gyration approach infinity, they are by no means identical at moderate values of those quantities. Both plots exhibit scaling behavior in that both $\langle R^2 \rangle$ and A (or $\langle A \rangle$) scale with $N^{2\nu}$.

Finally, we turn to the asphericity parameter A_2 (*8, 9*), which provides a direct measure of the deviation from a sphere of the vesicles' shapes. In terms of the eigenvalues of $\mathbf{T}$, $A_2 = (\lambda_1 - \lambda_2)^2/(\lambda_1 + \lambda_2)^2$. In the constant-pressure ensemble, after a rather tedious calculation, we find

$$A_2 = \left[\frac{8}{p^4} - \frac{N^2}{6p^2} - \frac{2N}{p^3 \tan\left(\frac{Np}{4}\right)}\right] \Bigg/ \left[-\frac{8}{p^4} - \frac{N^2}{6p^2} + \frac{N^2}{2p^2 \sin^2\left(\frac{Np}{4}\right)}\right] \quad (15)$$

In the limit $p = 0$, the asphericity approaches 1/3. This limiting value is consistent with earlier results for the average shape of a closed, non–self-avoiding random walk. As the pressure approaches its critical value ($p \rightarrow p_c$), the right side of Eq. 15 approaches zero, as expected. A similar calculation can be done for the constant-area ensemble.

Although the results presented here are new and interesting in their own right, they become particularly pertinent when used as the starting point for an excluded-volume calculation, a problem that has proven to be analytically intractable. The approach outlined here is useful for studying the dynamic behavior of vesicles under pressure (*6*).

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A Virus-Encoded "Superantigen" in a Retrovirus-Induced Immunodeficiency Syndrome of Mice

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The development of an immunodeficiency syndrome of mice caused by a replication-defective murine leukemia virus (MuLV) is paradoxically associated with a rapid activation and proliferation of CD4$^+$ T cells that are dependent on the presence of B cells. The responses of normal spleen cells to B cell lines that express the defective virus indicated that these lines express a cell surface determinant that shares "superantigenic" properties with some microbial antigens and Mls-like self antigens. This antigen elicited a potent proliferative response that was dependent on the presence of CD4$^+$ T cells and was associated with selective expansion of cells bearing $V_\beta 5$. This response was markedly inhibited by a monoclonal antibody specific for the MuLV *gag*-encoded p30 antigen.

INFECTION OF CERTAIN STRAINS OF mice with a mixture of replication-competent and replication-defective MuLV induces the disorder murine acquired immunodeficiency syndrome (MAIDS) (*1–6*), which is characterized by polyclonal activation and proliferation of T and B cells (*1*), severe immunodeficiency (*1*), aberrant regulation of cytokines (*1, 3*), enhanced susceptibility to infection (*4*), and late B cell lineage lymphomas in some animals (*5*). The defective virus in this mixture is required for development of disease (*6*) and can induce the syndrome if administered without helper MuLV (*7*).

Previous studies showed that complex interactions between T and B cells were required for induction of the full spectrum of immunologic abnormalities that characterize mice with MAIDS. T cells of the CD4$^+$ subset are required for induction of B cell activation, differentiation to immunoglobulin (Ig) secretion, and impaired responses to mitogenic and antigenic stimuli as well as for functional abnormalities of CD8$^+$ T cells (*8*). Conversely, mature B cells are required in vivo for induction of CD4$^+$ and CD8$^+$ T cell dysfunction (*9*).

We suggested previously that this interlocking activation of different components of the immune system may be due to stimulation of T cells by determinants expressed on the surface of B cells that have features of "superantigens" with the Pr60^{gag} of the defective virus as one candidate (*9*). Superantigens of microbial origin (*10–12*) (such as staphylococcal toxins and *Mycoplasma arthriditis* mitogen) or self origin (*10, 13–15*) (Mls-like products) have the ability to stimulate large numbers of T cells by virtue of their capacity to engage T cell receptors (TCRs) bearing particular variable region sequences of the TCR β chain (V_β), almost regardless of the contributions of other portions of the β or α chain to the structure of the TCR (*10–16*). The activity of these antigens is also dependent on the simultaneous expression of class II proteins on antigen presenting cells (APC) (*10–16*).

