CD4 T Cells in Murine Acquired Immunodeficiency Syndrome: Polyclonal Progression to Anergy

By Girija Muralidhar, Susanne Koch, Martin Haas, and Susan L. Swain

From the Department of Biology and the University of California, San Diego, Cancer Center, University of California, San Diego, La Jolla, California 92093

Summary

We have examined the kinetics of changes that occur in the helper T cell subset during murine acquired immunodeficiency syndrome, which occurs after infection with the mix of viruses known as BM5. We find that there is expansion of the CD4 T cells by 2 wk, 50% of the CD4 T cells become large as the disease progresses, and the CD4 T cell population is increasingly comprised of cells with a memory/activated phenotype. These effects are apparent by 2 wk postinfection, and the change is nearly complete by 6–8 wk. The phenotypic shift is paralleled by the loss of the ability of the CD4 T cells to proliferate or to produce interleukin 2 (IL-2), IL-3, IL-4, and interferon γ in response to stimulation with mitogens, superantigen, or anti-CD3. There is no obvious expansion or deletion of CD4 T cells expressing particular Vβ genes, as might be expected if a conventional superantigen were driving the changes. The results suggest, however, that the total CD4 population has been driven to anergy by some potent polyclonal stimulus directly associated with viral infection.

Infection of C57BL/6 mice with the LP-BM5 (BM5) mixture of retroviruses (1) causes a fatal immunodeficiency syndrome whose similarity to human AIDS has prompted the disease to be called murine acquired immunodeficiency syndrome (MAIDS)1 (2). The disease is characterized by dramatic splenomegaly and lymphadenopathy, by hypergammaglobulinemia and by loss of T cell and later B cell function (3, 4). During the late stages of the disease, mice may develop B cell lymphomas and are susceptible to opportunistic infections. The disease in B6 mice is invariably fatal. The virus responsible for MAIDS is a defective murine leukemia virus, termed BM5a (5) or DuSH (6), which may or may not require the presence of helper virus for disease induction (7).

The CD4 T cell subset is dramatically altered during the course of MAIDS. As the disease progresses, there is a loss of helper T cell response to mitogens, with both proliferation and IL-2 production virtually disappearing by the time the disease is full-blown (3, 8). It has also been shown that CD4 T cells undergo a phenotypic change losing expression of both SM3G11 and SM6C10 (8). Paradoxically, as the syndrome progresses and mice infected with BM5 become immunodeficient and as CD4 T cell function begins to decline, there is a dramatic increase in size of peripheral lymphoid organs, including an increase in the total number of CD4 T cells. Of particular interest is the fact that mice depleted either of T cells, or of only the CD4 subset of T cells, fail to develop MAIDS (2, 9). The CD4 dependence of the disease suggests that CD4 T cells play an important positive role in the disease process despite their apparent immunodeficiency.

The expression of distinct patterns of cell surface antigens has been useful in discriminating among different subsets of T cells and also in suggesting the way such subsets interact with other cells, with lymphokines, and with various components of the extracellular matrix. For instance, like other resting T cells, antigen-inexperienced naive T cells express no IL-2R p55 (10), and they express low levels of CD44 (11, 12), which binds to hyaluronate (13), LFA-3 (CD11a/CD54), which binds to CD2 (14), and VLA-β1 integrins (15). Naive T cells express the high molecular weight form of CD45R identified by antibody to CD45RA in humans (16) and CD45RB (17) in mice. Naive cells also express high levels of the lymph node homing receptor t-selectin, identified by Ab to MEL-14 (18), and they are apparently short-lived in that they are depleted after adult thymectomy (TX) (19). In contrast, resting, long-lived memory cells are CD44high, LFA-3high, express CD45RO, but not CD45RA, or B, and are MEL-14~ (11–19). Most memory cells are resting and express little IL-2R p55 and are retained for >40 wk after TX

