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A Recessive Defect in Lymphocyte or Granulocyte Function Caused by an Integrated Transgene

David Lo,* Helen Quill,† Linda Burkly,‖ Bernadette Scott,* Richard D. Palmiter,¶ and Ralph L. Brinster‡

From the Department of Immunology, The Scripps Research Institute, La Jolla, California, the Department of Pathology and Laboratory Medicine,† and the Laboratory of Reproductive Physiology,‡ School of Veterinary Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, Biogen,‖ Cambridge, Massachusetts, and the Department of Biochemistry,¶ Howard Hughes Medical Institute, University of Washington, Seattle, Washington*

A line of transgenic mice has been identified with a recessive defect in lymphocyte or granulocyte function, presumably as a result of insertional mutagenesis by the integrated transgene. Transgenic mice homozygous for the transgene integrant showed nearly complete absence of lymphocytes in peripheral lymph nodes and Peyer's patches, a severely diminished thymus medulla, and a greatly enlarged spleen. These animals also developed a syndrome characterized by granulocyte and mononuclear infiltrates in numerous tissues, including skin, liver, and lung, and immunoglobulin deposits in kidney glomeruli. Lung infiltrates were specifically localized around large blood vessels and bronchi, accompanied in some cases by destruction of arterial walls. The light scatter profile of spleen lymphocytes suggested an extremely high percentage of blast cells. Because tissue development and morphology appears to be normal in all other tissues observed, the genetic lesion appears to specifically affect the regulation of lymphocyte or granulocyte activation. (Am J Pathol 1992, 141:1237–1246)

Transgenic technology has a wide variety of applications to the study of gene regulation *in vivo*. In most situations, transgenic mice are studied for dominant gain of function as a result of transgene integration. For example, transgene constructs can be tested for the presence of tissue specific enhancer sequences. In other situations, heterologous promoters and enhancers can be used to direct the tissue-specific expression of desired structural genes. Because transgenes appear to integrate into random sites in the genome, however, some integrants may disrupt a gene important to development ("insertional mutagenesis"). As a consequence, a recessive defect in development is generated, having little relationship to the specific DNA sequences integrated. In a few well-characterized cases reported recently, recessive mutations have affected limb development[1] and collagen synthesis.[2]

In the example presented here, insertional mutagenesis appears to have altered directly or indirectly the regulation of lymphocyte or granulocyte activation, resulting in an autosomal recessive syndrome characterized by inflammatory infiltrates in a wide variety of tissues. The autosomal recessive syndrome present in transgenic mice may be similar to human genetic diseases involving chronic bacterial infections. There are a number of human disorders of granulocyte function that are either autosomal recessive or X-linked.[3] Notable among them is chronic granulomatous disease (CGD), a disease resulting from impaired peroxide production by granulocytes.[4] Patients with this syndrome suffer from recurrent infections with catalase-positive microorganisms. This syndrome can be caused by defects in a number of enzymes necessary for peroxide production, so although it is most often X-linked, autosomal recessive inheritance also has been noted. Another syndrome resulting in chronic bacterial infections, leukocyte adhesion deficiency (LAD), is caused by an autosomal recessive defect in the β2 integrin gene.[5] Thus, if the syndrome discovered in the transgenic mice is related to any of the known genetically inherited human disorders, it should help provide a valuable animal model for studying the pathogenesis and potential therapies in human patients.

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Address reprint requests to Dr. David Lo, Department of Immunology IMM-16, The Scripps Research Institute, 10666 North Torrey Pines Rd., La Jolla, CA 92037.

Materials and Methods

Transgenic Animals

Transgenic mice were generated as previously described by Brinster et al.[6] Briefly, fertilized mouse embryos were microinjected with a mixture of DNAs, including a 7.8-kb fragment containing the complete mouse K^k structural gene along with 2 kb of 5′ flanking sequence (generously provided by Dr. S. Kvist[7]) and a 2.1-kb portion of the human growth hormone gene (hGH) known to be inert as a transgene.[8] The line 272–4 was generated from microinjection of C57BL/6 embryos, and lines 2922-4 and 2920-6 were generated from microinjection of (C57BL/6 × SJL)F_2 embryos. Lines 272–4 and 2920-6 have been given the following designations as transgenic mouse lines: line 272–4 is Tg(H-2K + GH1)Bril06, and line 2920–6 Tg(H-2K + GH1)Bri164. Transgenic offspring were identified by either DNA dot analysis using an hGH probe, or by staining of peripheral blood lymphocytes using a monoclonal antibody specific for K^k. Homozygous animals were identified by quantitatve DNA dot analysis. Mice were bred and maintained in the animal facility at the University of Pennsylvania School of Veterinary Medicine, and under specific pathogen-free conditions in the vivarium at Scripps Clinic. Clinical features described were consistent among mice maintained in both facilities.

Antibodies, Flow Cytometry Analysis

For flow cytometry analysis of various cell populations, dispersed single-cell suspensions of bone marrow or spleen were washed in buffered salt solutions containing fetal bovine serum, then stained and washed in buffered saline containing 1% bovine serum albumin and 0.03% sodium azide. Cells were stained using biotin-conjugated monoclonal antibodies 12.2.2s (anti-K^k), 28-13-3 (anti-K^b), BP107 (anti-I-A^b), (anti-Thy1), (anti-CD4), (anti-CD8), followed by streptavidin conjugated to either fluorescein (A-FITC) or phycoerythrin (SA-PE). In other experiments, cells were stained with unconjugated antibodies RA3-6B2 (against the pre-B cell antigen B220) or R1-2 (anti VLA-4), followed by biotinylated goat anti-rat, and SA-PE, along with goat anti-mouse IgM conjugated to FITC. Flow cytometry was performed on a FACScan. Cells were not fractionated by density gradient before staining, so various bone marrow and spleen cell subpopulations could be analyzed separately on the FACScan by gating on cells based on forward and side scatter characteristics.