To evaluate this model, we took advantage of cultured cell lines derived from B cell lineage lymphomas that developed late in the course of MAIDS. Two lines, B6-1153 and B6-1710, were recovered from tumors of B6 mice and B6xCBA/N-2252 from an infected (B6 × CBA/N)F_1 mouse (*5*). All three lines have acquired multiple copies of the defective virus, but differ in the extent to which these genomes are expressed (*17*). For the lines studied most intensively, B6-1710 expressed *gag* polyproteins encoded by defective and nondefective viruses at concentrations readily detectable with monoclonal antibodies to p12 (anti-p12) and p30 (anti-p30) in flow cytometry analyses (Fig. 1A, inset), whereas B6-1153 expressed very little, if any (Fig. 1B, inset); B6xCBA/N-2252 was quite similar to B6-1710 for expression of *gag*-encoded antigens (*18*). All three lines expressed major histocompatibility complex (MHC) class II proteins comparably and at amounts higher than on most normal B cells (*18*).

After irradiation and cocultivation with normal B6 spleen cells, B6-1710 stimulated a vigorous proliferative response in a cell dose-dependent fashion (Fig. 1A) and at concentrations equivalent to those obtained with mitogenic lectins. Similar results were obtained when irradiated B6xCBA/N-2252 cells were used to stimulate normal F_1 spleen cells (*18*). In contrast, the low p12- and p30-expressing line, B6-1153, induced

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much less potent responses (Fig. 1B). These results suggest that the ability of the cell lines to stimulate proliferation of normal spleen cells correlated with the density of *gag* gene products expressed at the cell surface.

The T cell responses to superantigens in vitro primarily involve $CD4^+$ cells (*10*). The cellular response to stimulation with B6-1710 was also found to depend predominantly on this T cell subset (Fig. 2). Elimination of $CD4^+$ spleen cells before cocultivation with B6-1710 led to near total abrogation of the response, whereas deletion of $CD8^+$ cells had a much reduced inhibitory effect.

Two hallmarks of $CD4^+$ T cell responses to both conventional and superantigens are the requirement for MHC class II proteins on APC and the recognition of the antigen–class II complex through the TCR-CD4 complex on the responding cells (*10*). The proliferative response to B6-1710 was highly sensitive to inhibition with antibodies to $I\text{-}A^b$ and CD4, but was not appreciably affected by antibodies to surface molecules widely expressed on hematopoietic cells (CD11a and CD44), on all T cells (Thy-1), or on a restricted population of spleen cells (NK1.1) (Fig. 3). Thus, like other responses of $CD4^+$ T cells, the response to B6-1710 involves class II molecules on APC and the TCR-CD4 complex on T cells.

Cells responding to specific superantigens are dominated by T cells that use restricted sets of V_β genes. For example, the murine T cell response to Mls-2a is restricted to cells expressing $V_\beta 3$ (*14, 15*). T cells in macrocultures responding to B6-1710 contained $CD4^+$ and $CD8^+$ (mean = 41%) cells. In ten independent experiments, the responding cells (over 95% Thy-1^+) were found to contain from 24% to 41% $V_\beta 5^+$ T cells (Fig. 4A), whereas the frequency of $V_\beta 5^+$ cells among unstimulated spleen cells averaged 9% (*19, 20*). The expanded population of $V_\beta 5^+$ cells contained both $CD4^+$ and $CD8^+$ cells that expressed $V_\beta 5.1$ and $V_\beta 5.2$ in variable proportions (*18*). In macrocultures, the frequencies of T cells expressing $V_\beta 11$ were sometimes increased over their frequencies among normal T cells (Fig. 4C), whereas those for $V_\beta 6$, $V_\beta 8$, $V_\beta 13$ (Fig. 4, B and C), $V_\beta 2$, $V_\beta 7$, $V_\beta 9$, and $V_\beta 14$ (*18*) were either unaffected or, most often, reduced in relation to their distribution among normal T cells. Similar distributions of V_β specificities were detected in macrocultures obtained from spleen cells depleted of $CD8^+$ T cells before stimulation with B6-1710 (*18*).