1 Abbreviations used in this paper: MAIDS, murine acquired immunodeficiency syndrome; PI, propidium iodide; SAG, superantigen; TX, thymectomy.
When stimulated, naive cells synthesize and secrete only IL-2, while memory cells can secrete small amounts of IL-3, IL-4, IL-5, and IFN-γ in addition to IL-2 (17–21). The phenotype of activated, responding CD4 T cells is less clear, although they are invariably IL-2R p55 positive and often have lost CD45RB and MEL-14. Activated T cells often have increased expression of adhesion molecules such as CD44, LFA-3, and CD2 (17–20). Cell lines were used to generate Ab for indirect immunofluorescence with fluoresceinated RG7/9/1 (FLRG7, mouse anti-IL-4). Other mAbs used for staining were to LFA-1 (FD44.1), ICAM-1 (31), L-PAM (32), 3G11 and 6C10 (22), IL-2R (7D4), and Ab specific for TCR VB chains included Vβ5, Vβ8, and Vβ11 (23). Early studies of the MAIDS suggested that expansion of both B and T cells during disease was broadly polyclonal (24, 25), with oligoclonal populations of B cells developing later in disease in concordance with B cell lymphoma generation (4). However, recent studies from Hügin et al. (26) have suggested that BM5 may encode a superantigen (SAG) that preferentially stimulates the proliferation of CD4 T cells with TCR having either Vβ5 or Vβ11. They found that two B cell lymphomas, originally isolated from MAIDS animals, stimulated the proliferation of normal splenic Vβ5+ and Vβ11+ CD4 T cells. The ability of two of the three lymphomas to stimulate the response correlated with the expression of the defective viral gag product and was partially blocked by mAbs directed to that product.

Here we report studies on the phenotypic and functional changes in CD4 T cells that accompany the progression of MAIDS. We find that the CD4 T cell population shifts from one with a heterogeneous phenotype characteristic of a mixed population of cells of naive and memory phenotype, to a more homogeneous population with a phenotype that broadly shares characteristics of memory and activated cells. We suggest that this phenotypic shift of CD4 T cells differentiated to a more homogeneous population with a phenotype that is less frequently expressed on CD4 T cells, which have memory phenotype (22, 23). Cells expressing 3G11 are also less often expressed on Thy-1 molecules, appears to increase as CD4 T cells differentiate (23).

Materials and Methods

Mice and Viruses. C57BL/6 (B6) mice were either obtained from The Jackson Laboratory (Bar Harbor, ME) or bred in our own facility. LP-BM5 (BM5) viral stocks were obtained as cell free supernatants of chronically infected SC-1 cells (24). Mice between 6 and 8 wk of age were injected intraperitoneally with 0.5 ml of BM5 viral stock. A minimum of three mice per group were analyzed individually and compared with age-matched, uninjected control B6 mice.

Antibodies and Recombinant Lymphokines. Cell lines were maintained for mAb production as previously described (27) and were used for cell depletions. These mAbs were to: Thy-1.2 (F7D5 and HO 13.14), CD8 (HO2.2), CD4 (RL172.4), class II (4B12) (D3.137) (a gift of Dr. Susan Tonkonogy, North Carolina State University, Raleigh, NC), and J11D. Ab used for FACS® analysis include anti-CD4 PE-labeled GK1.5 (PE-GK1.5) (Becton Dickinson & Co., Mountain View, CA), and a panel of antibodies to phenotype CD4 cells including: anti-CD44 (Pgp-1) (28), anti-CD45RB (23G2) (a gift of Dr. Ellen Pure, The Rockefeller University, New York, NY) (29), and anti-thy-1-selectin (MEI-14) (30). Cell lines were used to generate Ab for indirect immunofluorescence with fluoresceinated RG7/9/1 (FLRG7, mouse anti-IL-4). Other mAbs used for staining were to LFA-1 (FD44.1), ICAM-1 (31), L-PAM (32), 3G11 and 6C10 (22), IL-2R (7D4), and Ab specific for TCR Vβ chains included Vβ3, Vβ5, Vβ8, Vβ11 (31, 32, 33, 6.7, 8.1–8.3, 9.11 (Pharmingen, San Diego, CA). For lymphokine assays, the following antibodies were obtained as ascites from nude mice (33): 11B11 (anti-IL-4), 7D4 and PC61 (anti-IL-2R), TRFK4 and TRFK5 (anti-IL-5), and R46A2 and XMG1.2 (anti-IFN-γ). Lymphokines were obtained as follows: human rIL-2 from Cetus Corp. (Emeryville, CA), murine rIFN-γ from Amgen Biologicals (Thousand Oaks, CA), rIL-4 and rIL-5 from X63.Ag6-653 line that was transfected with the murine cDNA for either IL-4 or for IL-5 (34), (a kind gift of Dr. Fritz Melchers, Basel Institutes for Immunology, Basel, Switzerland).

Preparation of Cells. Whole spleen cells depleted of erythrocytes by hypotonic lysis were used for staining. For functional analysis, CD4 T cells obtained from spleens of either uninfected or BM5-infected animals were used. CD4 T cells were purified by treatment with anti-CD8, anti-J11D, and anti-class II plus complement. Antibody treatment was carried out on ice for 30 min, followed by a combination of rabbit and guinea pig complement at 37°C for 45 min as previously described (35).