Histology

Immunohistochemical analysis was done using 10 μ thick cryostat sections of fresh tissues, or on paraffin sections of formalin-fixed tissues. In the case of cell suspensions, cytospin slides were prepared, and processed as with cryostat sections for staining. In assays for autoantibodies against anti-DNA or blood vessels, sections of liver, kidney, aorta, and lung were fixed and incubated with dilutions of serum from transgenic mice, followed by secondary antibodies against mouse immunogloulin. Control anti-DNA serum was obtained from autoimmune mice carrying the gld gene. Antibodies for immunoglobulin were biotin conjugated goat anti-mouse immunoglobulins, followed by SA-HRP, and diaminobenzidine as a chromagen. Counterstaining was done using Mayer's hematoxylin.

Bone Marrow Chimeras and Adoptive Transfer Studies

Bone marrow chimeras were generated by lethal irradiation (900 rad) of C57BL/6 mice with rescue by intravenous injection of 90×10^6 cells of a pool of spleen and peritoneal exudate cells taken from a homozygous 272–4 animal. Adoptive transfer of normal spleen cells into homozygous 272–4 mice was done by intravenous injection of 10^8 washed spleen cells from hemizygous 272–4 mice into homozygous 272–4 mice at approximately 3 weeks of age.

Lymphocyte Proliferation Assays

Lymphocytes from mice were made into single-cell suspensions, and cultured at 2×10^6/ml in the presence of various mitogens in RPMI 1640 plus 10% fetal bovine serum, 2-mercaptoethanol, antibiotics, and glutamine. Proliferation was assessed by adding ^{3}H-thymidine 18 to 24 hours before harvesting cultures onto glass fiber filters for liquid scintillation counting. The mitogens used were anti-CD3 epsilon antibody (145-2C11), concanavalin A (Con A), interleukin-2 (IL-2), and lipopolysaccharide (LPS, *S. Typhosa 0901*).

Results

Recessive Syndrome in K^k/hGH Transgenic Mice

Transgenic mice were generated on an inbred C57BL/6 background using a normal class I major histocompatibility complex (MHC) gene K^k. Because an integrated K^k transgene would be difficult to distinguish from endogenous class I genes by simple hybridization of genomic DNA, a nonfunctional fragment of hGH was added to the

injections as a marker gene. Previous studies have demonstrated that when two different DNA fragments are microinjected into embryos, more than 90% of the transgene integrants contain both fragments (Brinster et al, unpublished). From the original microinjection experiment, a single founder animal was identified and bred to establish line 272–4. Later, other lines were developed by injection of the same genes into (B6 × SJL)F_2 eggs (lines 2920–6 and 2922–4).

Brother–sister matings were initiated to establish a homozygous line of 272–4 transgenic mice, but among a series of seven litters from brother–sister transgene-positive matings (40 mice born), only 26 were transgene positive (65% *versus* an expected 75%), and only five of these were homozygous (12% *versus* an expected 25%). Of the homozygotes that survived to weaning, 100% (in this group and all subsequent groups) spontaneously developed a wasting syndrome. At weaning, homozygotes were often but not always smaller than littermates. As the syndrome progressed, the fur became ruffled, and the skin on the tail and around the eyes appeared edematous. On necropsy, the liver was pale and very firm, and the lung was mottled. The spleen was greatly enlarged, and unusually firm. Lymph nodes and Peyer's patches were extremely difficult to find in any of the animals tested. This syndrome could be detected in mice as young as 3 weeks of age, with death occurring anywhere between 3 weeks and several months. Because homozygotes constituted less than 25% of the progeny of brother–sister matings, some loss also probably occurred before 3 weeks of age. By contrast, hemizygous 272–4 and homozygote transgenic mice from both 2920–6 and 2922–4 lines appeared normal.

The syndrome seen in 272–4 mice was probably not due to differential levels of transgene K^k expression, because analysis of spleen cells from transgenic mice showed similar levels of surface K^k expression in all three lines and B10.BR controls (Figure 1). Cryostat sections of thymus from hemizygous 272–4 mice stained for K^k also showed expression primarily limited to the thymic medulla, as expected for normal K^k (not shown). Functionally, the transgene K^k was capable of acting as a strong alloantigen. Thus, spleen cells from 272–4 transgenic mice were strongly stimulatory to T cells from B6 mice (Table 1).

Histology of Homozygous 272–4 Transgenic Mice

The liver contained inflammation that primarily consisted of granulocytes and histiocytes, with some significant lymphocyte involvement as well (Figure 2A). In the most severe cases, the liver infiltrates appeared to involve the

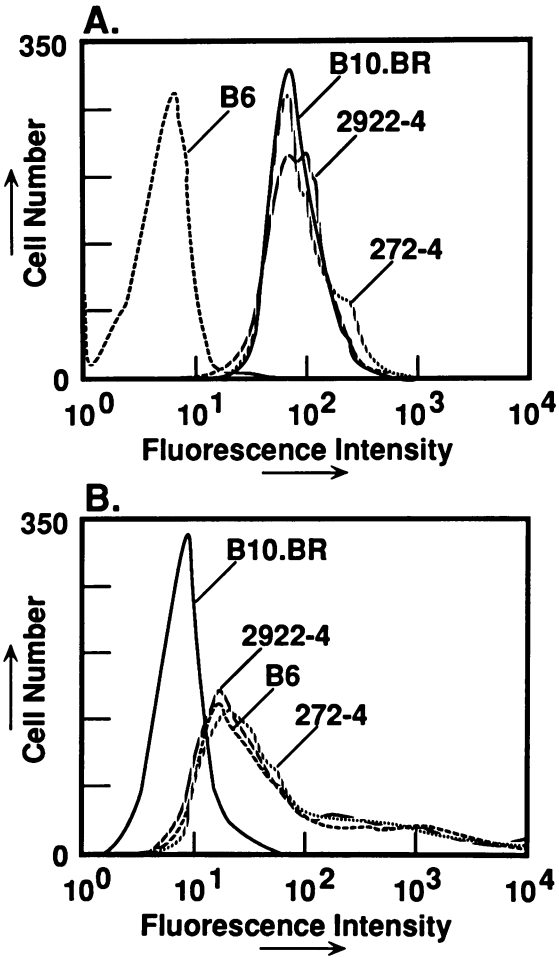

Figure 1. *Expression of transgene K^k and endogenous K^b by splenocytes from control and transgenic mice.* A: *Spleen cells stained for K^k. All spleen cells from the transgenic mice stained positive for K^k at levels indistinguishable from the B10.BR control.* B: *Spleen cells stained for K^b. Expression of the transgene did not affect expression of endogenous class I.*

parenchyma, but usually the accumulations of inflammatory cells were concentrated around portal tracts, with some accompanying fibrosis. The portal infiltrates did not appear to cause any significant cholestasis, however, and bile ducts were found intact. Increased numbers of