The expansion of $V_\beta 5^+$ T cells in this system was unexpected, as the only superantigen known to affect T cells with these specificities is not stimulatory in H-2–identical mixed lymphocyte cultures and acts as a cotolerogen with MHC class II I-E molecules to induce deletion of these cells in the thymus (*19, 20*). However, the observation that nearly 50% of T cells in macrocultures generated in response to stimulation with B6-1710 were $CD8^+$ is consistent with studies of normal T cells from E_α^- mice that revealed a relative paucity of $CD4^+$ $V_\beta 5^+$ T cells despite significant numbers of $V_\beta 5^+$ cells in the $CD8^+$ subset (*19, 20*). The preferential use of T cells with these specificities may provide, in part, an explanation for the H-2–determined differences in susceptibility to MAIDS exhibited by inbred strains of mice. While $H\text{-}2D^d$ has been defined as a major determinant of MHC-mediated resistance to MAIDS (*2*), the most resistant strains are MHC class II E_α^+ (*2*) and would thus experience somatic deletions of many T cells with the V_β specificities involved in the response to B6-1710. In contrast, the strains most susceptible to disease bear the E_α^- haplotypes b, q, and s (*2*) that are not generally associated with similar deletions.

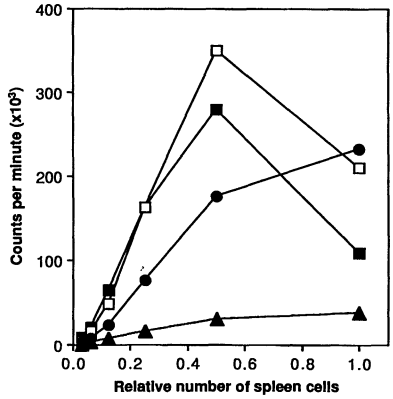

Fig. 2. Analyses of T cell subsets responding to stimulation with B6-1710. B6 spleen cells (10^7 per milliliter) were treated with antibody to CD4 (RL172; triangles), antibody to CD8 (53-8.7; circles), or with medium (open squares) for 30 min on ice, washed, and then incubated with complement (Low Tox-M; Accurate Chemicals, Westbury, New York) for 30 min at 37°C. After washing, the cells were resuspended to their initial volume and serial twofold dilutions were prepared. Proliferative responses to B6-1710 (4×10^5 cells per well) were determined as described in Fig. 1. Untreated spleen cells (closed squares) were included as a control. Treatments with antibodies and complement resulted in >95% depletions of the appropriate cell populations. The data shown are representative of four separate experiments.

We next determined whether the antigenic determinant responsible for T cell proliferation was virus-related by treating cultures with monoclonal antibody specific for the p12 or p30 products of *gag* (Fig. 3C). Anti-p12 that recognizes the 65-kD products of ecotropic or mink cell focus-inducing viruses, or both (*17*), but fails to react with the *gag*-encoded product of the defective virus on the surface of B6-1710 had no major effect on proliferation induced by the cell line. In contrast, the response was markedly inhibited by an anti-p30 that reacts with both the 60- and 65-kD *gag* products of the defective and competent viruses, respectively, expressed on the surface of B6-1710 (*17*). The specificity of inhibition induced by anti-p30 is suggested by the absence of any effect of this antibody on the proliferative response induced by concavalin A (*18*).

We conclude that a MuLV *gag*-encoded molecule with many characteristics compatible with its definition as a superantigen is expressed on the surface of B cell lineage tumors recovered from mice with MAIDS. In B6-1710, the potential sources of *gag* proteins that could act as a superantigen include the disease-causing defective virus as well as nonpathogenic ecotropic and poly-