Preparation of APC. Spleen cells from either uninfected or BM5-infected animals were depleted of T cells by treatment with anti-CD4, anti-CD8, and two anti-Thy-1 antibodies followed by complement treatment as described above. The resulting cell population was treated with 50 μg/ml of mitomycin C.

Culture Conditions. Medium used for all cultures was RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 200 μg/ml penicillin, 200 μg/ml streptomycin, 4 mM L-glutamine, 10 mM Hepes, 5 × 10−5 M 2-ME, and 7.5% FCS (HyClone Laboratories, Logan, UT). To determine the ability of CD4 T cells to produce lymphokines, purified CD4 T cells were cultured at 105/ml with control APC at 5 × 105/ml, and with 2 μg/ml Con A or 10 μg/ml SEA (Toxin Technologies, Sarasota, FL), 10 μg/ml anti-CD3, and 5 ng/ml PMA in 24-well plates. Culture supernatants were harvested after 36 h, and α methylmannoside was added to neutralize any residual Con A. Supercells were harvested at 36 h, and α methylmannoside was added to neutralize any residual Con A. Supernatants were stored at −20°C before analyzing lymphokine concentration. Proliferation was determined by mixing 105/ml of CD4 T and 5 × 105/ml APC in triplicate cultures. [3H]-UDR, was added for the last 18 h of a 72-h culture.

Lymphokine Assays. Bioassays for IL-2, IL-3, and IL-4, and the analysis of the data have been previously described (27, 33, 35). Briefly, IL-2 and IL-4 were assayed using the NK indicator line, which is responsive to IL-2 and IL-4. To assay IL-2 content of the supernatants, IL-4 was inhibited by addition of 11B11. In parallel cultures, IL-2 was blocked by addition of two anti-IL-2R antibodies to assay for IL-4.

IL-3 was assayed using the 32Dc15 line, which does not respond to other lymphokines under our assay conditions. IL-5 and IFN-γ were assayed by ELISA as described previously (18). Briefly, 96-well plates (Nunc, Roskilde, Denmark) were coated with 1 μg/ml of TRFK.5 anti-IL-5 or R46A2 anti-IFN-γ anti-
bodies and stored at 4°C overnight. Plates were blocked with PBS containing 1% BSA and 0.05% Tween-20 for 1 h at 37°C followed by the incubation of samples and standards for 1 h at room temperature. The plates were washed and further incubated with biotinylated TRFK.4 for IL-5 or XMG1.2 for IFN-γ at room temperature for another 1 h, and peroxidase-conjugated streptavidin was added for 1 h at room temperature. Substrate containing 100 μg/ml O-phenylenediamine dihydrochloride and 200 μg/ml urea hydrogen peroxide in citrate phosphate buffer was added to plates and incubated for 30 min at room temperature in the dark, after which the reaction was terminated by the addition of 25% sulfuric acid to a final concentration of 10%. Plates were read at 492 nm using a Multiskan MCC/340 ELISA reader (Titertek, McLean, VA). IFN-γ and IL-5 were quantitated in comparison to known standards.

**FACS® Analysis.** Freshly derived splenocytes were resuspended in red blood cell lysis medium (Sigma Chemical Co., St. Louis, MO), washed in BSS with 5% FCS, and the resultant cells were resuspended in PBS containing 5% FCS and 0.1% sodium azide. A 100-μl aliquot containing 106 cells was used for staining. Cells were stained with appropriate antibodies on ice for 30 min, washed, and counterstained with a secondary labeled antibody. All cells were stained with PE-conjugated anti-CD4. Cells were either fixed with 1% methanol-free formaldehyde in PBS or analyzed immediately on a FACScan® flow cytometer (Becton Dickinson & Co.).

For propidium iodide (PI) staining, cells were prepared as described above and stained with PE-conjugated anti-CD4. PI was added to cells to a final concentration of 5 μg/ml, and PI– and CD4 cells were analyzed immediately. Data are shown as dot plots of gated CD4 or as histograms obtained using Consort 30 software.

**Results**

In our preliminary studies it was clear that significant changes in the CD4 population were already apparent within 2 wk of inoculation with the BM5 virus mixture. Therefore, we have focused our studies on the CD4 T cells taken within the first few weeks of infection, and we have analyzed the early kinetics of the changes in function and phenotype as a way to investigate the mechanisms leading to immunodeficiency.

