increased frequencies of B cells in lymph nodes of mice infected with LP-BM5 eco were similar to those observed after infection of B6 mice with other B-tropic or NB-tropic MuLV (Lee, J. S., et al., manuscript in preparation), suggesting that both components of the virus mixture can influence B cell proliferation. The frequencies of $Ia^+$ cells (Fig. 2 $c$) and $Mac-1^+$ cells (Fig. 2 $e$) in nodes of mice infected with LP-BM5 eco + MCF were significantly and persistently increased at 4–12 wk after infection. Many of the cells expressing high levels of Ia (see below) were B cell blasts simultaneously expressing low levels of membrane Ig, and most $Mac-1^+$ cells were also $Ia^+$ (data not shown). The frequencies of cells expressing Ia and Mac-1 remained within normal ranges in mice infected with LP-BM5 eco (Fig. 2, $c$ and $e$).

Parallel studies of mice treated with mAb GK1.5 before infection with LP-BM5 eco + MCF showed that complete depletion of $CD4^+$ T cells (Fig. 1 $f$) prevented the development of each of these alterations in lymph node cell subpopulations. In comparison to normal mice depleted of $CD4^+$ cells, depleted mice infected with the virus mixture showed no significant changes in the frequencies of $Thy-1^+$ or $CD8^+$ T cells (Fig. 1, $b$ and $d$) or $\kappa^+$, $Ia^+$, or $Mac-1^+$ cells during the 12-wk observation period (Fig. 2, $b$, $d$, and $f$). Depletion of $CD4^+$ cells before infection thus prevented the development of lymphoproliferation and changes in lymphoid populations that are characteristic of MAIDS.

It should be noted that the effects of depleting $CD4^+$ cells cannot be attributed to elimination of macrophages. In both man and rats, macrophages as well as T cells express cell surface antigens homologous to CD4 (22, 23). However, we (data not shown) and others (24) have demonstrated that mouse macrophages are $CD4^-$, indicating that the preventive effects of treatment with mAb GK1.5 were due to depletion of $CD4^+$ T cells.

*Inhibition of B Cell Abnormalities in Virus-infected Mice by Depletion of $CD4^+$ Cells.* Abnormalities of B cells in mice infected with LP-BM5 eco + MCF include explosive follicular hyperplasia, enhanced differentiation to Ig-secreting cells, and failure to generate antigen-specific responses on immunization with Th-dependent or Th-independent antigens (1–3). In lymph nodes and spleens of mice infected with LP-BM5 eco + MCF, B cell hyperplasia was marked by an early expansion of lymphoid follicles, followed by distortions of normal follicular structure, and finally, by nearly complete effacement of normal architecture (data not shown). By comparison, lymph nodes and spleens of mice infected with LP-BM5 eco or LP-BM5 eco + MCF but depleted of $CD4^+$ cells before infection exhibited none of these perturbations of lymphoid structure (data not shown).

Polyclonal B cell activation in mice infected with LP-BM5 eco + MCF was also evidenced by the appearance of B cell blasts that expressed reduced levels of surface Ig. At 8 wk after inoculation, the majority of $\kappa^+$ cells in lymph nodes of mice infected with LP-BM5 eco + MCF (Fig. 3, $c$ and $d$) stained with lower intensity and were considerably larger, as judged by forward angle light scatter, than $\kappa^+$ cells in lymph nodes of mice infected with LP-BM5 eco (Fig. 3, $a$ and $b$). By comparison,
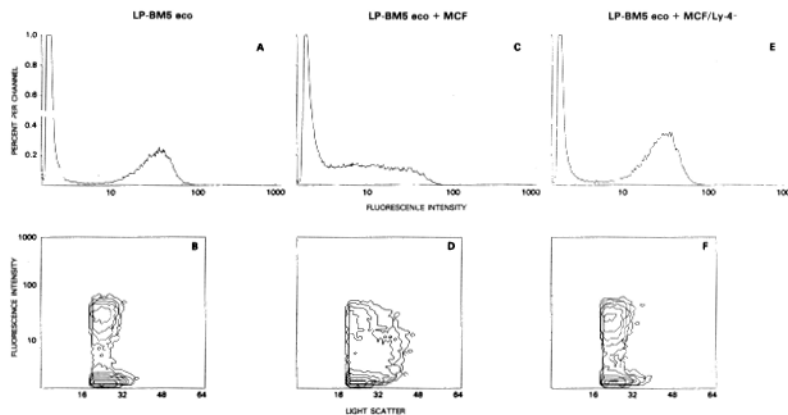
FIGURE 3.   FMF analyses of κ⁺ cells in lymph node from infected mice. Single cell suspensions prepared from peripheral lymph nodes of mice infected 8 wk previously with LP-BM5 eco (*a*, *b*), LP-BM5 eco+MCF (*c*, *d*), or infected mice depleted of CD4⁺ cells (*e*, *f*) were reacted with FITC-labeled goat anti–mouse κ light chain antibodies and analyzed on a FACS. Single parameter fluorescence profiles (*a*, *c*, *e*) demonstrate increased frequencies of dull κ⁺ cells in lymph nodes of mice infected with LP-BM5 eco+MCF (*c*). Two-parameter (fluorescence vs. light scatter) contour maps of lymph node cells (*b*, *d*, *f*) demonstrate the presence of dull κ⁺ cells of increased size (*d*) present in lymph nodes of mice infected with LP-BM5 eco+MCF that are not seen in cells from infected mice depleted of CD4⁺ cells.