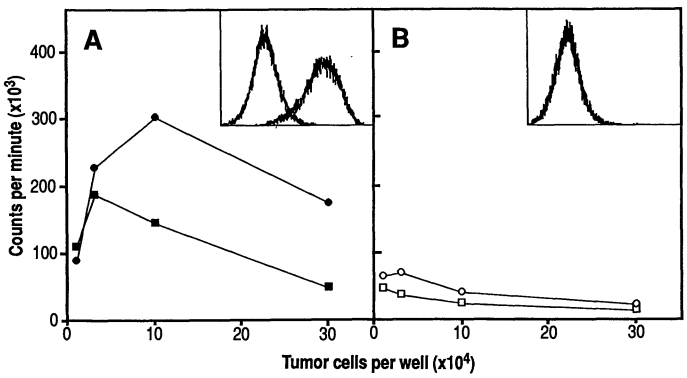

Fig. 1. Proliferative responses of B6 spleen cells to stimulation with B6-1710 and B6-1153. Spleen cells from normal B6 mice were cultured in 96-well microtiter plates at 2×10^5 (squares) or 4×10^5 (circles) cells per well with graded numbers of irradiated (3000 R) B6-1710 (**A**) or B6-1153 (**B**) tumor cells. After 72 hours, the cultures were pulsed for 6 hours with [^{3}H]thymidine and harvested for counting. Numbers indicate the mean counts per minute minus background for triplicate cultures. Standard errors were <15% of the means. The insets show p12 expression on the two cell lines as determined by flow cytometry. The cells were incubated sequentially with a broadly reactive mouse anti-p12, 548 (*24*), or an anti-p30, R187 (*24*) (not shown), with biotin-labeled protein G (Genex Corporation, Gaithersburg, MD), and with allophycocyanin-labeled avidin (Caltag Laboratories, South San Francisco, California). Analyses were performed on a FACS 440 (Becton Dickinson, Mountain View, California) with established techniques.

tropic viruses present in the LP-BM5 virus mixture (*1, 2, 6*). The failure to inhibit the cellular response to this line with the anti-p12 that clearly reacts with the cell surface forms of the *gag* products of the nondefective viruses but not that encoded by the defective virus is evidence against the ecotropic or polytropic *gag* precursors acting as superantigens. In addition, cell lines that express high amounts of ecotropic or polytropic MuLV but not the defective virus fail to elicit superantigen-like responses (*18*). These results implicate the defective virus as the source of the *gag*-encoded superantigen. The similarities between the T cell responses to this antigen and those to Mls-like determinants are striking and raise the possibility that *Mls* products may be encoded by related endogenous retroviral sequences. In this regard, other studies have suggested a superantigen-like role for the products of an endogenous mouse mammary tumor virus (*19*).

The expression of a virus-encoded superantigen in mice with MAIDS would provide a ready explanation for the rapid and extensive activation of T cells characteristic of this disorder (*1*), as well as the ability of a limited number of cells expressing the defective virus to induce the full syndrome (*7*). Consequent release of lymphokines (*3*) could then lead to a generalized activation of other T cells and B cells, resulting in a diminution in the frequencies of resting cells capable of cognate interactions in response to infectious agents (*4*) or tumors (*5*). The observation that cyclosporine A blocks the response of normal spleen cells to B6-1710 (*18*) and the demonstration that in vivo treatment with this drug greatly restricts the progression of MAIDS (*21*) are in keeping with this model. Although the relevance of this scheme to other retrovirus-induced immunodeficiency syndromes is uncertain, it is noteworthy that regions of the human immunodeficiency virus type-1 (HIV-1) envelope capable of stimulating proliferative responses by normal cells or by human or mouse cells primed by gp120 or gp160 have been described (*22*), and that one HIV-1 sequence (*23*) has features of MHC class II proteins, which are among the most potent stimuli for $CD4^+$ T cells. If these HIV-1 epitopes are stimulatory for $CD4^+$ T cells in vivo, they would have the added deleterious effect of generating an expanded pool of activated cells susceptible to infection and lysis by the virus.