**Kinetics of Changes in CD4 Number and Function during MAIDS.** We analyzed groups of three individual mice at 1, 2, 4, 6, and 8 wk postinfection, and evaluated the early changes in the number of CD4 T cells. We determined total white cell recovery and the proportion of cells expressing CD4 as detected by staining with PE-labeled anti-CD4. Results of this analysis are shown in Fig. 1.

The total number of CD4 T cells recovered per spleen rose steadily from 1 to 6 wk postinfection, but leveled off after that time. The ratio of CD4 T cells in infected over uninfected B6 mice at 8 wk postinfection ranged from 1.2 to 2.0, with a mean of 1.7 for four experiments. Thus, the population of CD4 T cells clearly underwent moderate, but significant expansion. To begin to assess the fraction of the T cells involved in response at different times after inoculation of virus, we examined the size of individual CD4 T cells by examining their forward vs. side scatter in FACS® analysis, reasoning that cells must enlarge before they divide. A large forward scatter indicates a larger cell volume. An example of such an analysis of fresh, unseparated splenic cells gated on CD4 cells that exclude PI (live cells) from mice infected 1, 2, and 8 wk previously with BM5 vs. control age-matched animals is shown in Fig. 2. Only 5% of CD4 cells in control mice (A) gave a forward scatter significantly higher than the majority of cells (to the right of the dotted line). In contrast, in this experiment >10% of CD4 cells had higher forward scatter at 1 wk (B), and by 2 wk (C), 18% CD4 T cells were larger. By 8 wk, 50% of the CD4 T cells were larger than the bulk of control cells (D). These results indicate that an increasing proportion of CD4 T cells in the BM5-infected animal are apparently participating in the response to virus, at least by undergoing enlargement. It is interesting that this larger cell size is not accompanied by the conversion of the same proportion of cells to an IL-2R p55-positive state, as shown below (see Fig. 4).

**CD4 T Cells in MAIDS Do Not Show an Altered Distribution of TCR Vβ Expression.** If the proliferation of CD4 T cells were due to the action of a Vβ-selective element or SAG encoded by the BM5 viral mixture, the Vβ usage of the CD4 T cells in infected mice should shift to reflect the selective outgrowth of CD4 T cells using TCR with the responsive Vβ(s). In parallel, TCR using other nonselected Vβs should progressively decrease. As the responding population expanded, the changes in representation of Vβs should become more and more dramatic. Therefore, we analyzed the expression of a panel of Vβ chains by staining and fluorescent analysis (Tables 1 and 2). We examined mice at 1, 3, 8, and 11 wk after BM5 infection. Splenocytes from groups of two to three mice at each time point were pooled and compared with cells from control mice. Because activated T cells might be differentially damaged by some purification techniques, whole spleen cells were used. To detect each Vβ, we stained first with Ab to the individual Vβ, followed by fluoresceinated Ab specific for the first Ab. After washing, we counterstained with PE anti-CD4 and analyzed only CD4+ T cells. Results of several experiments are summarized in Table 1.

We saw no striking, consistent preference in Vβ usage, although there was some variability in expression of Vβs from experiment to experiment. Shown in Table 2 is a summary of the mean ratio of different Vβs with standard errors (infected/control determined at 8 wk) from all four experiments performed at this time point. There were no differences in Vβ usage that would be strong enough to indicate a truly Vβ-selective effect. There were slight increases in the ratio in BM5 infected vs. control mice for Vβ5 (mean 1.75) and Vβ7 (mean 2.0). However, there were no significant decreases
Forward Scatter

in other Vβs (ranging from 0.76 to 1.30). Since 50% of CD4 T are large at this time, indicating that they have responded in some fashion, it must be concluded that cells bearing many Vβs are represented in the compartment of responding cells.

Phenotype of CD4 T Cells in MAIDS. Since CD4 T cells at different stages of differentiation and with different histories of antigen exposure show very different expression of many functionally relevant cell surface markers (reviewed in

