The Journal of Immunology

RESEARCH ARTICLE | MARCH 01 2000

NOD/LtSz-*Rag1*^{null} Mice: An Immunodeficient and Radioresistant Model for Engraftment of Human Hematolymphoid Cells, HIV Infection, and Adoptive Transfer of NOD Mouse Diabetogenic T Cells[1] FREE

Leonard D. Shultz; ... et. al

J Immunol (2000) 164 (5): 2496–2507.
https://doi.org/10.4049/jimmunol.164.5.2496

NOD/LtSz-*Rag1*^{null} Mice: An Immunodeficient and Radioresistant Model for Engraftment of Human Hematolymphoid Cells, HIV Infection, and Adoptive Transfer of NOD Mouse Diabetogenic T Cells[1]

Leonard D. Shultz,[2]* Pamela A. Lang,* Sherri W. Christianson,* Bruce Gott,* Bonnie Lyons,* Syuji Umeda,* Edward Leiter,* RuthAnn Hesselton,† Eric J. Wagar,† Jean H. Leif,† Órit Kollet,‡ Tsvee Lapidot,‡ and Dale L. Greiner†

Development of a small animal model for the in vivo study of human immunity and infectious disease remains an important goal, particularly for investigations of HIV vaccine development. NOD/Lt mice homozygous for the severe combined immunodeficiency *(Prkdc*^{scid}*)* mutation readily support engraftment with high levels of human hematolymphoid cells. However, NOD/LtSz-*scid* mice are highly radiosensitive, have short life spans, and a small number develop functional lymphocytes with age. To overcome these limitations, we have backcrossed the *null* allele of the recombination-activating gene *(Rag1)* for 10 generations onto the NOD/LtSz strain background. Mice deficient in RAG1 activity are unable to initiate V(D)J recombination in Ig and TCR genes and lack functional T and B lymphocytes. NOD/LtSz-*Rag1*^{null} mice have an increased mean life span compared with NOD/LtSz-*scid* mice due to a later onset of lymphoma development, are radioresistant, and lack serum Ig throughout life. NOD/LtSz-*Rag1*^{null} mice were devoid of mature T or B cells. Cytotoxic assays demonstrated low NK cell activity. NOD/LtSz-*Rag1*^{null} mice supported high levels of engraftment with human lymphoid cells and human hemopoietic stem cells. The engrafted human T cells were readily infected with HIV. Finally, NOD/LtSz-*Rag1*^{null} recipients of adoptively transferred spleen cells from diabetic NOD/Lt+/+ mice rapidly developed diabetes. These data demonstrate the advantages of NOD/LtSz-*Rag1*^{null} mice as a radiation and lymphoma-resistant model for long-term analyses of engrafted human hematolymphoid cells or diabetogenic NOD lymphoid cells. *The Journal of Immunology,* 2000, 164: 2496–2507.

The establishment of a functional human immune system reconstituting a small animal model is a goal of many workers investigating human immunity and human-specific infectious agents such as HIV-1 (1–3). Since the first reports of low levels of engraftment of human hematolymphoid cells in C.B-17 mice homozygous for the severe combined immunodeficiency *(Prkdc*^{scid}*)* mutation (hereafter abbreviated as *scid)* (3–5), we have focused on optimizing this model by manipulating the host strain background to increase levels of human cell engraftment. We have provided evidence that NOD/LtSz-*scid* mice support levels of human hematolymphoid engraftment that are ~5-fold higher than those observed in C.B-17-*scid* mice (1, 6–10). Recently, we have demonstrated that NOD/LtSz-*scid* mice homozygous for the β2-microglobulin *null* allele (*B2m*^{null}) support even higher levels of human cell engraftment than do NOD/LtSz-*scid* mice (11). The data support the hypothesis that the host strain background strongly modulates the level of achievable human cell engraftment in this model system.

We have continued to focus on the NOD/Lt genetic background in our attempts to further optimize engraftment with human cells because of the numerous defects in innate immunity characterizing this strain. NOD/Lt (and NOD/LtSz-*scid*) mice lack hemolytic complement, have defects in myeloid development, display abnormalities in Ag-presenting function, and have low NK cell activity (7). It is unknown which specific innate immune defect, or combination of defects lead to enhanced engraftment with high levels of human hematolymphoid cells. Markedly decreased levels of human lymphoid cell engraftment are associated with increased levels of innate immunity in other strains of *scid* mice (1, 12). Based on our published data, NODLtSz-*scid* mice are now the strain of choice for most laboratories studying engraftment of human hematolymphoid cells in immunodeficient mice (1, 8–10, 13–18). Despite its ability to support high levels of human cell engraftment, numerous limitations still exist in this model system. Unlike NOD/Lt mice, which are very radioresistant, NOD/LtSz-*scid* mice are highly radiosensitive (9). Furthermore, they become leaky and generate mature lymphocytes with age, and develop a high incidence of thymic lymphomas at an early age, resulting in a short life span (7, 9, 19). These characteristics have been ascribed predominately to the combined defects in adaptive immunity and DNA repair due to the *scid* mutation accompanied by deficiencies in innate immunity caused by the NOD/Lt strain background.