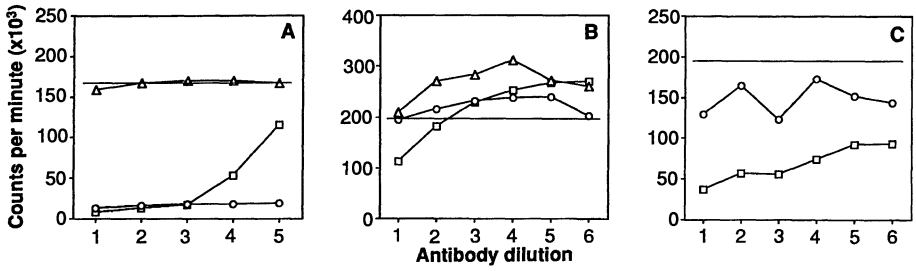

Fig. 3. Effects of antibodies to cell surface antigens on the proliferative responses of normal spleen cells to stimulation with B6-1710. Cultures of normal B6 spleen cells and B6-1710 (4×10^5 cells per well) were prepared as described for Fig. 1. Serial two- or fourfold dilutions of ascites or hybridoma supernatants containing monoclonal antibodies to the indicated cell surface antigens were added to the cultures, and proliferative responses were determined after 48 or 72 hours. Points indicate the mean value for triplicate cultures as a function of antibody dilution. Standard errors were <15% of the means. Proliferation of cultures prepared in the absence of antibody is indicated by a horizontal line. Antibodies to cell surface antigens were present at supersaturating concentrations at the lowest dilutions as determined by flow cytometry analysis. Data are representative of results obtained in at least two experiments. Monoclonal antibodies to $I\text{-}A^b$ (Y3P), CD4 (GK1.5), NK1.1 (PK136), p30 (R187), and p12 (548) were prepared as ascites or as hybridoma supernatants [CD44 (1M7), CD11a (M1/69)] in our laboratory or were purchased from Accurate Chemicals (Thy-1.2). (**A**) I-A, □; CD4, ○; NK1.1, △. (**B**) CD11A, □; CD44, ○; Thy-1, △. (**C**) p12, ○; p30, □.

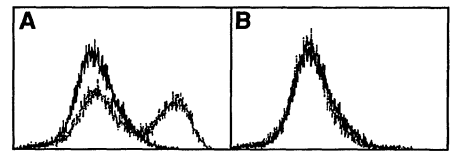

Fig. 4. Analyses of V_β expression on normal T cells and cells from bulk cultures stimulated with B6-1710. Normal B6 spleen cells (4×10^6 per milliliter) were stimulated with irradiated B6-1710 cells (2×10^6 per milliliter) in 2 ml for 4 days before addition of ribosomal interleukin-2 (rIL-2) (50 U/ml; Genzyme Corp., Boston, Massachusetts) for 1 day. Cultures were harvested the next day and propagated for an additional 4 to 8 days in medium containing rIL-2 at the same concentration. For analyses of V_β expression, the cultures or fresh lymph node cells were stained with monoclonal antibodies to various V_β specificities (*25*) and analyzed by flow cytometry on a FACS 440. (**A**) The FACS profile of a macroculture incubated with unlabeled antibody to $V_\beta 5.1$ and $V_\beta 5.2$ (MR9-4) and counter-stained with FITC-labeled antibody to mouse (anti-mouse) IgG or treated with the counterstain alone. (**B**) Profiles for cells from the same culture stained with unlabeled anti-$V_\beta 13$ (MR12-4) counterstained with anti-mouse IgG or stained with anti-mouse IgG alone. The frequencies of cells expressing various V_β specificities among normal T cells and in individual macrocultures are given in (**C**). The values for normal T cells represent the mean for values obtained with four individual mice. Northern hybridization analyses of mRNA prepared from bulk cultures of cells responding to B6-1710 confirmed expansion of cells that use $V_\beta 5$ and $V_\beta 12$; specificities for $V_\beta 1$, $V_\beta 4$, $V_\beta 10$, $V_\beta 15$, and $V_\beta 16$ were not increased over normal (*18*).

C

Cell culture	Percent of cells expressing					
	Thy-1	$V_\beta 5$	$V_\beta 6$	$V_\beta 8$	$V_\beta 11$	$V_\beta 13$
Normal T lymphocytes	100	9.3	10.2	19.3	6.6	6.3
M3	99	31.5	1.6		1.0	0.3
M5	95	35.7	3.3	2.7	4.5	3.0
M7	99	29.3	2.0	5.0	17.6	1.1
M8	99	34.9		1.4	16.4	
M12	98	40.4	4.3	10.7	7.3	
M16	99	27.7	5.4	6.9	10.4	3.3

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25. Unlabeled monoclonal antibodies with specificity for $V_\beta3$ [KJ25 (*14*)]; $V_\beta5.1$ and $V_\beta5.2$ [MR9-4; E. P. Reich *et al., Nature* **341**, 326 (1989)]; $V_\beta5.2$ only [MR9-8 (*19*)]; $V_\beta6$ [RR4-7; O. Kanagawa *et al., Cell. Immunol.* **119**, 412 (1989)]; $V_\beta7$ [TR-310; C. Y. Okada and I. L. Weissman, *J. Exp. Med.* **169**, 1703 (1990)]; $V_\beta8.1$, $V_\beta8.2$, and $V_\beta8.3$ [F23.1; U. D. Staerz *et al., J. Immunol.* **134**, 3994 (1985)]; $V_\beta11$ [RR3-15; J. Bill *et al., J. Exp. Med.* **169**, 1405 (1989)]; and $V_\beta13$ [MR12.4; D. M. Zaller *et al., J. Exp. Med.* **171**, 1943 (1990)] were used for staining of cells with appropriate fluorescein isothiocyanate (FITC)–labeled second antibodies. Some studies were performed with FITC- or biotin-labeled antibodies to $V_\beta3$ specificities $V_\beta5$, $V_\beta9$, and $V_\beta13$ (PharMingen, San Diego, CA). Analyses were performed on a FACS 440.
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Engraftment and Development of Human T and B Cells in Mice After Bone Marrow Transplantation