Table 1. Vβ Usage in MAIDS Animals

| Vβ | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 |
|----|-------|-------|-------|-------|
|    | Control | Week 1 | Control | Week 3 | Week 11 | Control | Week 8 | Control | Week 8 |
| Vβ3 | 1.5 | 5.7 | ND | ND | ND | 4.2 | 5.5 | 2.8 | 4.9 |
| Vβ5.1, 5.2 | 2.6 | 4.2 | 2.7 | 6.3 | 3.8 | 2.2 | 3.3 | 3.7 | 6.6 |
| Vβ6 | 6.6 | 8.3 | 7.2 | 7.6 | 3.2 | 6.8 | 5.2 | 10.9 | 14.7 |
| Vβ7 | 0.5 | 3.5 | ND | ND | ND | 1.7 | 1.8 | 2.3 | 5.8 |
| Vβ8.1, 8.2 | 7.2 | 8.7 | 16.2 | 14.2 | 11.4 | 10.3 | 10.8 | 10.3 | 10.3 |
| Vβ9 | ND | 4.3 | ND | ND | ND | 2.3 | 3.0 | 4.5 | 4.5 |
| Vβ11 | 5.8 | 4.4 | 4.4 | 5.3 | 3.1 | 4.8 | 3.4 | 9.4 | 6.0 |

10^6 Splenocytes from control or infected animals at different weeks postinfection were double stained with PE-labeled anti-CD4 and Vβ-specific antibodies. The percentage of Vβ-positive cells among CD4 cells was calculated based on FACS® analysis.
Table 2. The Ratios of Vβ-positive Cells in Infected/Control Spleens at 8 wk

| Vβ-specific CD4 cells | BM5/control ratio |
|-----------------------|------------------|
| Vβ3                   | 1.30 ± 0.28      |
| Vβ5.1, 5.2            | 1.75 ± 0.35      |
| Vβ6                   | 0.84 ± 0.33      |
| Vβ7                   | 2.00 ± 0.63      |
| Vβ8.1, 8.2            | 1.05 ± 0.25      |
| Vβ9                   | 0.94 ± 0.16      |
| Vβ11                  | 0.76 ± 0.16      |

Same as Table 1, except ratio of Vβ+ CD4 cells of infected over control animals is shown as mean ± SE.

reference 19), it seemed likely that examination of the phenotype of CD4 T cells in MAIDS animals would be informative. An example of expression of such a panel of markers by CD4 T cells in 6-wk BM5 infected (solid line) versus control mice (dotted line) is shown in Fig. 3. Fig. 4 shows results with additional markers from a separate experiment at 8 wk postinfection. Both are representative of profiles seen in more than three experiments. The histograms reflect the staining intensities among the gated CD4+ population. CD4 T cells from normal animals were heterogeneous with respect to expression of CD44, CD45RB, and the t-selectin, MEL-14 (Fig. 3, left, middle, and right, respectively). Routinely, a majority of the peripheral CD4 T cells (60-80%), shown with dotted lines, had a naive phenotype (CD44\textsuperscript{hi}, CD45RB\textsuperscript{hi}, CD45RB\textsuperscript{hi}, 3G11\textsuperscript{hi}) rather than a memory phenotype (CD44\textsuperscript{lo}, CD45RB\textsuperscript{lo}, CD45RB\textsuperscript{lo}, 3G11\textsuperscript{lo}) (Figs. 3 and 4). Most resting T cells (both naive and memory) also express moderate levels of LFA-1 and ICAM-1, and low levels of LPAM and ICAM-1, and they are almost all IL-2R negative (Fig. 4).

In MAIDS animals, the majority of cells express a phenotype similar to that of memory cells or of activated T cells. The majority of T cells from BM5-infected animals became CD44\textsuperscript{hi}, CD45RB\textsuperscript{lo}, and MEL-14\textsuperscript{lo} (Fig. 3), a phenotype that characterizes both memory (19) and many activated CD4 T cell populations. In addition, the MAIDS CD4 T cells expressed higher levels of LFA-1 and ICAM-1 than either memory or naive cells (Fig. 4). Their expression of LPAM and IL-2R was also slightly higher than that of resting cells. Although many cells appeared to be activated on the basis of forward scatter, the increase in IL-2R expression was quite slight in BM5-infected animals, and was considerably below the levels expressed on activated Con A blasts or in vitro generated effectors (not shown), which express very high levels of IL-2R p55. As indicated previously (8), CD4 T cells in BM5-infected animals express little 3G11 or 6C10, a phenotype largely associated with a population of apparently nonfunctional CD4 cells (23).

To better understand these phenotypic shifts, we have analyzed the kinetics of the changes in expression of CD44, CD45RB, and MEL-14. In Fig. 5, the marker-positive BM5 cells as a percent of positive control cells has been determined for three individual animals per group at 1, 2, 4, 6, and 8 wk after infection. The mean percent of CD4 cells positive for each marker in individual infected mice versus the average in control mice with standard error of that mean is shown. This shift in expression of CD44, CD45RB, and MEL-14 after BM5 infection phenotype was progressive, but was already clearly detectable by 2 wk indicating a rapid change. Thus, the CD4 T cells in BM5-infected animals almost all have a unique phenotype that is significantly different from other well-characterized subsets of CD4 T cells.