Table 1. *Stimulation of B6 Lymphocytes by 272-4 Splenocytes in Mixed Lymphocyte Culture*

	Spleen stimulators		
Day of culture	C57BL/6 (H-2^b)	B10.BR (H-2^k)	272-4 (H-2^b + K^k)
d4	2.9	86.0	42.4
d5	1.6	88.0	50.7

They expressed class I transgene is a functional alloantigen in mixed lymphocyte culture. C57BL/6 lymph node cells (2×10^5 per well) were cultured with irradiated spleen cell stimulators (5×10^5 per well), and harvested on day 4 or 5. Cultures were pulsed with ^{3}H-TdR 18–24 hours before harvesting. Data presented as counts per minute (in thousands).

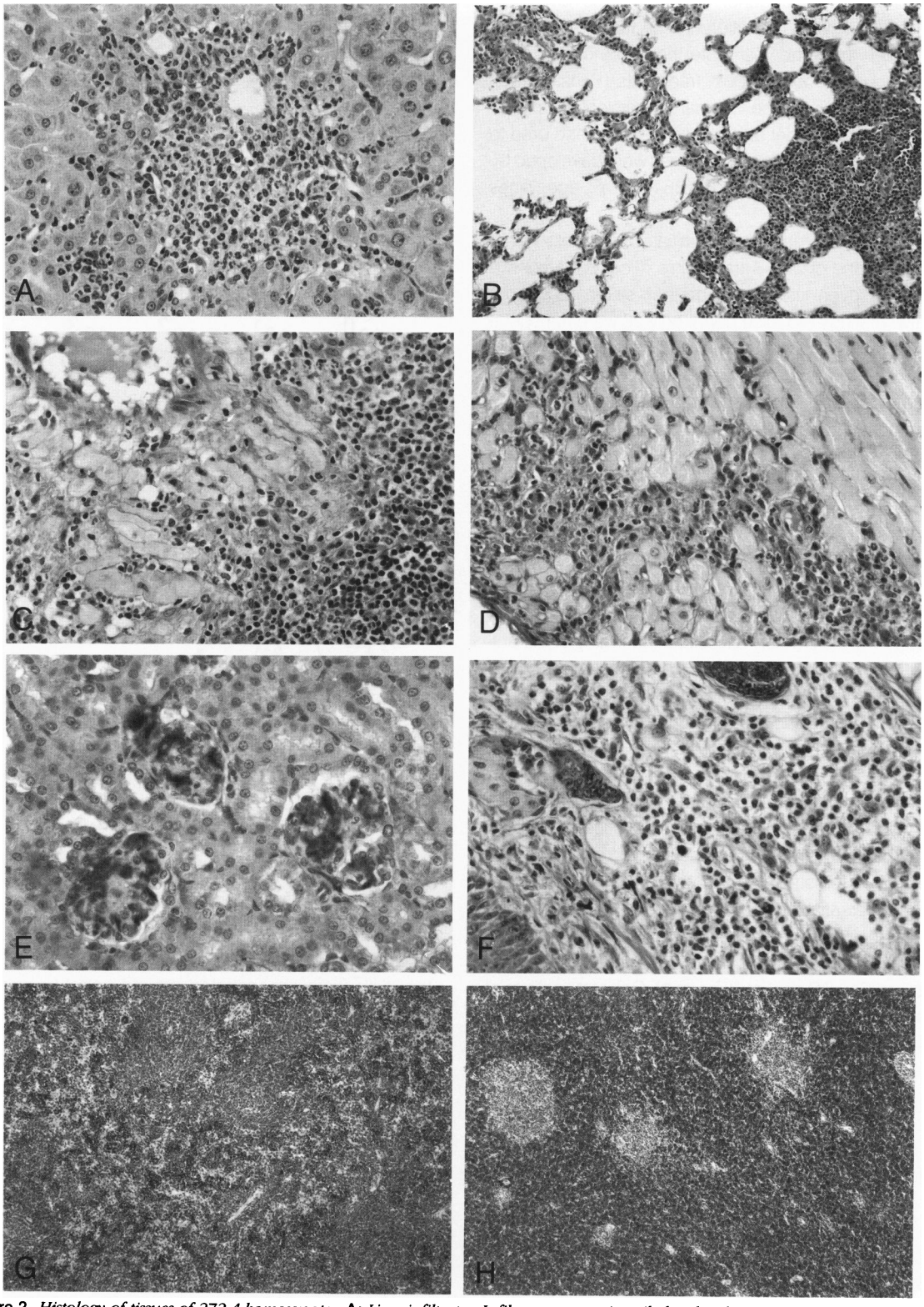

Figure 2. *Histology of tissues of 272-4 homozygotes.* **A**: *Liver infiltrates. Infiltrates were primarily localized to portal tracts (PAS, ×40).* **B**: *Lung infiltrates. Infiltrating cells accumulated primarily near large vessels (PAS, ×10).* **C**: *Lung infiltrates. Disruption of some blood vessels can be seen; in this case, the arterial muscular layer is infiltrated (PAS, ×40).* **D**: *Cardiac muscle. Inflammatory infiltrates have entered the muscle (PAS, ×40).* **E**: *Kidney stain for Ig. Immunoglobulin deposits were found in a patchy pattern within glomeruli (peroxidase labeled immunocytochemical stain for Kappa light chain, hematoxylin counterstain, ×40).* **F**: *Ear skin infiltrates. Infiltrating cells were mostly found in the dermis (H&E, ×40).* **G**: *Spleen. The spleen was dominated by granulocytes, with a sparse white pulp (PAS, ×10).* **H**: *Thymus. Thymus medulla was extremely sparse (PAS, ×10).* **I**: *Mesenteric lymph node. Lymph node consisted primarily of mesenchymal cells and granulocytes (PAS, ×20).* **J**: *Mesenteric lymph node stained for Ig. Plasma cells were present within the medullary areas of lymph nodes, but B cells could not be detected in the cortex (peroxidase labeled immunocytochemical stain for Kappa light chain, hematoxylin counterstain, ×40).*