the staining levels and sizes of κ⁺ cells in lymph nodes of infected mice depleted of CD4⁺ T cells (Fig. 3, *e* and *f*) were comparable to κ⁺ cells from uninfected mice or from mice infected with ecotropic virus alone.

Lymph node cells from the same mice were also studied for levels of Ia expression. The levels of class II antigens were greatly increased on B cells of mice infected with LP-BM5 eco+MCF. In contrast, levels of Ia expression on B cells of infected mice depleted of CD4⁺ T cells were similar to the levels on B cells from mice infected with LP-BM5 eco (data not shown).

Hypergammaglobulinemia is another hallmark of B cell dysfunction in MAIDS (1–3). Studies of sera from infected mice showed that IgM levels of animals inocu-
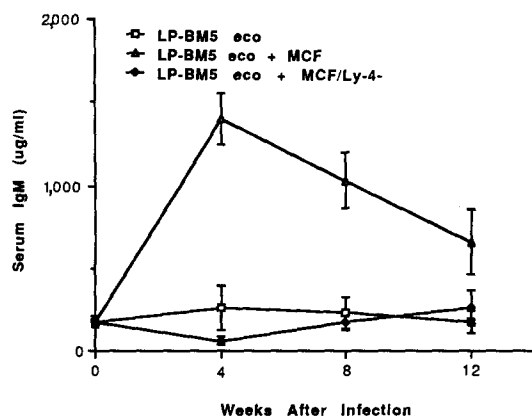


FIGURE 4.   IgM levels in sera from infected mice. Sera collected from infected mice were tested for IgM levels by an ELISA assay (Lee, J. S., et al., manuscript in preparation) using purified TEPC 183 IgM as a standard.
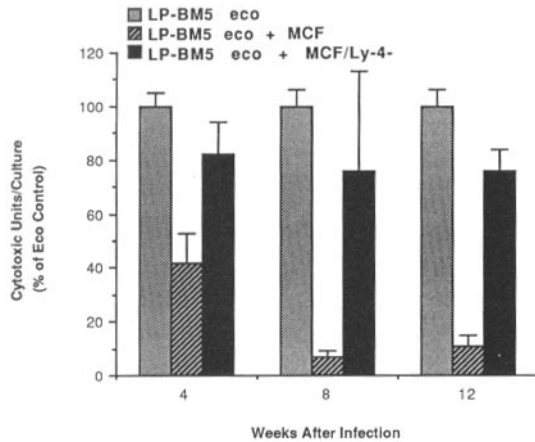
FIGURE 5.   Allogeneic CTL responses of mice infected with LP-BM5 MuLV. Mice infected with LP-BM5 eco, LP-BM5 eco + MCF, or LP-BM5 eco + MCF after depletion of CD4$^+$ cells (LP-BM5 eco + MCF/CD4$^-$) were killed at 4, 8, and 12 wk after infection for assays of splenic allogeneic CTL reactivity. Bars indicate the means ($\pm$ 1 SE) of cytotoxic units/culture (21) for spleen cells from three to eight mice stimulated for 5 d in vitro with irradiated (2,000 rad) BALB/c spleen cells and assayed on $^{51}$Cr-labeled, Con A-stimulated BALB/c spleen cell blasts.

lated with LP-BM5 eco + MCF were increased more than fivefold at 4 wk after infection and gradually decreased at 8–12 wk (Fig. 4). By comparison, IgM levels in sera of mice infected with LP-BM5 eco or LP-BM5 eco + MCF after depletion of CD4$^+$ cells remained at essentially normal levels at all times after infection.