Proliferative Response of CD4 T Cells during MAIDS. We examined the proliferation of CD4 T cells from control mice and from mice infected with BM5 8 wk previously in response to a variety of stimulants. The results, presented in Fig. 6a, compare the responses of two individual control and BM5-infected animals. CD4 T cells from control mice proliferated vigorously to Con A and to anti-CD3, and gave
modest response to SEA. The addition of PMA somewhat improved the responses to TCR-directed reagents. Regardless of the stimulant, BM5 CD4 T cells proliferated poorly.

Since the MAIDS CD4 T cells had slightly elevated levels of IL-2R (Fig. 5), we also tested whether addition of IL-2 would reconstitute the proliferation of the CD4 T cells. The presence of 20 U/ml exogenous IL-2, a dose that in our hand supports maximum expansion of CD4 T cells, and maximum proliferation of activated CD4 T cells (33), had very little effect on the proliferation of CD4 T cells from BM5-infected animals stimulated by Con A or Con A plus PMA (Fig. 6 b). IL-2 also had little effect alone or with SEA (not shown).

Kinetics of Changes in Lymphokine Secretion by CD4 T Cells during MAIDS. To confirm the loss of CD4 T cell function and to further investigate the extent of the immunodeficiency among CD4 T cells after BM5 infection, CD4 T cells were purified from the spleens of the individual mice analyzed in Fig. 5 and mixed with APC from control animals. Cultures of cells from individual spleens were stimulated with Con A and PMA, and supernatants harvested 36 h later. The titers of IL-2, IL-3, IL-4, IL-5, and IFN-γ were determined in each supernatant. The mean values with standard errors from three individual mice are shown in Fig. 7. By 2 wk postinfection, mitogen-stimulated IL-2 production by the CD4 cells of
MAIDS animals was already significantly reduced to ~50% of control levels. By 6–8 wk postinfection, IL-2 production by CD4 T cells from MAIDS mice was barely detectable. IL-4 and IL-3 production was also lost with time, but the kinetics of these changes were somewhat delayed with a twofold reduction obvious only at 4–6 wk. IL-5 production was too low to be reproducibly detected in controls and is therefore not shown, but little or no IL-5 production was seen at any time point. IFN-γ production appeared somewhat more resistant to the disease, and significant decreases in IFN-γ were not detected until 6–8 wk. The levels of IFN-γ, however, were extremely low and quite variable between animals. Moreover, IFN-γ may be made by APC and possibly by the few contaminating CD8 T cells. The kinetics of changes, in mitogen-stimulated lymphokine production after viral infection, especially of IL-2, which is the major lymphokine produced by CD4 T cells directly derived from animals, thus appears quite similar to those changes in cell surface marker expression.

Since one report had suggested that IL-5 and IL-4 production was not lost in BM5-infected animals (36), we further examined the IL-4 production of infected animals to a variety of stimulants, and results are presented in Fig. 8. As expected, IL-2 production (Fig. 8a) was completely absent by 8 wk postinfection regardless of whether Con A, anti-CD3 with or without PMA, or SEA was used to stimulate the cells. Similarly, IL-4 production was dramatically reduced and low IL-4 production was independent of the stimulant (Fig. 8b). The low levels of IFN-γ produced by control cells was absent in the BM5-infected mice and levels of IL-3 were reduced with similar effects seen regardless of the mode of stimulation (not shown). No IL-5 production was detected in any case.

Discussion

This analysis of the changes in CD4 T cells associated with MAIDS, induced by BM5 retroviral infection, suggests that the initial resting immunocompetent CD4 population is quite rapidly replaced by a polyclonal population of CD4 T cells that might best be described as anergic. The CD4 population, which comes to predominate, largely has a memory or activated phenotype as shown here, but responds poorly to mitogenic stimuli such as Con A, anti-CD3, or SEA. This anergy includes a loss of the ability to proliferate or to produce IL-3, IFN-γ, and IL-4, as well as IL-2.