Mice defective in either of two additional genes that perform a critical function in Ag receptor gene rearrangements in T and B cells have been generated by knockout technology (20, 21). Mice

*The Jackson Laboratory, Bar Harbor, ME 04609; †Departments of Medicine and Pediatrics, University of Massachusetts Medical Center, Worcester, MA 01605; and ‡Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

Received for publication September 16, 1999. Accepted for publication December 21, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by U.S. Public Health Service Grants AI30389, DK57199, DK32520, RR07068, DK36175, DK2772, AI38757, AI24544, DK/AI53006, and CA34196; the Hood Foundation; the Balfur Peisner Bone Marrow Cancer Research Fund (O.K.); and a grant from the Israel Academy of Science (T.L.).

[2] Address correspondence and reprint requests to Dr. Leonard D. Shultz, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609. E-mail address: lds@jax.org

0022-1767/00/$02.00

homozygous for the *null* allele at the recombination-activating gene-1 (*Rag1*^{null}) or the recombination-activating gene-2 (*Rag2*^{null}) lack functional T and B lymphocytes. Mice deficient in RAG1 or RAG2 do not become leaky with age due to their complete inability to initiate V(D)J recombination of Ig or TCR genes. Previous attempts to engraft human hematolymphoid cells into *Rag1*^{null} or *Rag2*^{null} mice on a mixed genetic background led to disappointingly low levels of engraftment (22, 23). Based on our studies on the effect of background-modifying genes on the phenotype of *scid* mice, we hypothesized that the observed low levels of human cell engraftment in *Rag1*^{null} mice were due to effects of the host strain background and accompanying high level of innate immune activity in these strains. We further predicted that NOD/Lt mice bearing the *Rag1*^{null} allele would engraft with high levels of human hematolymphoid cells, do not become leaky with age, and display a longer life span than do NOD/LtSz-*scid* mice. The longer life span would be expected due to an anticipated delay or prevention of thymic lymphomas associated with the DNA repair defect in NOD/LtSz-*scid* mice (24–27).

To test this hypothesis, we backcrossed the disrupted *Rag1*^{null} gene from the (C57BL/6, 129) segregating stock onto the NOD/Lt strain background. At the N10 backcross generation, NOD/Lt +/*Rag1*^{null} heterozygotes were intercrossed to produce a homozygous NOD/LtSz-*Rag1*^{null} genetic stock for study. In this work, we describe the phenotypic characteristics of NOD/LtSz-*Rag1*^{null} mice and document the ability of these mice to support high levels of engraftment with human T cells and hemopoietic stem cells. The human T cells supported infection with HIV-1. We further document that NOD/LtSz-*Rag1*^{null} mice are suitable as hosts for the adoptive transfer of insulitis and diabetes by spleen cells from spontaneously diabetic NOD/Lt +/+ donors.

Materials and Methods

Mice

NOD/Lt +/+, NOD/LtSz-*scid*, C57BL/6-*scid*, and C57BL/6-*Rag1*^{null} mice were raised in our research colony at The Jackson Laboratory (Bar Harbor, ME), as previously described (7). The *Rag1*^{null} mutation on chromosome 2 was originally made by insertion of a neomycin-resistance cassette into a coding region of the *Rag1* gene (20). One copy of this disrupted gene was backcrossed 10 generations from the (C57BL/6, 129) segregating stock onto the NOD/Lt strain background. This backcross was initiated by mating a (C57BL/6, 129)-*Rag1*^{null} female with a NOD/Lt male. The female F_1 +/*Rag1*^{null} offspring were backcrossed to NOD/Lt males. For each successive backcross, the +/*Rag1*^{null} female offspring were identified by PCR for the neomycin insert. Genomic DNA was prepared from tail snips (28). For PCR amplification, the following primers were used: IMR013, 5′-CTTGGGTGGAGAGGCTATTC-3′, and IMR014, 5′-AGGTGAGATGA CAGGAGATC-3′. This PCR amplifies a 280-bp product. At the tenth backcross generation, NOD/Lt +/*Rag1*^{null} mice were intercrossed. NOD/Lt-*Rag1*^{null} homozygous mice were identified by the lack of serum IgM, as determined by immunodiffusion in micro-Ouchterlony plates (The Binding Site, Birmingham, U.K.) using affinity-purified $F(ab')_2$ fraction of rabbit anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL). The NOD/LtSz-*Rag1*^{null} breeding colony was raised in microisolator boxes that were housed in laminar flow hoods. After weaning, the NOD/LtSz-*Rag1*^{null} mice were housed in a conventional specific pathogen-free mouse room that was maintained at 68°F with 14:10 h of light:dark cycle. The animals were fed NIH-31 6% fat diet and received acidified (HCl; pH 2.8–3.2) water ad libitum. Mice housed under specific pathogen-free conditions received acidified water containing sulfamethoxazole-trimethoprim (Goldline Laboratories, Ft. Lauderdale, FL) on 7 consecutive days per week, every other week, to protect the mice from infection with *Pneumocystis carinii*.

Cumulative percent survival

Twelve female and seven male NOD/LtSz-*Rag1*^{null} mice were monitored weekly from 8 wk of age and necropsied when moribund.

