B CELLS ARE REQUIRED FOR INDUCTION OF T CELL ABNORMALITIES IN A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY SYNDROME

By ANDREAS CERNY,* AMBROS W. HÜGIN,‡ RICHARD R. HARDY,§ KYOKO HAYAKAWA,§ ROLF M. ZINKERNAGEL,‖ MASAHIKO MAKINO,* AND HERBERT C. MORSE III*

*From the *Laboratory of Immunopathology and ‡Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; the §Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111; and the ‖Institute for Experimental Pathology, University Hospital, Zurich, CH-8091 Switzerland*

A retrovirus-induced immunodeficiency syndrome of mice (1–4), termed MAIDS, shares many features with HIV-induced disease in man (5). Studies of host–virus interactions contributing to the pathogenesis of this syndrome showed that abnormalities of B cells, including polyclonal activation and impaired responses to mitogenic and specific antigenic stimuli, are dependent on the presence of activated T cells (6) of $CD4^+$ phenotype (7). Cells contributing to $CD4^+$ T cell activation under normal conditions include MHC class II (Ia)-bearing monocytes/macrophages, dendritic cells, or B cells. In the present study, the contribution of B cells to activation of $CD4^+$ T cells in MAIDS was evaluated in mice depleted of these cells by treatment from birth with rabbit anti–mouse IgM antibodies (8). We report here that induction of phenotypic and functional abnormalities of T cells in this syndrome requires the presence of mature B cells.

Materials and Methods

Mice.   C57BL/6J (B6) and BALB/cJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and B6 mice were bred in our laboratory. Induction, maintenance, and monitoring of B cell suppression were performed as described (8). Briefly, B6 mice were injected from birth repeatedly with high titer rabbit anti–mouse IgM antiserum. Treated mice had <1.5% B cells in FACS analyses of spleen and lymph node cells using mAb to Ly-5(B220) (9), undetectable mouse serum IgM levels by ELISA tests, and high titers of free rabbit anti–mouse IgM by immunodiffusion. Moreover, spleen cells did not proliferate in response to LPS, and histopathologic studies of spleen and lymph nodes showed hypoplastic follicles devoid of germinal centers.

Viruses.   Cell-free virus stocks of LP-BM5 MuLV, prepared and titered as previously described (2–4), were inoculated intraperitoneally in a volume of 0.1 ml at 4 wk of age. Tech-

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niques used in infectious center tests of mitomycin C-treated spleen cells for detection of ecotropic and mink cell focus-inducing (MCF) MuLV are detailed elsewhere (10, 11).

FACS Analyses. Single cell suspensions prepared from spleen were stained with labeled antibodies and analyzed on FACStar Plus machines (Becton Dickinson & Co., Mountain View, CA) as described (12, 13). Reagents included recently described mAbs SM6C10 and SM3G11 (12) that define subsets of CD4$^+$ T cell recognized by mAb GK1.5 (13), and mAb 6B2 reactive with Ly-5 (B220) (9).

Proliferative Responses and CTL Assays. Responses to Con A were induced in cultures of spleen cells (2 × 10^5/well) in 96-well plates treated with 2 μg of Con A for 24 h, pulsed with [^{3}H]thymidine, and harvested. MLR were induced in 96-well plates by cocultivation of 1 × 10^5 B6 spleen cells with 10^5 irradiated (2,500 rad) BALB/c spleen cells for 5 d before pulsing with [^{3}H]thymidine. CTL activity of MLC was also determined at 5 d of culture using ^{51}Cr-labeled, Con A-stimulated BALB/c splenic blast cells targets in a 4-h release assay.

Results and Discussion

Adult mice of susceptible strains (4) infected with a unique mixture of murine leukemia viruses (MuLV), designated LP-BM5 MuLV (1), develop a syndrome characterized by lymphoproliferation and progressive impairments of immune function, resulting in enhanced susceptibility to otherwise innocuous infection (2) and appearance of B cell lineage lymphomas (3). T cells of the CD4$^+$ subset are central to induction of B cell dysfunction as mice depleted in vivo of CD4$^+$ cells do not exhibit B cell proliferation, hypergamma globulinemia or severely depressed responses to Th-independent antigens (7). Studies of CD4$^+$ Th in infected animals demonstrated rapid alterations in both phenotype and function that were disproportionate to the decreased frequency of these cells in lymph node and spleen (13). In normal mice, CD4$^+$ cells in spleen are resolved into four subsets, termed fractions (Fr.) I to IV, (Fig. 1 *a*) based on expression of cell surface determinants SM3G11 and SM6C10
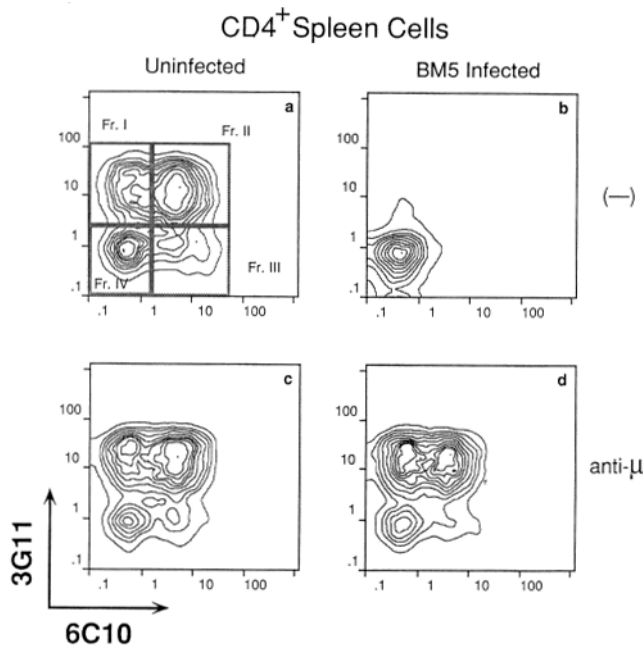