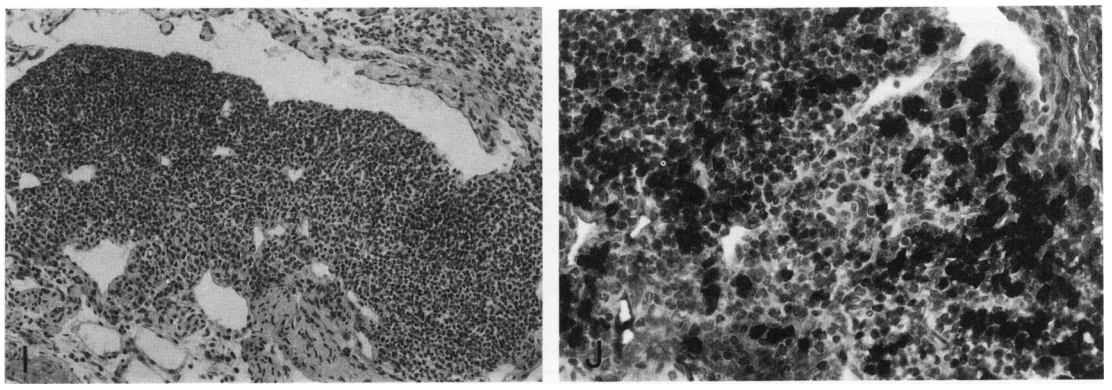

Figure 2. *(Continued).*

bile ducts near infiltrated portal tracts were sometimes seen.

In the lung, granulocytes and lymphocytes also were seen throughout the parenchyma, but the most impressive accumulations of inflammatory cells were localized near bronchi and major blood vessels (Figure 2B). In some cases, arterial walls were disrupted by inflammation (Figure 2C). Despite the accumulations of inflammatory cells, however, the lung alveolar spaces showed no evidence of exudates or pneumonia.

Other nonlymphoid tissues found to have similar granulocytic infiltrates included epididymis, pancreas, skeletal muscle (not shown), and cardiac muscle (Figure 2D).

In older homozygous mice, the kidney showed signs of glomerulonephritis, with accumulation of large amounts of periodic acid-Schiff (PAS)-positive material. Immunohistochemical staining showed immunoglobulin deposits containing kappa, IgG, and IgM. The staining pattern was consistent with immune complex deposition (Figure 2E). In addition, serum from homozygous mice did not stain sections of normal kidney, lung, or aorta (not shown), suggesting that the immunoglobulin deposits in the glomeruli were not due to basement-membrane–specific autoantibodies. Because the presence of immune complex glomerulonephritis is often correlated with serum autoantibodies specific for nuclear antigens, serum from homozygotes were tested for antinuclear antibody titers, but were found to be negative (not shown).

The dermal layer of skin from older homozygous mice showed infiltrates resembling acute inflammation. Thus, infiltrating cells were predominantly granulocytes, with some histiocytes and lymphocytes (Figure 2F). These changes could be found in skin from the ear, tail, and periorbital region. In the ear, the epidermis also showed signs of hyperkeratosis, possibly as a response to chronic irritation.

The spleens of homozygous mice were extremely large, and cell counts indicated a threefold to 10-fold increase over control littermates. Histologically, the spleen was dominated by granulocytes, but some white pulp organization was still evident (Figure 2G). Immunohistochemical staining suggested that T- and B-dependent regions of the white pulp retained their normal relationship; however, the B cell mantle layer was extremely sparse, and no germinal centers were seen.

Thymus sections were remarkable for the almost complete absence of a medulla (Figure 2H). Immunohistochemical staining for class I and class II MHC antigens confirmed the extremely small proportion of medulla (not shown).

Lymph nodes were extremely difficult to find in the periphery. Histologic sections of mesentery did show structures that appeared to be lymph node, with the characteristic capsule and subcapsular sinus, and mesenchymal cells normally found in lymph nodes (Figure 2I). Few mononuclear cells were found in these lymph nodes, whereas granulocytes were abundant. Interestingly, sections of mesenteric lymph node stained for immunoglobulin light chain did show plasma cells (Figure 2J).

Syndrome Is Intrinsic to Bone Marrow-derived Cells

To determine if the defect was intrinsic to bone-marrow–derived cells rather than intrinsic to target tissues, spleen cells from homozygous 272–4 mice were injected into lethally irradiated B6 mice (not shown). After 2 months, mice developed similar symptoms including skin edema, inflammation in several tissues, and glomerulonephritis, suggesting that bone-marrow–derived cells were sufficient to produce the syndrome.