Antibodies

The following mAb used for characterization of spleen cells from unmanipulated 9- to 10-wk-old mice were purchased from PharMingen (San Diego, CA) as FITC or PE conjugates: anti-CD3, clone 500A2; anti-CD4, clone RM4-5; anti-Igκ, clone R5-240; and anti-pan granulocyte (Gr-1), clone RB6-8C5. Additional mAb were generated as ascites, precipitated with ammonium sulfate, purified by size exclusion chromatography, and labeled with biotin, PE, or FITC. These mAb included: anti-CD8α, clone 53-6.72 (29); anti-B220, clone RA3-6B2 (30); antipan macrophage, clone F4/80; anti-Mac-1, clone M1/70; anti-MHC class I, clone 28-14-8 (31); and anti-I-A^k that cross-reacts with NOD-MHC class II I-A^{g7}, clone 10-2.16 (32).

Flow cytometry analyses of splenocytes in nonengrafted mice

Preparation of spleen cells for single-label and dual-label flow cytometry analysis was performed, as previously described (7). Single cell suspensions were kept on ice in FACS medium (PBS containing 5% FBS (HyClone, Logan, UT) and 0.1% sodium azide) for subsequent staining. Before staining with fluorochrome-labeled mAb, nonspecific binding was blocked by incubation of the cells with rabbit IgG (100 μg/10^6 cells; Sigma, St. Louis, MO) (7). Cells (10^6/well) were stained and washed in V-bottom 96-well plates. After staining with mAb for 30 min on ice, cells were washed two times with FACS medium. All samples were incubated with propidium iodide to allow exclusion of dead cells from the final analysis. Nucleated cells were selected by light scatter. Flow cytometry analysis was conducted on 10^4 to 2×10^4 viable leukocytes using a Becton Dickinson FACScan (San Jose, CA).

Histopathologic analyses

Mice were euthanized in an atmosphere of 100% CO_2. Axillary, brachial, cervical, and inguinal lymph nodes, thymus, spleen, kidney, liver, brain, spinal cord, lungs, pancreas, and femurs were fixed in Bouin's fixative (33), embedded in paraffin, sectioned at 5 μm, and stained with Mayer's hematoxylin and eosin (H&E).[3]

Hematological analyses

Blood was collected from the retroorbital plexus of 8- to 10-wk-old mice using heparinized capillary tubes. Erythrocytes and leukocytes were counted using a model ZBI Coulter counter (Hialeah, FL). Blood smears were stained with Wright-Giemsa (Sigma, St. Louis, MO). Assessments of cell populations in the peripheral blood were conducted by flow cytometry, as follows. Approximately 150 μl of heparinized blood was diluted in 1 mM EDTA/0.85% saline and washed in HBSS. After lysing of red cells in ACK (ammonium chloride) lysis buffer, nucleated cells were incubated with rabbit IgG to block nonspecific staining, as described above. Peripheral blood cells were then incubated with FITC- or PE-labeled Abs, as indicated above for spleen cells. In addition to Abs used on splenocytes, PE-labeled antinucleated RBC, clone TER-119, was used to identify immature nucleated RBC (34).

Quantitation of mouse serum Ig

Sera were collected from 11 female and 12 male NOD/LtSz-*Rag1*^{null} mice aged 104 to 268 days. Levels of serum Ig were determined from individual mice by ELISA, as previously described (7). For quantitation of total mouse Ig, goat anti-mouse Ig (heavy and light chain specific) (Southern Biotechnology Associates) was used as the first layer. Alkaline phosphatase-labeled Ab to mouse κ-chain (Southern Biotechnology Associates) was used as the second layer. Total IgGκ (Calbiochem, San Diego, CA) standard curves were run with each assay, and the Ig levels were determined from the curves. Absorbances of ELISA samples were read at 405 nm on an EL312e ELISA plate reader (Bio-Tek Instruments, Winooski, VT).

NK cell assays

NK cell activity of splenocytes was determined as described (7). Mice of each genotype (four to five males) were injected at 6–8 wk of age i.p. with 100 μg of poly(I:C) (Sigma) 36 h before recovery of spleen cells for analyses. ^{51}Cr-labeled YAC-1 cells (American Type Culture Collection, Manassas, VA) were used as targets. Various E:T ratios were set up in triplicate in V-bottom 96-well microtiter plates. After a 4-h incubation of

[3] Abbreviations used in this paper: H&E, hematoxylin and eosin; FCC, follicular center cell; RAG, recombination-activating gene; SCR, SCID-repopulating cell.

effector and target cells at 37°C, supernatants were recovered and the amount of released radioactivity was quantitated using a gamma counter. Percent specific ^{51}Cr release was calculated as follows, where X = mean experimental release from triplicate wells. Total release (T) was determined from wells receiving ^{51}Cr-labeled YAC-1 target cells and 2% SDS. Spontaneous release (S) was determined from wells receiving ^{51}Cr-labeled YAC-1 targets in growth medium: percent-specific release = $[(X - S)/(T - S)] \times 100$.