The ability of B cells to generate antigen-specific PFC responses to Th-dependent antigens is absent in mice depleted of CD4$^+$ cells (13, 14), whereas responses to at least some Th-independent antigens may be much less affected (data not shown). To evaluate the function of B cells in MAIDS, we immunized mice with the Th-independent antigen, TNP-Ficoll (20). At 8 wk after inoculation, the responses of mice infected with LP-BM5 eco + MCF were negligible (11 PFC/10$^6$ spleen cells), but a substantial response (63 PFC/10$^6$ cells) was generated by infected mice depleted of CD4$^+$ T cells.

We conclude that depletion of CD4$^+$ cells before infection with LP-BM5 MuLV inhibits the development of most if not all of the B cell abnormalities associated with MAIDS including lymphoproliferation, polyclonal B cell activation, hypergammaglobulinemia and impaired specific antibody responses to a Th-independent antigen.

*Inhibition of Allogenic CTL Dysfunction in Virus-infected Mice.*    We reported elsewhere that T cells of mice infected with LP-BM5 eco + MCF exhibited a variety of abnormalities, including markedly depressed in vitro CTL repsonses to TNP-modified autologous cells (self + x) (3; Yetter, R. A., G. Shearer, M. Miller, H. C. Morse, III, manuscript in preparation) and alloantigens (2) and in vivo CTL responses to ectromelia virus (4). Since CD4$^+$ Th cells are not required for induction of allogenic CTL responses (25, 26), we studied the ability of spleen cells from infected B6 (*H-2$^b$*) mice to generate CTL after cocultivation with irradiated BALB/c (*H-2$^d$*) spleen cells (Fig. 5). In agreement with other studies (2, 3), B6 mice infected with LP-BM5 eco + MCF showed an early and progressive loss of allogenic CTL reactivity in comparison to mice infected with LP-BM5 eco. In contrast, allogenic CTL responses of infected mice depleted of CD4$^+$ cells did not differ significantly from those of mice infected with LP-BM5 eco. These results indicate that depletion of CD4$^+$ cells before infection prevents development of one of the major T cell dysfunctions evidenced in mice infected with LP-BM5 eco + MCF.

TABLE II

*Expression of Infectious MuLV in Spleen Cells of Normal and CD4+
T Cell-depleted Mice Infected with LP-BM5 eco + MCF*

| Weeks after infection | Treatment | Virus expression* | |
| --- | --- | --- | --- |
| | | Ecotropic MuLV ($\log_{10}$ PFU/$10^7$ cells) | MCF MuLV ($\log_{10}$ FFU/$10^7$ cells) |
| 4 | None | $4.3 \pm 0.1$ | $1.0 \pm 0.2$ |
| | GK1.5 | $4.8 \pm 0.1$ | $0.9 \pm 0.2$ |
| 8 | None | $4.5 \pm 0.1$ | $1.9 \pm 0.1$ |
| | GK1.5 | $4.7 \pm 0.1$ | $1.5 \pm 0.2$ |

* Infectious center assays in tissue culture were performed by cocultivation of mitomycin C–treated spleen cells with SC-1 cels for assays of ecotropic MULV by the XC plaque test (16) and with mink lung cells for assays of mink-infectious viruses by a fluorescent antibody focus-forming test (17). Titers of ecotropic virus are expressed as $\log_{10}$ PFU/$10^7$ cells and titers of mink infectious virus as $\log_{10}$ FFU/$10^7$ cells. Numbers indicate the mean ($\pm$ 1 SE) for assays of spleen cells from three mice per group.

*Expression of Infectious MuLV in Virus-infected Mice.*    To examine the possibility that differences in immune function between mice infected with LP-BM5 eco + MCF and infected mice depleted of CD4+ cells could be explained by alterations in virus expression, spleen cells from inoculated mice were tested for production of ecotropic and MCF MuLV. As shown in Table II, there were no significant differences between the proportions of spleen cells producing ecotropic or MCF viruses in either set of mice at 4 or 8 wk after infection. These results suggest that CD4+ T cells are not the primary targets for infection by LP-BM5 viruses, and are not required for infection of other cell types.