How does this change in the overall population occur? There are several possibilities. First, the bulk of the naive cells could be induced to proliferate in a limited fashion and then become anergic. Alternatively, a smaller population of memory or naive cells could be stimulated to proliferate more extensively and their progeny could become the predominant population. Recently a number of studies have indicated that certain bacteria, especially of the Staphylococci and Streptococci...
families, and mouse mammary tumor viruses (reviewed in references 37 and 38), each encode so-called SAG with similar properties. These SAG are β select, stimulate strong proliferative responses and lymphokine production by T cells in vitro, and induce initial proliferation followed by donor proliferative responses and lymphokine production by T cells

The results in this study are more compatible with a large polyclonal response than with either a small or Vβ selective response. First, the increase in CD4 T cell numbers (Fig. 1) and changes in phenotype (Figs. 2–4) can already be detected by 1–2 wk postinfection, and the changes plateau by 6–8 wk, indicating that even by 2–4 wk a majority of the CD4 T cell population has been affected. Even if a small fraction of virus-specific T cells or virally infected T cells were proliferating rapidly enough to come to

represent a major proportion of the CD4 population, there is no a priori reason that the nonresponder population should change phenotype and/or lose function. A broadly polyclonal response is also suggested by the lack of significant changes in the spectrum of Vβ TCR utilized by the CD4 T cells in MAIDS vs. control animals (Tables 1 and 2). If only a small number of CD4 T cells expressing just a few Vβs were responsible for the net increase in CD4 T cells, there should be a considerably more dramatic shift of Vβ usage. This would be particularly obvious by 6–8 wk, when the total CD4 population has roughly doubled in number (Fig. 1), when ~50% of cells show increased forward scatter (Fig. 2), and when the phenotypic and functional changes suggest that the majority of CD4 T cells have become anergic (Figs. 3, 6, and 8). CD4 cells expressing Vβs that selectively responded should show a dramatic increase in proportion, while those that had not participated in response should show a substantial reduction. No consistent changes in ratio of that magnitude were detectable either at 1, 3, 8, or 11 wk of infection, and the changes in Vβ ratio at 8 wk were very modest with no Vβ showing more than a twofold increase or decrease (Table 2).

Especially surprising, in light of the results from Morse's laboratory, was the fact that Vβ- and Vβ1-positive cells, which are those that have been reported to selectively respond to a B cell lymphoma from BM5-infected mice (26), were neither selectively expanded nor deleted. This suggests that the conditions that lead to a Vβ selective expansion in vitro are not present in vivo. It should be noted that although these results argue against a conventional Vβ-selective SAG model, they do not rule out the possibility that a viral component or product could have broader mitogen activity that is not detectably Vβ selective but that causes a response similar to known SAGs.

A surprisingly dramatic shift in phenotype of the majority of CD4 T cells takes place after infection with BM5. The CD4 T cells in normal uninfected B6 animals include both naive and memory T cells. In young, healthy animals cells of naive phenotype usually predominate (60–80%). These cells are included in a population with the following characteristics inferred both from cell separation studies and from studies with adult thymectomized mice (reviewed in reference 19). Naive cells are small, resting, relatively short-lived cells without IL-2R, they express moderate levels of LFA-1, low levels of CD44 (Pgp-1), ICAM-1, L-PAM, and 6C10, and high levels of CD45RB, 3G11, and the t-selectin Mel-14. In contrast, resting, long-lived memory cells, which make up the other predominant component of peripheral CD4 cells, display a very different phenotype of high CD44 expression and low expression of CD45RB and MEL-14 and 3G11. However, like the naive cells, memory T cells express moderate levels of LFA-1 and low levels of ICAM and L-PAM, and are IL-2R negative. Activated or effector cells, which are represented in very low numbers in the peripheral CD4 T cells from healthy mice (<5%) can be induced by polyclonal stimulation. Such population often express memory levels of CD44, CD45RB, and MEL-14 (18, 19), and display enhanced levels of LFA-1 and ICAM-1, and high levels of IL-2R.

The cells in MAIDS animals progress within 6 wk to a
unique phenotype that has characteristics of both memory and activated cells. Most of the CD4 T cells in BM5-infected animals expressed increased levels of CD44, uniformly low levels of CD45RB and ME1:14 (Fig. 3), and a majority of cells expressed no 3G11 or 6C10 (Fig. 4; and see reference 8). There was a dramatic increase in the expression of LFA-1 and ICAM-1 and a lower but significant increase in expression of L-PAM. Only a small population of cells expressed the p55 IL-2R, and they did so at low levels. This phenotype is unusual, including the expression of many activation markers, but without the expression of the high levels of IL-2R normally found on activated T cells. The lack of expression of both 3G11 and 6C10 has been previously reported in MAIDS and has been associated with the CD4 population with little function in previous studies (8, 22, 23).