Lymphocyte Subpopulations in Homozygous Mice

Using light scatter characteristics to gate on various nonlymphocyte cells in the spleens of homozygote 272–4 mice (Figure 3), most of the cells appeared to be either

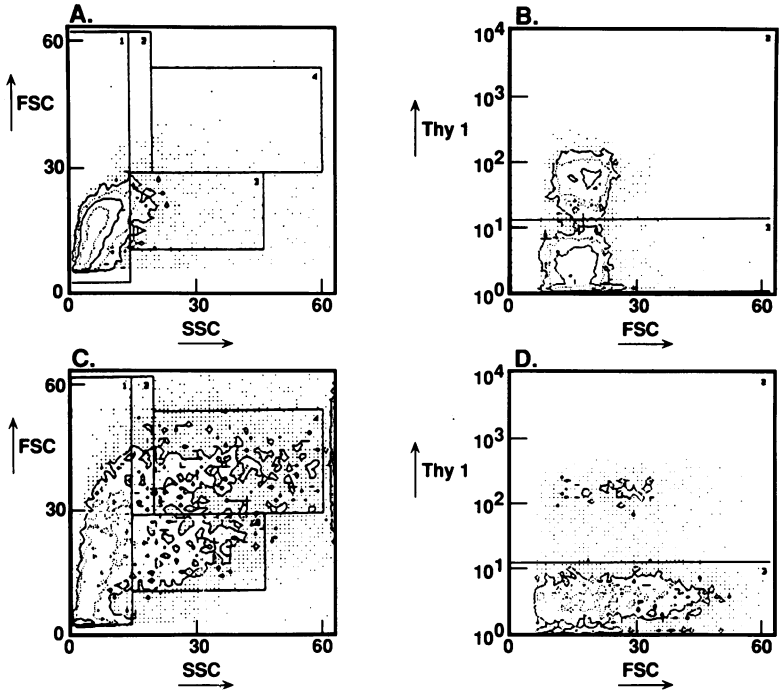

Figure 3. *Light scatter characteristics of spleen cells from control 272-4 homozygotes.* A: *Littermate control showing forward (FSC) and side scatter (SSC) profile. Most cells fell into the category of small lymphocytes (box 1), with some cells showing characteristics consistent with macrophages (box 3).* B: *FSC profile of T cells (Thy 1 positive cells) from littermate control spleen. Mean FSC channel for Thy 1 positive cells is 17.3.* C: *FSC versus SSC profile of spleen cells from 272-4 homozygote. Most of the spleen cells show characteristics consistent with granulocytes (Box 4) or macrophages (Box 3).* D: *FSC profile of T cells from 272-4 homozygote. Mean FSC channel for Thy 1-positive cells is 25.7, much higher than for control spleen.*

granulocytes (about 23% of total spleen) or macrophages (13% to 18% of total spleen). Using the same gates, control spleen yielded only 0.5% granulocytes and 3% to 5% macrophages.

Flow cytometry analysis of 272–4 homozygote spleen lymphocytes showed that the percentage of T cells was significantly reduced, down to an average of 18% (range, 8% to 32%) as compared with an average of 36% (range, 32% to 41%) in control littermate spleens (Table 2). The CD4/CD8 ratio was slightly below 1 in homozygous mice, whereas littermate controls showed the ratios between 1 and 1.3 as expected for normal C57BL/6 mice. Considering the enlarged spleen size (three to 10 times normal), and the absence of lymph node lymphocytes, the total T cell numbers per animal was estimated to be roughly normal in homozygous mice (depending on estimates for normal total lymph node T cell numbers). The forward light scatter profile of spleen lymphocytes showed that the T cells were much larger than normal resting cells (Figure 3), suggesting that the cells were activated blasts. Consistent with the notion that the lymphocytes were activated, lymphocyte homing receptor MEL-14 levels were found to be lower in homozygous spleen cells as compared with hemizygous spleen cells. That is, the percentage of MEL-14–positive cells was lower, and the mean fluorescence intensity of the positive cells was lower relative to controls (Figure 4).

Whereas the total number of T cells was estimated to be normal, the numbers of B cells in homozygotes appeared to be reduced, even when the increase in spleen

Table 2. *FACS Analysis of 272-4 Splenocytes*

	% of total spleen			
Animal	CD4	CD8	Thy 1.2	Surface Ig
Expt. 1				
272-4 hemizygote	nd	nd	35	nd
272-4 homozygote	nd	nd	32	nd
Expt. 2				
NonTg littermate	25	14	41	56
272-4 hemizygote	19	19	40	55
272-4 homozygote	3	3	8	3
Expt. 3				
NonTg littermate	18	(14)*	32	50
NonTg littermate	16	(16)	32	52
272-4 homozygote	7	(6)	13	<1
272-4 homozygote	8	(10)	18	9

* (), derived by subtracting CD4% from total Thy 1.2%.

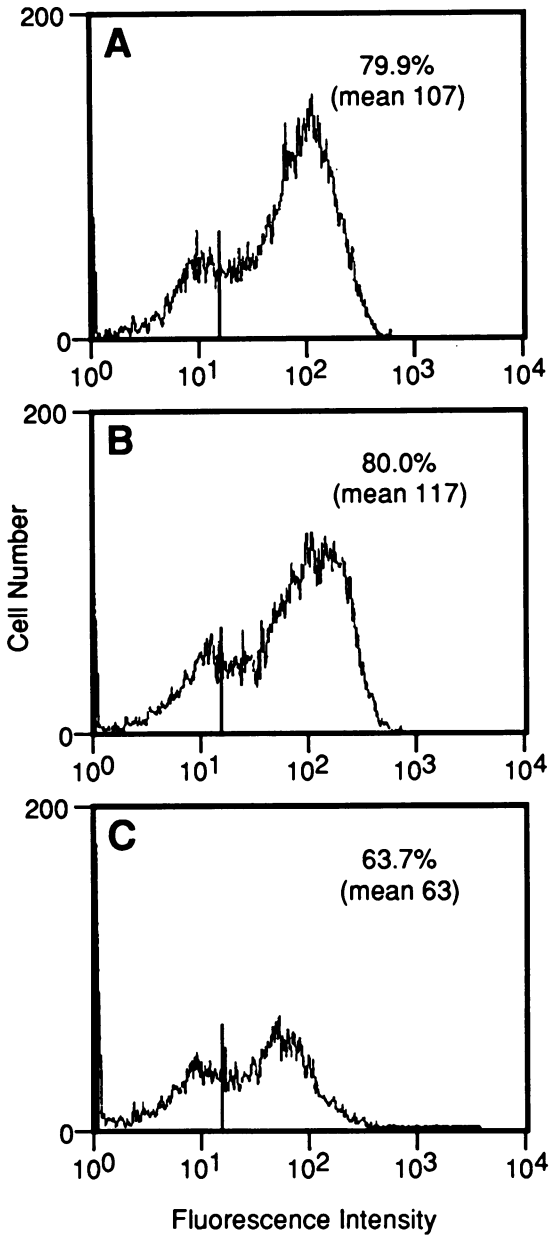

Figure 4. *Loss of MEL-14 expression in spleen lymphocytes of homozygous 272-4 mice. By comparison with negative control (no primary antibody), the percentage of MEL-14+ cells was reduced in homozygotes, and the mean fluorescence in the remaining positive cells was reduced. Spleen cell suspensions were analyzed by flow cytometry, gating by light scatter characteristics on lymphocytes. In each histogram, the percentage of MEL-14+ cells is indicated, with the mean fluorescence of the positive peak in parentheses.* **A,** *nontransgenic;* **B,** *272-4 hemizygous littermate;* **C,** *272-4 homozygote.*