*Treatment of MAIDS with mAb GK1.5.*    The results presented above indicated that depletion of CD4+ cells before infection with LP-BM5 eco + MCF prevented the development of most of the major manifestations of MAIDS. To determine if CD4+ cells were required not only to initiate but also to sustain the immunologic abnormalities characteristic of the syndrome, mice were infected with LP-BM5 eco + MCF, and the disease was allowed to develop for 2 wk before treatment with mAb GK1.5 was initiated using the protocol diagrammed in Fig. 6 *a*. After 4 wk of treatment, 16 of 17 mice in two experiments had <1% CD4+ cells in lymph nodes and <4% anti-rat Ig+ cells (data not shown).

Additional FMF studies of lymph node cells from depleted mice showed that they closely resembled cells from mice depleted of CD4+ cells before infection; they included few dull $\kappa^+$ blast cells, and the intensity of Ia staining on B cells was equivalent to that detected on cells from uninfected mice. In addition, the frequencies of Thy-1+, $\kappa^+$, Ia+, and CD8+ cells were similar to those of mice treated with antibody before infection (data not shown).

Further studies of mice completely depleted of CD4+ T cells after infection showed that they exhibited minimal lymphadenopathy and had spleen weights similar to those of uninfected mice or mice infected with LP-BM5 eco (Fig. 6 *b*). Serum IgM levels were in the range for uninfected mice and were considerably lower than untreated mice infected with LP-BM5 eco + MCF (Fig. 6 *c*). In this experiment, spleen cells from mice were assayed for generation of allogeneic CTL in vitro and for TNP-
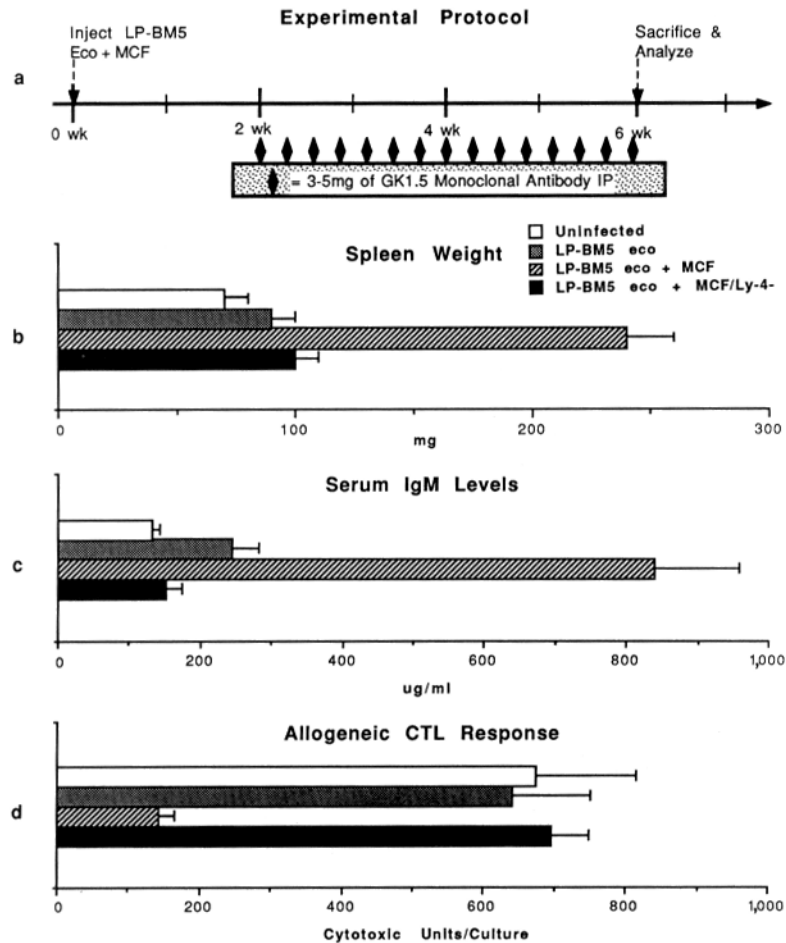
FIGURE 6.    Treatment of LP-BM5 MuLV-induced disease by depletion of CD4$^+$ T cells. The experimental protocol is outlined in (a). At 6 wk post infection, 9 mice in each experimental group were sacrificed and examined for spleen weight (b), serum IgM levels (c) and CTL responses to allogeneic (BALB/c, $H$-$2^d$) stimulator cells. Bars indicate the mean ($\pm$ 1 SE) for mice in each group.