CD4$^+$Spleen Cells

FIGURE 1. Distribution of CD4$^+$ T cells subsets in spleens of normal mice (*a*), mice infected for 9 weeks with LP-BM5 MuLV (*b*), mice depleted of B cells (*c*), and mice depleted of B cells and infected for 9 wks with LP-BM5 MuLV (*d*) defined by multicolor FACS analyses using SM3G11 and SM6C10 mAb. Induction, maintenance, and monitoring of B cell suppression were performed as described (8). The mAbs SM3G11 and SM6C10 and the techniques adopted for FACS analyses have been detailed elsewhere (11).

recognized by two mAbs (12). These phenotypically distinct subsets also differ functionally such that following stimulation with Con A, cells from Fr.I produce IL-2 but not IL-4, cells from Fr.II produce both lymphokines, cells from Fr.III produce IL-4 but not IL-2, and cells from Fr.IV fail to produce either of these mediators (12). Infection with LP-BM5 MuLV results in the accumulation of CD4$^+$ cells of predominantly Fr.IV phenotype (Fig. 1 *b*) (13). Functional assays showed that these changes are associated with marked impairments in the ability of CD4$^+$ cells to provide help for induction of CTL responses, to proliferate in response to stimulation with the mitogen Con A or to soluble antigen, and to produce IL-2 after stimulation with autoantigen or Con A (13).

The reduced responsiveness of CD4$^+$ cells from infected mice to a variety of stimuli resembles the antigen-unresponsive state of Th clones soon after antigenic restimulation of maintenance cultures (14), following addition of high concentrations of antigen at the beginning of culture (15), or after exposure to antigen in the absence of adequate costimulatory activity (16). APC are required for induction of a nonresponsive state in each of these in vitro systems, suggesting that they could also contribute to induction of T cell dysfunction in vivo. To study a role for B cells versus other APC in this phenomenon, mice were depleted of B cells from birth (μ-suppressed) by chronic administration of rabbit anti–mouse IgM antibodies (8) and infected with LP-BM5 MuLV at 4 wk of age.

Multicolor FACS analyses of CD4$^+$ spleen cells from uninfected μ-suppressed mice showed that their distribution among Fr.I–IV was essentially unchanged from that of uninfected controls (Fig. 1, *c* vs. *a*; Table I), indicating that chronic administration of rabbit antisera had little if any effect on the phenotype of these cells. Strikingly, parallel studies of CD4$^+$ subsets in spleens of μ-suppressed mice infected with LP-BM5 MuLV for 9 wk also showed a near normal distribution of cells (Fig. 1 *d*; Table I), indicating that B cells are required for development of phenotypic abnormalities in mice infected with LP-BM5 MuLV.

Functional correlates of phenotypic alterations in CD4$^+$ cells after induction of MAIDS include impaired proliferative responses to Con A or alloantigens (1), and at 7–9wk post infection, inability to provide help for CTL responses to modified-self or alloantigens (13). Although allogeneic CTL responses can be obtained at this stage of disease, they do not require functional CD4$^+$ T cells for their induction (13). Analyses of these responses in μ-suppressed mice showed that proliferative responses to Con A and to alloantigens in a MLR were increased over normal (Fig. 2 *A*), whereas allogeneic CTL responses were indistinguishable from those of untreated mice (Fig. 2 *B*). Parallel studies of mice infected for 8 or 9 wk with LP-BM5 MuLV demonstrated that the impaired responses characteristic of untreated, infected mice were completely normalized in μ-suppressed animals (Fig. 2, *A*, *B*). These results demonstrated that B cells were required for induction of functional as well as phenotypic alterations of T cells in MAIDS.