size was taken into consideration. Less than 10% of total spleen lymphocytes stained for surface Ig, and staining of cytospin slides detected only a small percentage with cytoplasmic kappa. Despite the decreased numbers of B cells in homozygote spleens, total serum IgG was 3 to 10 times higher than control levels, and staining of histologic sections for kappa demonstrated that plasma cells were present in spleen, mesenteric lymph node, and tissue inflammatory infiltrates.

Because decreased numbers of peripheral B cells may be due to decreased output from the bone marrow, bone marrow from homozygous animals were stained for IgM and the pre-B cell marker B220. In two separate experiments on homozygotes and littermate controls, the homozygous animals had a much lower proportion of B220 + cells (Table 3, Figure 5). Interestingly, although the homozygous bone marrow had very few B220 + sIgM− cells ("pre-B cells"), the B220-sIgm-appeared to be split into a true negative peak and a $B220^{lo}$ $sIgM^{lo}$ peak, suggesting that development of pre-B cells was blocked at this stage. As a result, although the B220 + IgM + cells constituted less than 30% of total B220 + cells in controls, B220 + IgM + cells constituted more than 60% of the B220 + cells in the homozygotes. We interpret this to mean that although there is a relative decrease in the generation of pre-B cells in homozygotes, the few cells generated may be rapidly driven to maturation.

Some studies have suggested that pre–B-cell development is dependent on stem cell–stromal cell interaction mediated by VLA-4.[9] Because this molecule is also involved in lymphocyte homing to Peyer's patches,[10] bone marrow and spleen cells from control and homozygous mice were stained with the antibody R1-2 (anti-VLA-4). Cells from both groups gave equivalent staining, ruling out any abnormalities in VLA-4 expression (Figure 5), although the function of VLA-4 may be impaired.

Functional Analysis of 272-4 Lymphocytes

Immune function was initially assessed by testing for antibody responses to Murine Hepatitis Virus (MHV). Some of the mice were maintained in a mouse colony where MHV was endemic, so nearly all normal animals tested positive for serum titers against MHV. Under these conditions, the two homozygotes tested had no detectable titers against MHV, whereas littermates housed in the same cages tested positive.

Spleen cells were tested *in vitro* for their ability to respond by proliferation in response to T cell mitogens such as IL-2, Con-A, and anti-CD3 antibody, and the B cell mitogen LPS (Figure 6). The spleen cells from homozygote mice were less sensitive to IL-2, but maximal responses approached those of normal cells. Responses to all other stimuli were well below control levels. In the case of T cell mitogens, the reduced frequency of T cells seen on FACS analysis was not sufficient to explain the reduced proliferative responses to Con-A and anti-CD3

Table 3. *Decrease in Pre-B-cells (B220+ IgM-) in Bone Marrow of Homozygotes*

Animal	% of total bone marrow			% of total spleen		
	All B220+	B220+ IgM−	B220+ IgM+	All B220+	B220+ IgM−	B220+ IgM+
Expt. 1						
NonTg littermate	56	46	10	52	9	43
272-4 homozygote	13	5	8	13	5	8
Expt. 2						
272-4 hemozygote	57	42	15	53	3	50
272-4 homozygote	17	5	12	11	1	10

Mice in experiment 1 were 4 weeks old, in experiment 2, 3 weeks old.

antibody, especially in view of the near-normal responses to IL-2. Interestingly, despite the activated phenotype based on light scatter characteristics, there was no increased background proliferation from spleen cells cultured alone. By contrast to the results with T cell mitogens, the poor response to LPS was probably a direct consequence of the drastically reduced numbers of B cells.

Considering the apparent inability to generate specific immune responses, it is possible that the syndrome of tissue inflammation was due to an inability to respond to bacterial pathogens (the mice developed the syndrome even under virus-free conditions). In an attempt to remedy such a defect, two young homozygous mice were injected with 10^8 spleen cells from normal healthy hemizygous 272-4 mice. Because the line was generated on an inbred C57BL/6 background, and both donor and recipient cells would share the same K^k transgene, rejection of donor cells was unlikely. After 1 month, the homozygous recipients still developed the full-blown inflammatory syndrome, indicating that the immune defect could not be prevented by the presence of normal splenocytes. To confirm the persistence of the injected normal cells, spleen cells from one of the injected homozygotes were included in the functional assays in Figure 6. Injection of normal cells resulted in partial restoration of T and B cell responses to mitogen and essentially normal responses to IL-2. In this experiment, the injected animal

