Brief Definitive Report

FUNCTIONAL T LYMPHOCYTES ARE REQUIRED FOR A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY DISEASE (MAIDS)

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We have recently described (1–3) a non-neoplastic lymphoproliferative disease in C57BL/6 mice infected as adults with the LP-BM5 murine leukemia virus (MuLV) mixture originally isolated by Laterjet and Duplan (4). This disease has many of the features of the acquired immunodeficiency syndrome (AIDS), including polyclonal B lymphocyte activation, hypergammaglobulinemia, lymphadenopathy, and profound immunodeficiency (1–6). The retroviral origin of the murine disease and the striking similarities in immunologic abnormalities to AIDS lead us to use the abbreviations MAIDS (murine acquired immunodeficiency syndrome) for the LP-BM5 MuLV-induced immunodeficiency disease. In man, the human immunodeficiency viruses (HIV) are thought to cause immunologic abnormalities by depleting $CD4^+$ helper T cells (7, 8) and by directly activating B cells (9). In mice, LP-BM5 MuLV infection is known to decrease helper T cell function (1), but it is unclear whether alterations in T cell function are related to the observed B cell hyperactivity. Since polyclonal activation of B cells normally precedes loss of helper T cell function in both AIDS and MAIDS, and because normal B cell activation is dependent upon a series of T cell–derived lymphokines (10–13), it is possible that the initial effects of retroviral infection include a polyclonal activation of helper T cells that in turn act to stimulate B cells. We have examined these issues by studying the course of MAIDS in T cell–deficient athymic C57BL/10 *nu/nu* mice.

Materials and Methods

Mice. C57BL/10N mice heterozygous for the nude mutation were obtained from the Division of Research Resources, NIH, and maintained under specific pathogen-free conditions. Homozygous *nu/nu* offspring or *nu/+* littermates were used at 8–16 wk of age.

Virus. A freshly-thawed suspension of LP-BM5 MuLV containing 3×10^4 PFU ecotropic MuLV and 4×10^3 mink cell focus–forming units (MCF) MuLV was injected intraperitoneally in a volume of 0.5 ml. This dose of virus is ~10-fold greater than that required to cause disease in 100% of normal C57BL/10 mice.

T Cell Reconstitution. 10^7 nylon wool–passed splenic T cells from C57BL/10 mice were

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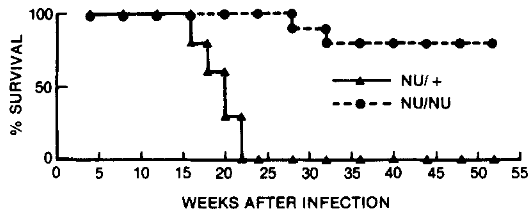

FIGURE 1. Survival of normal (*nu*/+) and athymic *nu*/*nu* C57BL/10 mice infected with LP-BM5 MuLV. 10 mice (5 of each sex) were injected intraperitoneally with 0.5 ml LP-BM5 MuLV containing 3×10^4 PFU ecotropic and 4×10^5 FFU MCF virus at 6–8 wk of age. The two *nu*/*nu* mice that died at 28 and 32 wk of age showed no histopathologic evidence of MAIDS.

injected intravenously into C57BL/10 *nu*/*nu* recipients. The splenic T cells were >95% Thy-1^+ by flow cytometric analysis. All such T cell–reconstituted *nu*/*nu* mice showed evidence of T cell function (see Fig. 3).

Virus Assays. Mitomycin C–treated suspensions of spleen or lymph node cells were tested in infectious center assays as described (14). Briefly, expression of infectious ecotropic MuLV was quantitated by the XC plaque assay of SC-1 cells cocultured with lymphocytes. Expression of infectious MCF MuLV was quantitated by detection of infectious centers in mink lung cells using fluorescein-labeled anti-MuLV antibodies. Values are expressed as $\log_{10}$ FFU per 10^7 cells.

Assays of Immunologic Function. Serum IgM levels were determined by an ELISA technique using goat anti–mouse IgM–coated microtiter plate wells. IgM binding was detected with a peroxidase-labeled goat anti–mouse Ig antibody. B cell proliferation was stimulated with lipopolysaccharide from *E. coli* 0111:B4 or with affinity-purified goat anti-IgM antibodies. [^{3}H]Thymidine incorporation was determined after a 4-h pulse on the third day of culture. In vitro antibody responses to the T cell–independent antigens trinitrophenyl (TNP)-lipopolysaccharide, TNP–*B. abortus*, and TNP-Ficoll were determined by the plaque-forming cell assay using TNP–sheep erythrocyte targets as described (15).