One explanation for the effects of B cell depletion would be elimination of a major target for virus replication. This possibility was explored by determining the frequencies of spleen cells from inoculated control and μ-suppressed mice producing infectious ecotropic and MCF MuLV. At 9 wk after infection, equivalent frequencies of cells producing virus of either class were detected in spleens of both intact and μ-suppressed mice (Table I). These results indicate that LP-BM5 viruses have

TABLE I

Analysis of Spleen Cells for Lymphocyte Distribution and Virus Expression

Treatment[*]		Percent CD4+ cells[‡]	Percent CD4+ cells in:[‡]			
Anti-μ	LP-BM5		Fr. I	Fr. II	Fr. III	Fr. IV
−	−	15	26	49	16	18
−	+	13	8	3	6	82
+	+	22	43	33	8	16
+	−	19	34	38	12	17

		Percent Ly-5 (B220)[§] cells[‖]	Virus recovery[¶]	
			Eco	MCF
−	−	48	ND	ND
−	+	21	5.4	2.1
+	+	<1	5.4	2.4
+	−	<1.4	ND	ND

* Mice were treated from birth with rabbit antibodies to IgM (Anti-μ +) as described and were infected at 4 wk of age with LP-BM5 MuLV (LP-BM5 +). Spleen cells were tested for frequencies of lymphocyte populations and virus-producing cells 9 wk after infection.

‡ Frequencies of splenic CD4+ cells were determined in FACS analyses using mAb GK1.5.

§ Representation of CD4+ cells among the four fractions (Fr.I–IV) defined by reactivity with mAb SM3G11 and SM6C10 in multicolor FACS analyses.

‖ Frequencies of splenic Ly-5(B220)+ cells were determined in FACS analyses using mAb 6B2.

¶ Spleen cells from virus-infected mice were tested for frequencies of cells producing infectious ectotropic (Eco) or mink cell focus-forming (MCF) MuLV using infectious center assays performed as previously described. Numbers indicate the frequency (log_{10}) of virus producing cells/10^7 spleen cells.

no direct effect on T cell phenotype or function, and that in mice susceptible to MAIDS, T cells are ineffective in controlling virus replication.

Our data demonstrate that complex interactions between B and T cells are required for induction of the full spectrum of immunologic abnormalities characteristic of mice infected with LP-BM5 MuLV; B cells are critical for development of CD4+ T cell dysfunction, while CD4+ T cells are needed for induction of abnormalities of B cells, CD8+ T cells, and macrophages (7). Means by which B cells in
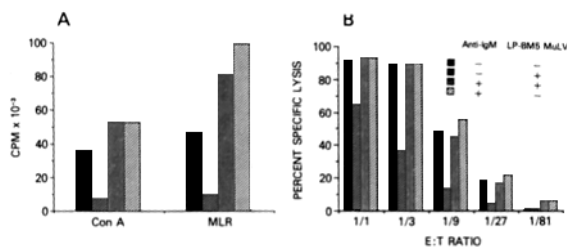

FIGURE 2. Proliferative responses of spleen cells to stimulation with Con A or irradiated BALB/c spleen cells (A) and CTL responses to allogeneic cells (B). For Con A proliferative responses and MLR, numbers indicate mean cpm × 10^{-3} for triplicate cultures minus background. CTL activity of MLC was also determined at day 5 using ^{51}Cr-labeled Con A-stimulated BALB/c splenic blast cell targets in a 4-h release assay. Numbers indicate mean percent specific lysis from triplicate wells for different E/T ratios minus spontaneous release.

intact, infected mice could alter phenotype and function of CD4$^+$ T cells include production of anti-T cell autoantibodies (17) or changes in APC activity. The observation of generalized T cell activation from early in disease (1) is consistent with the latter suggestion, and raises the possibility that a molecule with features of "superantigens" of self or microbial origin is presented to T cells by B cells (18). The variant Pr60^{gag} produced by the replication-defective component of LP-BM5 MuLV required for disease (19) is an attractive candidate for such a molecule.

Summary

The role of B cells in induction of phenotypic and functional abnormalities of T cells in a murine retrovirus–induced immunodeficiency syndrome, MAIDS, was evaluated in mice depleted of mature B cells from birth with anti-IgM antibodies (μ-suppressed) and infected at 4 wk of age. Multicolor FACS analyses of CD4$^+$ T cell subsets showed that development of phenotypic abnormalities of these cells at 9 wk after infection was completely inhibited by μ-suppression. Furthermore, induction of impaired proliferative responses to Con A and alloantigens and CTL responses to alloantigens was fully blocked in antibody-treated animals. The extent of virus replication was comparable in spleens of untreated and μ-suppressed mice. Retroviral induction of T cell dysfunction in MAIDS is thus dependent on the presence of B cells, and high level virus expression in mice without B cells has little or no effect on T cell function.

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