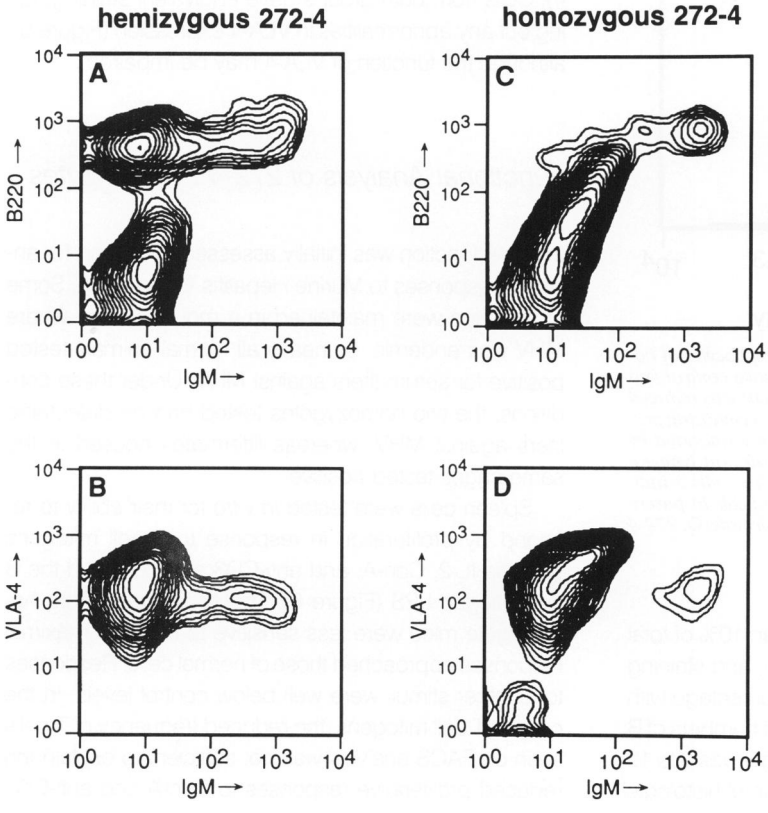

Figure 5. *Lymphocyte subpopulations in the bone marrow. Bone marrow cells from 272-4 homozygotes show reduced percentage of cells with a pre–B-cell phenotype (B220+ sIgM-), but normal levels of VLA-4. Bone marrow from 272-4 hemizygous littermate* **(A,B)** *and 272-4 homozygote* **(C,D)** *analyzed for B220 versus sIgM* **(A,C)** *and VLA-4 versus sIgM* **(B,D)**. *Data is same as shown in Table 3, Expt. 2.*

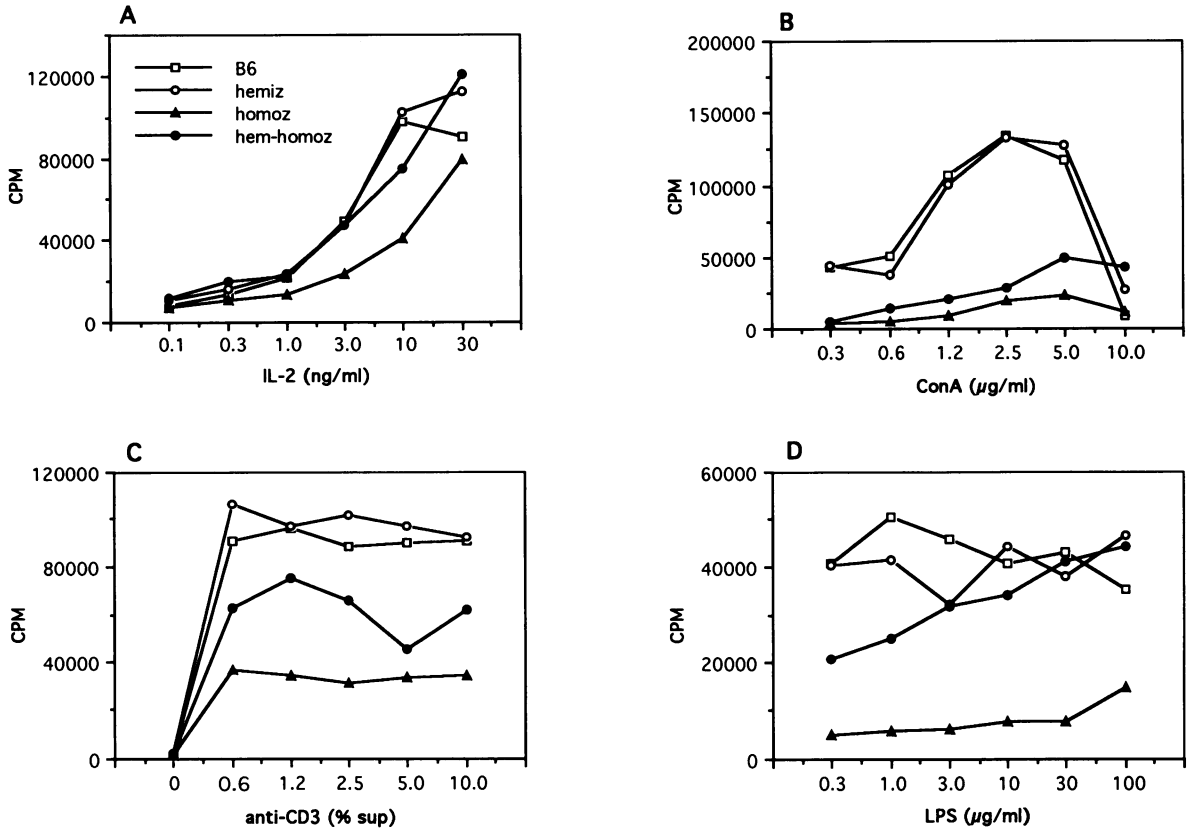

Figure 6. *Proliferative response of 272-4 lymphocytes to mitogen stimulation. Splenocytes from homozygous (solid triangles) or hemizygous (open circles) 272-4 mice were compared with cells from parental C57BL/6 (open squares), or from homozygous 272-4 mice that were injected with 10^8 hemizygous spleen cells 1 month before assay (solid circles). Cells were cultured with mitogens for optimal times before pulsing with ^{3}H-thymidine. Data is presented in CPM;* (A) *IL-2, 70 hr;* (B) *ConA, 50 hr;* (C) *anti-CD3 antibody, 26 hr;* (D) *LPS, 50 hr. Cells from all four mice cultured without mitogens gave background proliferation below 2000 CPM for 26 hr and 50 hr of culture, and below 3200 CPM for 70 hr of culture.*

also generated serum titers against MHV, whereas the uninjected homozygous animal was negative.