Results

Adult C57BL/10 *nu*/*nu* mice or their *nu*/+ or +/+ littermates (hereafter *nu*/+) were infected with LP-BM5 MuLV by intraperitoneal injection (10). The mice were observed for lymph node enlargement, elevation of serum immunoglobulin levels, and death. Fig. 1 shows the survival of virus-infected mice. All mice with normal T lymphocyte function had died by 24 wk after LP-BM5 MuLV inoculation. In marked contrast, 8 of 10 virus-infected *nu*/*nu* mice have continued to survive for over a year. The two *nu*/*nu* mice that died at 28 and 32 wk after LP-BM5 MuLV infection had shown no lymphadenopathy or hypergammaglobulinemia. At necropsy, the first mouse showed a diffuse interstitial pneumonitis and no evidence of lymphoid hyperplasia. The cause of death is presumed to be viral pneumonitis. The second animal was found to have an enlarged thymus and mesenteric lymph nodes at necropsy. Immunochemical staining demonstrated T cell proliferation in these areas. The presumptive cause of death is a T cell lymphoma in a *nu*/*nu* mouse. Such tumors are occasionally found in older *nu*/*nu* mice, and it is impossible to determine whether or not LP-BM5 MuLV infection contributed to the generation of the lymphoma. No virus-infected *nu*/*nu* mice had detectable inguinal or axillary lymph node enlargement, whereas prominent lymphadenopathy was apparent by 4 wk after infection in thymus-bearing littermates. Not only do *nu*/*nu* mice survive LP-BM5 MuLV infection, they show no signs of immunologic abnormalities. Serum IgM levels, proliferative responses to B cell mitogens, and in vitro antibody responses to T

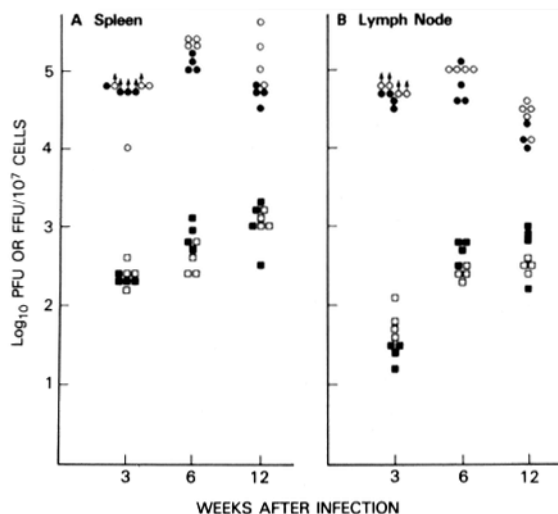

FIGURE 2. Recovery of infectious ecotropic or MCF MuLV from lymphoid tissues of LP-BM5 MuLV–infected *nu/nu* (*open figures*) or +/+ (*closed figures*) mice. Mitomycin C–treated suspensions of spleen (*A*) or lymph node (*B*) cells were tested in infectious center assays as described in Materials and Methods. Values are given as $\log_{10}$ PFU per 10^7 cells (*open or closed circles*) or $\log_{10}$ FFU per 10^7 cells (*open or closed squares*).

cell–independent antigens (12) were equivalent in virus-infected and uninfected *nu/nu* mice examined at 4, 8, 12, and 16 wk after infection (data not shown). The major features of MAIDS thus were absent in virus-infected *nu/nu* mice.

One potential explanation for the resistance of *nu/nu* mice to virus-induced immunodeficiency disease could be a reduced replication of either the ecotropic or MCF components of LP-BM5 MuLV in *nu/nu* mice. To evaluate this possibility, spleen and lymph node cells from C57BL/10 *nu/nu* and +/+ mice were examined in infectious center assays (13) at various times after inoculation of LP-BM5 MuLV for the expression of ecotropic and MCF viruses (Fig. 2). At each time, equivalent levels of ecotropic or MCF virus–producing cells were detected in lymphoid tissues of mice of either genotype. These results indicate that LP-BM5 MuLV have no direct effect on the function of B lymphocytes and, further, that T cells exert little or no control over virus replication.