Sensitivity of mice to whole body irradiation

NOD/LtSz-*scid*, NOD/LtSz-*Rag1*^{null}, and NOD/Lt +/+ mice were exposed to varying doses of whole body irradiation at a rate of 165 rad/min from a Shepard Mark I irradiator loaded with 10,000 Ci of ^{137}Cs (J. L. Shepard and Associates, San Fernando, CA). For life span studies, mice were irradiated at 6–8 wk of age, examined daily, and necropsied when moribund. To determine effects of irradiation on newborn mice, 1- to 2-day-old pups were irradiated as above and killed at 6–8 wk of age for flow cytometry analyses of spleen cell populations and histopathologic analyses of other tissues.

Human PBMC engraftment

Human PBMC were prepared from normal leukaphoresis donors, as described (1). Mice were injected i.p. with 20×10^6 human PBMC within 3 h of separation by density-gradient centrifugation of heparinized blood. At 4 wk following injection of human PBMC, peripheral blood leukocytes and spleen cells from engrafted mice were analyzed for expression of human CD45$^+$ cells by flow cytometry. Mice exhibiting <2% human CD45$^+$ cells in the spleen were excluded from the final analyses of mean percentage level of engraftment.

Human umbilical cord blood engraftment

Human cord blood samples were obtained from full-term deliveries after informed consent and were used in accordance with the procedures approved by the human experimentation and ethics committees of the Weizmann Institute of Science (Rehovot, Israel). The blood samples were diluted 1/1 in PBS without Mg^{2+}/Ca^{2+}, supplemented with 10% FBS. Low density mononuclear cells were collected after standard separation on Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden), and washed in RPMI with 10% FBS. Some samples were frozen in 10% DMSO, while the others were used fresh. Enrichment of CD34$^+$ cells was performed with a mini MACS separation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. The purity of the enriched CD34$^+$ cells was 60–80% after one passage. NOD/LtSz-*Rag1*^{null} mice at 8 wk of age were irradiated as indicated with a sublethal dose of 750–850 rad at 67 rad/min from a cobalt (^{60}Co) source 24 h before transplantation. Human cells were injected into the tail vein of irradiated mice in 0.5 ml of RPMI with 10% FBS. Mice were sacrificed 1 mo posttransplantation, and bone marrow cells were harvested. All the experiments were approved by the Animal Care Committee of the Weizmann Institute.

Flow cytometry analyses of splenocytes and peripheral blood from mice engrafted with human PBMC

Spleen and peripheral blood samples were prepared as lysed whole blood or as cell suspensions of lymphoid tissues in PBS with 0.2% sodium azide. mAb against human CD3, CD4, CD8, CD14, CD19, and CD45 were purchased from PharMingen or Becton Dickinson (San Jose, CA). Phenotypic analyses of engrafted human PBMC were performed by two- and three-color flow cytometry analyses with anti-human CD45 used to label cells of human origin. In addition, anti-mouse CD45 (PharMingen) was used to identify host cells. Dead cells and any remaining erythrocytes were gated out on the basis of light scattering. A minimum of 10^4 cells were analyzed for each sample. Cells were analyzed in a FACScan Instar (Becton Dickinson), as previously described (1).

Flow cytometry analyses of bone marrow from mice engrafted with human cord blood cells

Human and mouse FcR were blocked by using human plasma (1:50) and anti-mouse FcR blockers (anti-mouse CD16/CD32 mAb; PharMingen). Isotype control mAb were used to exclude false positive staining (Coulter). The levels of human myeloid CD45$^+$ cells and lymphoid pre-B CD19$^+$ cells in the marrow of engrafted mice were detected by double staining with anti-human CD45 FITC (Immuno Quality Products) together with anti-human CD19 PE (Coulter) for pre-B cell detection. Cells were washed with PBS supplemented with 1% FBS and 0.02% azide, suspended to a volume of 1×10^6 cells/ml, stained with directly labeled mAb, and incubated for 25 min on ice. After staining, cells were washed once in the same buffer and analyzed on a FACSort (Becton Dickinson). Analysis was performed using CELLquest software (Becton Dickinson).

Quantification of human DNA in bone marrow of mice engrafted with human cord blood cells

The levels of human cell engraftment were determined in parallel by quantification of human DNA in the marrow of transplanted mice, as previously described (35). Briefly, high m.w. DNA was obtained from the bone marrow of transplanted mice by phenol/chloroform extraction. DNA (5 μg) was digested with *Eco*RI, subjected to electrophoresis on 0.6% agarose gel, blotted onto a nylon membrane, and hybridized with a human chromosome 17-specific α satellite probe (p17H8) labeled with ^{32}P. The intensity of the bands in the samples was compared with artificial human/mouse DNA mixtures (0%, 0.1%, 1%, and 10% human DNA) to quantify the human DNA. Multiple exposures of the autoradiographs were taken to ensure sensitivity down to 0.1% human DNA.

Quantitation of human Ig levels in mouse serum

Blood was collected from the retroorbital plexus from individual engrafted mice at 4 wk postengraftment with human PBMC. Levels of human IgG and IgM were determined in serum samples by nephlometry using a Beckman ARRAY 360 CE Serology System (Beckman Instruments, Palo Alto, CA).