specific PFC 4 d after immunization with TNP-F. As expected, the CTL responses of mice infected with LP-BM5 eco+MCF were greatly impaired, whereas normal responses were generated by infected mice depleted of CD4$^+$ T cells (Fig. 6 $d$). In parallel with earlier studies, mice infected with LP-BM5 eco+MCF were markedly deficient in the generation of antigen-specific PFC after immunization with TNP-Ficoll; however, a low but significant PFC response was detected in spleen cells of infected mice depleted of CD4$^+$ T cells (data not shown). Histologic studies of spleens and lymph nodes from antibody-treated mice showed a return of distorted follicular areas towards normal (data not shown). Finally, spleen cells from mice infected with LP-BM5 eco+MCF or from infected mice depleted of CD4$^+$ cells were examined in infectious center assays for expression of infectious ecotropic and

MCF viruses. Equivalent frequencies of virus-producing cells were detected among spleen cells from mice of both groups (data not shown).

The results of our studies demonstrate that depletion of CD4$^+$ T cells was effective in preventing many of the immunologic abnormalities that develop within the first 12 wk after infection with LP-BM5 eco + MCF, and that established disease could be reversed by elimination of this T cell subset. These findings indicate that dysfunction of CD4$^+$ T cells is central to the induction and progression of both B and T cell abnormalities in MAIDS. The mechanisms by which infection with LP-BM5 MuLV activates and alters the functions of these cells is not known, although previous studies (6) suggest that infection of T cells by these viruses occurs at low frequencies if at all. In situ hybridization studies demonstrating virus in marginal zone macrophages at one week after infection (Mosier, D. E., R. Gulizia, D. Spector, unpublished observations) suggest that CD4$^+$ T cell/macrophage interactions may contribute to the activation process.

Although the present studies clearly indicate the pivotal role of activated CD4$^+$ T cells in MAIDS, the exact means by which these cells influence the functions of B cells and other T cells remains to be determined. Recent studies demonstrating greatly impaired IL-2 production (Yetter, R. A., et al., manuscript in preparation) in association with markedly stimulated gamma interferon production (Pitha, P. M., D. Biegel, R. A. Yetter, and H. C. Morse, III, submitted for publication) in mice infected with LP-BM5 MuLV suggest that imbalances among immunoregulatory products of CD4$^+$ cells may be of importance. Our studies do not exclude the possibility that CD8$^+$ cells may also contribute to the pathogenesis of MAIDS in untreated mice, but they strongly suggest that, in the absence of CD4$^+$ cells, CD8$^+$ cells have little if any effect on disease progression.

The effect of depleting CD4$^+$ cells on the late sequelae of virus infection (shortened life span, enhanced susceptibility to infection, and development of lymphomas) is not known. We have found that mice incompletely depleted of CD4$^+$ T cells before or after infection exhibit abnormalities that presage the appearance of oligoclonal populations of B cells and failure to generate virus-specific CTL responses (data not shown) indicating the induction or progression of MAIDS is inhibited only to the extent that CD4$^+$ T cells are depleted. Indeed, cessation of treatment with mAb was associated with development of fulminant disease.

The implications of our results for understanding the pathogenesis of the HIV-induced immunodeficiency syndrome of man are as yet unknown, although numeric and functional abnormalities of CD4$^+$ are prominent features of AIDS (27) as well as MAIDS. It seeems likely, however, that the development of detailed understandings of retroviral pathogenesis in immunodeficiency syndromes of the mouse, cat, and nonhuman primates will contribute to a dissection of the mechanisms operative in the human disease.

## Summary

Mice depleted in vivo of CD4$^+$ Th cells by treatment with mAb GK1.5 were found to be resistant to the lymphoproliferative/immunodeficiency disease (MAIDS) induced in intact mice by infection with the mixture of LP-BM5 murine leukemia viruses. Depleted mice did not develop lymphadenopathy or splenomegaly, had normal serum IgM levels, normal CTL responses to alloantigens, and were able to generate

PFC responses to Th-independent antigens even though frequencies of virus-producing spleen cells were comparable in depleted and intact mice. Depletion of CD4$^+$ Th cells after infection resulted in a reversal of many abnormalities exhibited by infected controls; spleen weights, serum IgM levels, and allogeneic CTL responses of treated mice were comparable to those of uninfected controls. These results demonstrate that dysfunction of CD4$^+$ Th cells is central to the induction and progression of both T and B cell abnormalities in MAIDS.

## References

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