Since the *nu/nu* mutation involves phenotypic manifestations other than the simple deletion of T lymphocytes (16, 17), the conclusion that T cells are required for the expression of LP-BM5 MuLV–induced immunodeficiency disease depends upon the demonstration that T cells are sufficient to reconstitute disease susceptibility of *nu/nu* mice. Accordingly, groups of *nu/nu* mice were injected intravenously with mature splenic T lymphocytes (15), and 2 wk later were infected with LP-BM5 MuLV. The immunologic status of the mice was determined 3 and 6 wk after infection. The T cell reconstitution of *nu/nu* mice restored T lymphocyte function, and LP-BM5 MuLV infection of such T cell–restored mice resulted in clear signs of immunodeficiency disease (Fig. 3). IgM levels were elevated, proliferative responses to mitogens were depressed, and antibody responses to specific antigens were absent in the T cell–reconstituted, LP-BM5 MuLV–infected group of mice. The in vivo antibody response to TNP-Ficoll, a T-independent antigen, was normal in all groups of *nu/nu* mice except the T cell–reconstituted, virus-infected group (data not shown). These data confirm the T cell dependency of MAIDS.

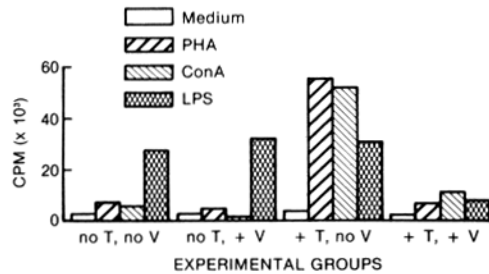

FIGURE 3. T cell reconstitution of susceptibility to MAIDS in *nu/nu* mice. C57BL/10 *nu/nu* mice were injected intravenously with 10^7 nylon wool–purified splenic T cells from C57BL/10 +/+ donors. 2 wk later, half of the T cell-reconstituted mice were infected with LP-BM5 MuLV. At 6 wk after infection, spleen cell suspensions were prepared from five mice in each experimental group and assayed for [^{3}H]thymidine incorporation after 3 d culture with PHA, Con A, or LPS. The designation of experimental groups is as follows: T-, V-, no T cell reconstitution, no LP-BM5 MuLV infection; T-, V+, no T cell reconstitution, LP-BM5 MuLV infected; T+, V-, T cell reconstituted, no LP-BM5 MuLV infection; T+, V+, T cell reconstituted, LP-BM5 MuLV infected. Data are the mean responses of each group, and the differences between the T+, V- and the T+, V+ groups are highly significant ($p < 0.001$).

Discussion

Our data demonstrate that development of MAIDS is totally dependent upon the presence of T cells in virus-infected animals. Because spleen and lymph node cells from T cell–deficient *nu/nu* mice produce LP-BM5 MuLV viruses at levels comparable to those of T cell competent +/+ mice, it appears that cells other than T lymphocytes are the primary targets for infection. However, infection of these cells (which are known to include at least some B lymphocytes and macrophages [R. A. Yetter, H. C. Morse, D. Spector, and D. E. Mosier, unpublished observations]) is insufficient to cause B cell polyclonal activation or the failure of B cells to generate specific antibody responses, hallmarks of B cell dysfunction in MAIDS and AIDS. Because the extent of LP-BM5 MuLV replication in T cells is not known, it remains uncertain whether or not the critical contribution to disease induction involves virus-infected or -uninfected T lymphocytes. Several products of activated T cells are required for normal B cell activation (11–13), e.g., IL-2, IL-4 (BSF-1), and BCGF-II, and it is likely that the polyclonal B cell activation that heralds the onset of MAIDS cannot proceed without such T cell–derived lymphokines. The suggestion that virus-induced B cell abnormalities in MAIDS are T cell–dependent differs from the conclusion that B cell dysfunction in AIDS reflects a direct effect of HIV on B cells (9). Many differences between MAIDS and AIDS, including the etiologic retroviruses and the infected species could explain this apparent discrepancy, but the observation that mice selectively depleted of $L3T4^+$ helper T cells do not develop MAIDS (Yetter and Morse, unpublished observations) confirms the required role of T cells in the murine disease. MAIDS therefore appears to result from a virally induced distortion of the normal pathways for B cell activation. These observations raise the question of whether there is an early stage in the progression of AIDS for which intact helper T cell function is required.

Summary

Athymic *nu/nu* mice were found to be resistant to the immunodeficiency disease and lethality induced in normal mice by the injection of the LP-BM5

mixture of murine retroviruses (LP-BM5 MuLV). Susceptibility to disease induction was reconstituted by injection of *nu/nu* mice with purified, mature T lymphocytes. The extent of viral replication of both the ecotropic and mink cell focus forming (MCF) components of LP-BM5 MuLV was equivalent in both *nu/nu* and normal animals. Retrovirally-induced immunodeficiency disease in mice (MAIDS) is thus dependent upon the presence of functional T lymphocytes, and high virus titers in athymic mice have little or no effect on the immune system.

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