HIV-1 infection of PBMC-engrafted mice

HIV-1 viral stock used in these experiments was a biological clone of a cytopathic patient isolate (RULDA). This isolate has been well characterized in vitro and in vivo in Hu-PBL-SCID mice (36). Virus stocks were aliquoted, maintained at −80°C, and used within 2 h of thawing. Stock virus was diluted in PBS supplemented with 20% FBS to a concentration of 20,000 tissue culture-infective dose in 50 ml of medium (TCID50/ml, which is approximately a 200 animal infectious dose) (37). Each mouse was given 0.5 ml of the virus by i.p. injection 2–3 wk after engraftment with 20×10^6 human PBMC. Three, 4, and 8 wk after HIV-1 infection, mice were killed and blood and spleen samples were collected. The samples were analyzed for percentages of human CD45$^+$ cells by flow cytometry and for the presence of HIV-1 DNA and RNA by PCR, as previously described (1, 36). Spleen cell samples for flow cytometry were analyzed with the panel of human mAb used for the PBMC-engrafted mice described above. Aliquots of 200 μl of plasma were frozen at −80°C within 3 h of being drawn from the mice for subsequent quantitative HIV-1 RNA analysis using Amplicor RNA PCR kits (Roche Diagnostic Systems, Branchburg, NJ). Samples of 100 μl of whole blood and 10^6 spleen cells were processed for DNA and frozen at −80°C. These samples were later analyzed quantitatively (spleen) or qualitatively (blood) using the Amplicor PCR Assay system for DNA. Controls were included to ensure that the sensitivity of the assay remained consistent at one to two proviral copies per sample.

Adoptive transfer of diabetes

Single cell suspensions of splenocytes obtained from spontaneously diabetic NOD/Lt +/+ female donors were passaged through Nitex 110 (Tetko, Elmsford, NY) to remove cell clumps. RBC were lysed using 10× Gey's solution. Spleen cells were washed in HBSS supplemented with HEPES (2.2 g/L; Life Technologies, Grand Island, NY). Viable cells were identified and enumerated by trypan blue exclusion using a hemacytometer. The donor spleen cells (2×10^7 viable cells) were injected i.v. via the tail vein into groups of 7-wk-old NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* female mice, as previously described (38). The recipients were monitored weekly for urinary glucose using Diastix, kindly provided by Bayer (Elkhart, IN), and killed when glucosuria reached >250 mg/dl. At necropsy, blood was collected from the retroorbital sinus, and plasma glucose levels were determined with a Glucose Analyzer II (Beckman Instruments). For histopathologic evaluation of insulitic lesions, pancreata from recipient mice were fixed in Bouin's solution overnight, embedded in paraffin, and stained with H&E for light-microscopic examination.

Statistics

All measures of variance are presented as SEM. Tests of significance of difference of independent means were performed with Student's t test, and significance was assumed for p values < 0.05.