This unique cell surface phenotype was associated with a concomitant and severe loss of responsiveness. The CD4 T cells no longer proliferated to mitogenic stimulation (3; and Fig. 6). This dramatic loss of proliferative response is seen with Con A, anti-CD3, and SEA, and is not reconstituted by addition of PMA or IL-2. Moreover, CD4 T cells made very little or undetectable levels of most lymphokines in response to stimulation with conventional mitogens. Here we show kinetic results of stimulation with Con A plus PMA (Fig. 6), and we obtain a similar pattern with anti-CD3 plus or minus PMA, SEA, or Con A alone (Fig. 8). The immunodeficiency of the CD4 T cells in response to stimulation included lack of production of IL-2, IL-3, IL-4, IL-5, and IFN-γ. The results of IL-2, IL-3, and IL-4 were most striking because the CD4 population from normal mice made substantial levels of those lymphokines when stimulated, while they made only low levels of IFN-γ and no detectable IL-5. In the experiments shown, purified CD4 T cells from both control and MAIDS animals were combined with fresh APC from normal animals so that the defect in MAIDS animals seen here is clearly in the CD4 T cell rather than in the APC population. A recent study by Gazzinelli et al. (36) suggested that whole spleen cells from MAIDS-infected animals secreted IL-4 and IL-10 in response to Con A from 4 to 12 wk postinfec tion. Our studies differ from theirs in that we used isolated CD4 T cells supplemented with APC from normal mice rather than whole spleen. It should also be mentioned that the levels of IL-4 and IL-10 seen by Gazzinelli et al. (36) were rather modest. We have seen loss of IL-4 production consistently in more than five experiments.

The changes in CD4 phenotype that accompany MAIDS suggest that the CD4 population has received signals that have driven all or at least most of the cells to an anergic state. We would suggest that the wholesale shift of CD4 cells to a phenotype characteristic of memory or activated cells supports the concept that most cells have participated in a response and have effectively differentiated to an anergic state. Recently, we have analyzed the phenotype of a population of CD4 T cells rendered anergic by treatment of mice with a bacterial superantigen, SEA. These anergic CD4 T cells have a phenotype indistinguishable from that of the MAIDS CD4 T cells in this study (Swain, S. L., D. Hall, and G. Huston, manuscript submitted for publication). Therefore, it seems likely that the phenotype described here for MAIDS CD4 T cells will be one generally representative of that of T cells driven to the state of differentiation that corresponds to anergy.

What kinds of mechanisms might lead to the polyclonal anergy seen in MAIDS? It has been suggested that virally infected T cells may be influenced by viral genes and/or products that could interfere with their function, and that if such T cells proliferated they might be responsible for the state of immunodeficiency (41). Several considerations argue against such a model. First, CD4 T cells have not been shown to be a major target of the virus, and so far virus has been detected primarily in macrophages (42) and in some B cells (26). Second, it would be expected that if direct viral infection was required to suppress each T cell, many uninfected CD4 T cells would continue to be responsive to stimulation, and the state of immunodeficiency would not be so profound. Third, when cloned defective virus is introduced into mice using the ψ-2 packaging system, so that virus cannot replicate and widely infect cells, it is nonetheless able to cause all or most manifestations of MAIDS (6, 7).

A rapid and broadly polyclonal response of the kind observed in MAIDS would be most compatible with the existence of a soluble, diffusible product capable of stimulating most or all CD4 T cells, rather than with a more limited event. It is for these reasons that some form of SAG-like mechanism is an attractive possibility. The lack of Vβ selectivity, however, would suggest either that a novel, non-Vβ-selective SAG, mitogen, or active lymphokine is involved, or that a few initially infected or stimulated cells make such a broadly stimulating product. Although it can not be ruled out at the present time, there is no precedence for the latter possibility.

Although the relationship of the murine BM5-induced disease to human AIDS, induced by HIV, is unclear, they share several informative features. Both involve initial splenomegaly and lymphadenopathy and hypergammaglobulinemia. In AIDS there is convincing evidence that there is significant T cell immunodeficiency before there is widespread infection of lymphocytes and selective CD4 depletion (e.g., reference 43). Recently, it has been suggested that there is a selective loss of T cells bearing certain Vβ, and this has been taken as evidence of a possible HIV-associated SAG (44). It is likely that further experiments in the murine MAIDS model will reveal in more detail the mechanism of CD4 immunodeficiency caused by infection with the BM5 retrovirus, and that this could provide insights into other immunodeficiency syndromes as well as describe important aspects of viral-host interactions.
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