Discussion

We have identified a recessive defect in a single line of mice harboring a normal class I MHC transgene. The defect results in an unusual pattern of tissue inflammation and defects in lymphocyte function and distribution. It is possible that the recessive defect involves granulocyte function in response to infection, but some specific features complicate the picture. First, the inflammatory sites are curiously limited to specific organs, and the gut and urinary tract are remarkably free of inflammation. This suggests that the granulocytes are at least capable of handling the threat from gut flora. Considering that the sites of inflammation may correlate with the presence or absence of gram-positive bacteria, there may be a granulocyte defect in superoxide production. In contrast to human chronic granulomatous disease, however, which is characterized by a defect in superoxide production by granulocytes, the 272–4 mice do not show evidence of extensive granuloma formation. Moreover, the infiltrates in the lung are not consistent with bacterial pneumonia, suggesting that other factors may have caused the accumulation of inflammatory cells. Further studies on granulocyte function in these mice are necessary to clarify whether the infiltrates are a normal response to infection or if they represent a defect in granulocytes themselves.

The spleen and bone marrow of 272–4 mice contained few B cells staining for surface immunoglobulin, probably because of a defect in bone marrow production of pre-B cells. This feature is all the more curious because the serum levels of immunoglobulin were high, and the kidney glomeruli developed what appeared to be immune complex deposits. Plasma cells were identified in tissue infiltrates, so it is clear that at least some B cells were able to develop into antibody-producing cells. It is possible that the genetic lesion affected the development of most B cells so that only a few progressed beyond the pre–B-cell stage. Those few cells that developed into mature B cells were probably then rapidly driven to the plasma cell stage by environmental (bacterial) antigens.

The immunoglobulin deposits in kidney glomeruli then might be explained as immune complexes. B cells were apparently not induced to produce titers against an endemic pathogen, MHV, although this also could be secondary to a T cell defect.

The complex phenotype presented by the 272–4 homozygotes has been a perplexing problem with no obvious explanation unifying all of the observed phenomena. Yet to stimulate further studies on this model system, we have considered a number of possible single-gene defects that could account for some of the peculiar features of these animals. Although these are purely speculative, they may lead to additional clues as to the pathophysiology of the 272–4 homozygotes.

One possible explanation is that the homozygous recessive allows for a spontaneous activation and maturation of T lymphocytes. This activation would secondarily result both in loss of homing receptor expression and in increased production of lymphokines such as GM-CSF. The few B cells produced would be rapidly driven to maturation into plasma cells either as a direct consequence of the gene defect or secondary to T cell lymphokine production. Consistent with this hypothesis, it has been reported that chronic injections of recombinant GM-CSF[11] into mice induces accumulation of granulocytes and macrophages in liver and lung, and the bone marrow increases granulocyte and macrophage production at the expense of lymphoid cells (eg, B cells). Unfortunately, this scheme does not explain why the activated lymphocytes are not spontaneously driven to proliferate or produce large amounts of IL-2 *in vitro.*

Another possibility is that the defect is in one of the genes associated with the α4 integrin (either β1, β7, or a gene regulating their function). Thus, although the α4 was identified on the cell surface, it is either associated with the wrong β integrin, or its function is somehow impaired. Based on studies by various groups, an α4-associated defect could explain the lymphocyte homing defect[12] and the bone marrow pre-B cell defect.[9]

It is noteworthy that the complex phenotype resulting from the integrated transgene appears to specifically affect cells of the lymphocytic or granulocytic lineages, and not cells in the erythroid lineage, nor cells in any other somatic tissue. Further functional studies are clearly necessary to determine the nature of the primary defect. An additional approach, however, will be to isolate the gene disrupted by the integrated transgene, using the integrated transgene sequences as rescue markers for cloning. Identification of such a gene responsible for regulation of lymphocyte development would be of great value for developing an understanding of tissue-specific gene regulation and development.

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References

1. Woychik RP, Stewart JA, Davis LG, D'Eustachio P, Leder P: An inherited limb deformity created by insertional mutagenesis in a transgenic mouse. Nature 1985, 318:36–40
2. Lohler J, Timpl R, Jaenisch R: Embryonic lethal mutation in mouse collagen I gene causes rupture of blood vessels and is associated with erythropoietic and mesenchymal cell death. Cell 1984, 38:597–614
3. Harrison's Principles of Internal Medicine. 12th edition. Edited by JD Wilson, E Brunwald, KJ Isselbacher, RG Petersdorf, JB Martin, AS Fauci, RK Root. New York, McGraw-Hill, 1991
4. Babior BM, Woodman RC: Chronic granulomatous disease. Seminars in Hematology 1990, 27:247–259
5. Dustin ML, Springer TA: Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. Annu Rev Immunol 1991, 9:27–66
6. Brinster RL, Chen HY, Trumbauer ME, Yagle MK, Palmiter RD: Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. Proc Natl Acad Sci USA 1985, 82:4438–4442
7. Arnold B, Burgert H-G, Archibald AL, Kvist S: Complete nucleotide sequence of the murine H-2Kk gene: Composition of three H-2K locus alleles. Nucleic Acids Res 1984, 12: 9473–9487
8. Behringer RR, Mathews LS, Palmiter RD, Brinster RL: Dwarf mice produced by genetic ablation of growth hormone-expressing cells. Genes Dev 1988, 2:453–464
9. Miyake K, Weissman IL, Greenberger JS, Kincade PW: Evidence for a role of the integrin VLA-4 in lympho-hemopoiesis. J Exp Med 1991, 173:599–607
10. Holzmann B, McIntyre BW, Weissman IL: Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an a chain homologous to human VLA-4a. Cell 1989, 56:37–46
11. Metcalf D, Begley CG, Williamson DJ, Nice EC, DeLamarter J, Mermod J, Thatcher D, Schmidt A: Hemopoietic responses in mice injected with purified recombinant murine GM-CSF. Exp Hematol 1987, 15:1–9
12. Holzmann B, McIntyre BW, Weissman IL: Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an α chain homologous to human VLA-4α. Cell 1989, 56:37–46