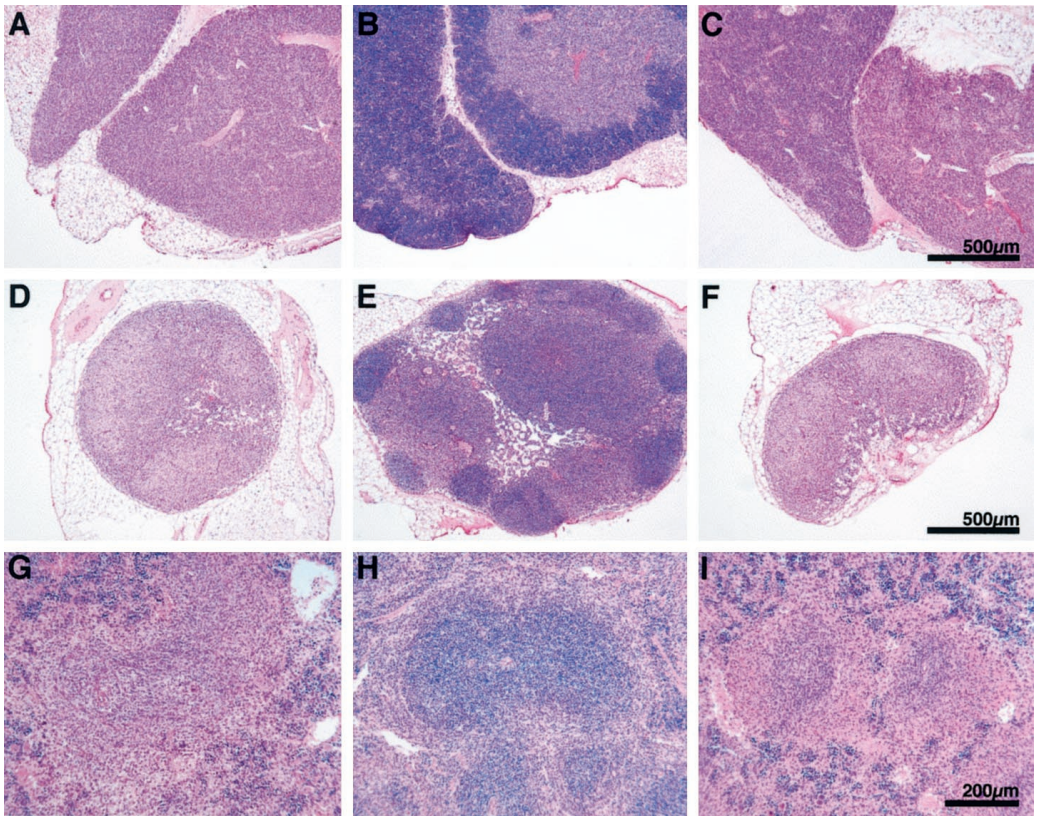

FIGURE 1. Histological sections of lymphoid tissues from 8- to 10-wk-old NOD/LtSz-*Rag1*^{null}, NOD/LtSz-*scid*, and NOD/LtSz +/+ mice. *A–C*, Thymi. Thymic lobes from NOD/LtSz-*Rag1*^{null} (*A*) and NOD/LtSz-*scid* (*C*) mice show absence of a defined cortex, while NOD/Lt +/+ thymus (*B*) has normal cortex and medulla. *D–F*, Lymph nodes. Severe reduction in lymphoid cellularity is evident in nodes from NOD/LtSz-*Rag1*^{null} (*D*) and NOD/LtSz-*scid* mice (*F*), while lymphoid follicles and normal cellularity are evident in the NOD/Lt +/+ node (*E*). *G–I*, Splenic lymphoid follicles. There is marked reduction in cellularity in follicles from NOD/LtSz-*Rag1*^{null} (*G*) and NOD/LtSz-*scid* (*I*) mice in contrast to well-developed normal follicles in NOD/Lt +/+ mice (*H*) (H&E).

Results

Histology of lymphoid tissues in young adult NOD/LtSz-Rag1^{null} *mice*

Histological examination of lymphoid tissues from 8- to 10-wk-old NOD/LtSz-*Rag1*^{null} mice ($n = 10$) showed lack of normal architecture accompanied by severe reduction of lymphoid cellularity (Fig. 1). Severe depletion of lymphoid cells in the spleen, lymph nodes, and thymus was evident in both NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice. In contrast, NOD/Lt +/+ mice showed normal lymphoid architecture.

Life span of NOD/LtSz-Rag1^{null} *mice*

Twelve female and seven male NOD/LtSz-*Rag1*^{null} mice were monitored weekly from 8 wk of age to assess longevity. Mice were necropsied when they appeared moribund. Tissues were fixed in Bouin's solution and prepared for histopathological examination. There was no significant difference in life span between male and female NOD/LtSz-*Rag1*^{null} mice. As shown in Fig. 2, the cumulative survival of NOD/LtSz-*Rag1*^{null} mice was ~50% by 46 wk of age compared with 36 wk in NOD/LtSz-*scid* mice (7) and 24 wk in NOD/LtSz-*scid B2m*^{null} mice (11). The mean life span of NOD/LtSz-*Rag1*^{null} mice was 319.5 ± 7.5 days. This was ~8 wk longer than the life span of NODLt/Sz-*scid* mice (7), and ~18 wk longer than NOD/LtSz-*scid B2m*^{null} mice (11).

*Histology of lymphomas in aged NOD/LtSz-*Rag1^{null} *mice*

The increased life span of NOD/LtSz-*Rag1*^{null} mice compared with NOD/LtSz-*scid* and with NOD/LtSz-*scid B2m*^{null} mice was associated with later onset of lymphomas. There were also differences between NOD/LtSz-*Rag1*^{null} mice and NOD/LtSz-*scid* mice in the cellular composition of lymphomas that developed. Histological examination of tissues from 15 NOD/LtSz-*Rag1*^{null} mice found to be moribund and killed at 26–64 wk of age revealed

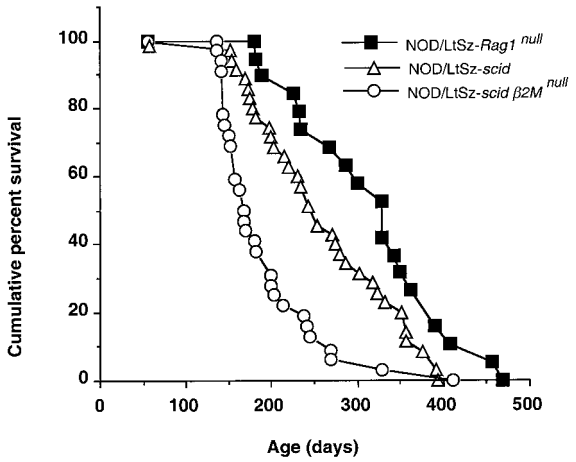

FIGURE 2. Cumulative percent survival of NOD/LtSz-Rag1^{null} mice (■), NOD/LtSz-scid mice (△), and NOD/LtSz-*scid B2m*^{null} mice (○) as a function of age. Twelve female and seven male NOD/LtSz-Rag1^{null} mice were monitored weekly from 8 wk of age. The survival data for NOD/LtSz-scid mice (7) and NOD/LtSz-*scid* B_2M^{null} mice (11) have been published previously.