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A model for human lymphocyte ontogeny has been developed in a normal mouse. Human bone marrow, depleted of mature T and B lymphocytes, and bone marrow from mice with severe combined immunodeficiency were transplanted into lethally irradiated BALB/c mice. Human B and T cells were first detected 2 to 4 months after transplantation and persisted for at least 6 months. Most human thymocytes (30 to 50 percent of total thymocytes) were CD3$^+$CD4$^+$CD8$^+$. Human immunoglobulin was detected in some chimeras, and a human antibody response to dinitrophenol could be generated after primary and secondary immunization.

XENOGENEIC BONE MARROW (BM) transplants are possible between closely related species, such as rat and mouse (*1*). Human hematopoietic stem cells can also be transplanted into mice, without the occurrence of graft-versus-host disease, if the mice are genetically immune-deficient; examples of such mice being those with severe combined immune deficiency (SCID) (*2, 3*) and Bg/Nu/Xid mice (*4*). Although the engraftment of human cells in these models permits the infection of mice with human immunodeficiency virus (HIV) (*5*), the degree of chimerism after transplantation is limited and transient. Transplants of hematopoietic stem cells from human fetal liver into SCID mice do not engraft or differentiate into T cells unless the transplant has been supplemented with a graft of human fetal thymic epithelium implanted under the kidney capsule. Furthermore, human lymphocytes are not detected in significant numbers in the peripheral blood beyond 2 to 3 months (*2, 3*).

After total body irradiation, normal mice die within 2 weeks if a BM transplant is not administered. T cell differentiation in mice occurs within approximately 14 to 21 days after BM transplantation. In clinical studies of transplants of T cell–depleted BM into SCID (*6*) or leukemic patients (7), differentiation of human stem cells into mature T cells is slow, requiring from 1 to 4 months. Clearly, in a murine microenvironment, human stem cell differentiation may be inefficient and even slower because of the low cross-reactivity between murine and human cytokines and other molecules that are important in cellular recognition. The conflict between the need for prompt hematopoietic reconstitution and the slow differentiation rate of human T and B cells was resolved in the present study by the use of SCID mice as BM donors rather than as recipients. BM cells from SCID mice can promptly reconstitute all hematopoietic lineages except T and B cells (*8*). To test whether the "empty space" in the thymus and BM could gradually be occupied by, and populated with, normal human T and B cells, we combined a transplant of SCID mouse BM with a transplant of human BM that had been depleted of both T and B cells by differential agglutination with soybean agglutinin (SBA$^-$) and by subsequent removal of residual CD2$^+$ lymphocytes that form rosettes with sheep erythrocytes (E$^-$) (*9*). Because SCID in mice does not always result in an absolute deficiency of T cells, BM from the SCID mice was also depleted of T cells to maximize the likelihood that the lymphoid compartment in the chimeric mice would be derived from human lymphocytes and their progenitors.

Our approach allowed human T and B cells to develop in the peripheral blood of lethally irradiated, normal BALB/c mice that were of the same H-2 haplotype as the SCID BM donors. A total of 31 mice in four series of experiments were transplanted with BM from humans and SCID mice. Of the 31 mice transplanted, 20 survived for three or more months. Infection killed 10 of the 31 mice, mainly during the first 3 weeks after transplantation. Sixteen of the 20 survivors contained human T cells or human B cells, or both. Fourteen of the 20 surviving mice had both human T and B cells in significant numbers in the peripheral blood.

The first series of mice was initially evaluated for T cell engraftment with the use of cytofluorometry to measure the appearance of human CD3$^+$ T cells in the peripheral blood. One mouse (no. 3) showed the presence of human T cells 7 weeks after the transplant (Fig. 1A, inset); a monoclonal

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