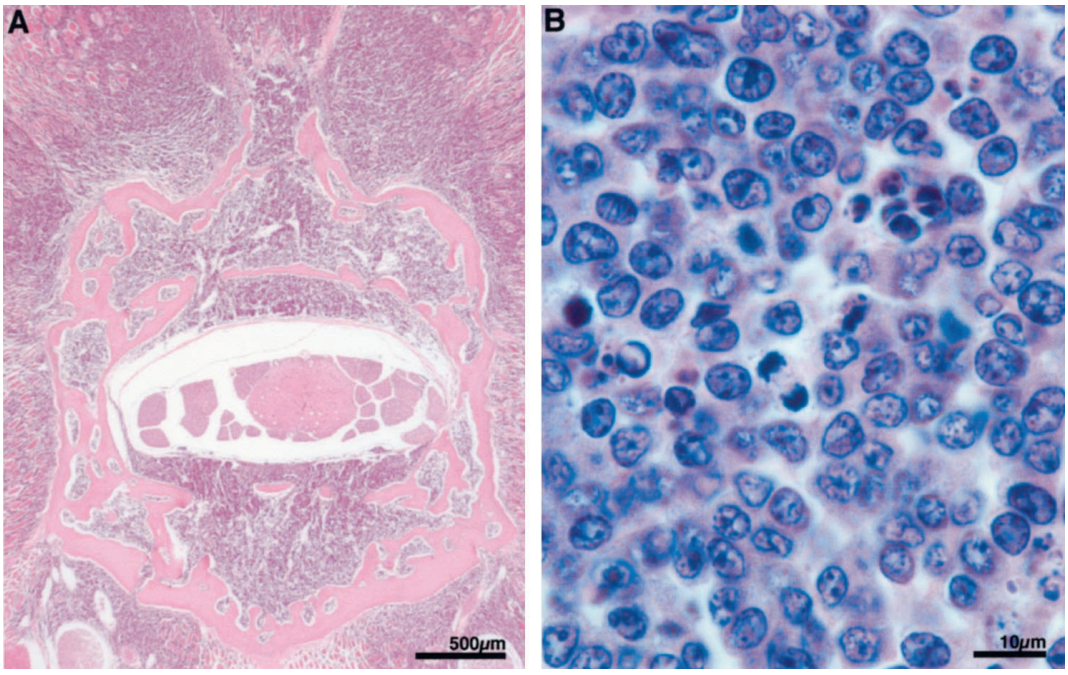

FIGURE 3. FCC lymphoma in a NOD/LtSz-*Rag1*^{null} mouse. *A*, Cross section through the spine of a 26-wk-old NOD/LtSz-*Rag1*^{null} mouse showing diffuse infiltrate of lymphoma throughout paraspinous skeletal muscles, spinal bone marrow, and epidural space. *B*, High magnification of a lymph node from the same mouse showing lymphoma cells of varying shape and size with slightly cleaved nuclei.

follicular center cell (FCC) lymphomas in 11 of 15 mice examined. These lymphomas were observed in the lymph nodes, spleen, kidney, liver, lung, and occasionally in the thymus. Approximately 50% of the mice with FCC neoplasms developed hind limb paralysis as a result of metastasis of the lymphoma to the vertebral bone marrow and spinal cord (Fig. 3). Two of fifteen mice had lymphoblastic lymphomas; one mouse had a mammary tumor; and the remaining mouse showed atrophy of the exocrine cells in the pancreas. Pancreatic islets were uniformly free of the insulitis characteristic of immunocompetent NOD/Lt +/+ mice. Certain lymphomas in NOD/LtSz-*Rag1*^{null} mice were classified as FCCs and were found in lymph nodes with no involvement of the thymus (Fig. 4, *A* and *B*). In contrast, aged NOD/LtSz-*scid* mice developed lymphoblastic lymphomas in the thymus with metastases to other organs consistent with previous studies (7, 19) (Fig. 4, *C* and *D*).

Phenotype of spleen cell populations

The phenotypic profile of spleen cell populations of young adult NOD/LtSz-*Rag1*^{null} mice was determined by flow cytometry. There was a severe deficiency of mature lymphocytes in spleens from NOD/LtSz-*Rag1*^{null} mice, similar to that observed in NOD/LtSz-*scid* mice (7). This lymphoid cell deficiency detected by flow cytometry was consistent with histological observations (Table I). There was no significant difference in nucleated spleen cell numbers between NOD/LtSz-*scid* ($1.95 \pm 0.3 \times 10^7$) and NOD/LtSz-*Rag1*^{null} mice ($1.96 \pm 0.3 \times 10^7$). As expected, NOD/LtSz-*Rag1*^{null} mice did have significantly fewer spleen cells than NOD/Lt +/+ control mice ($8.63. \pm 1.4 \times 10^7$, $p < 0.01$). The populations of spleen cells in NOD/LtSz-*Rag1*^{null} mice also differed significantly from those in NOD/Lt +/+ control mice in the percentages of cells bearing all surface markers examined (Table I). With the exception of Mac-1$^+$ or Gr-1$^+$ myeloid cells, all other cell populations were present on a much lower percentage of spleen cells in NOD/LtSz-*Rag1*^{null} mice than in NOD/Lt +/+ control mice. Although NOD/LtSz-*Rag1*^{null} mice lacked mature B and T cells, there was a small percentage of CD4$^+$ (2.1%) and CD8$^+$ (0.8%) spleen cells that lacked TCR-associated CD3 molecules. These percentages were significantly decreased from the CD4$^+$

Table I. *Flow cytometry analyses of spleen cells from 9- to 10-wk-old Rag1*^{null} *mice*[a]

Clone	Specificity	NOD/Lt +/+	NOD/LtSz-*scid*	NOD/LtSz-*Rag1*^{null}
500A2	CD3	52.1 ± 5.1	1.4 ± 0.5	0.4 ± 0.1[c]
Rm4-5	CD4	33.1 ± 4.7	6.2 ± 0.9	2.1 ± 0.3[b,c]
53-6.72	CD8	17.4 ± 2.1	2.4 ± 0.4	0.8 ± 0.2[b,c]
RA3-6B2	B220	33.6 ± 3.8	19.7 ± 3.6	13.0 ± 0.5[c]
R5-240	IgL,κ	28.1 ± 5.1	1.8 ± 1.2	0.5 ± 0.1[c]
M1/70	Mac-1	19.2 ± 2.0	60.1 ± 2.6	61.0 ± 3.5[c]
RB6-8C5	Gr-1	4.0 ± 0.2	22.1 ± 4.5	27.9 ± 3.3[c]
28-14-8S	H2-D^b	97.7 ± 2.1	98.7 ± 0.4	88.3 ± 3.3[b,c]
10-2.16	I-A^{k,g7}	35.9 ± 2.8	17.3 ± 4.8	16.5 ± 3.2[c]

[a] Data are expressed as mean percent ± SEM ($n = 6$ for NOD/LtSz-*scid* and for NOD/LtSz-*Rag1*^{null}, and $n = 4$ for NOD/Lt +/+)

[b] NOD/LtSz-*Rag1*^{null} vs NOD/LtSz-*scid* ($p < 0.05$).

[c] NOD/LtSz-*Rag1*^{null} vs NOD/Lt +/+ ($p < 0.05$).

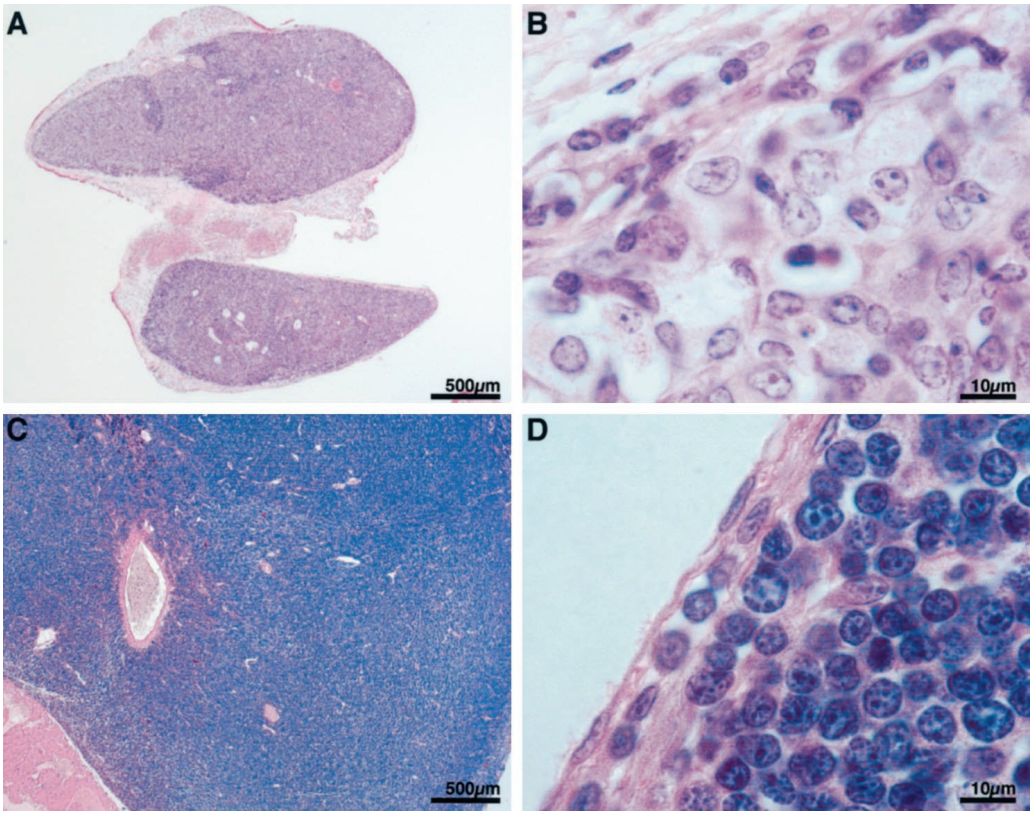

FIGURE 4. Thymi from aged NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice. *A* and *B*, 26-wk-old NOD/LtSz-*Rag1*^{null} mouse. *A*, Low power magnification showing absence of lymphoid cells. *B*, Higher power magnification of cortex showing epithelial and stromal cells. *C* and *D*, 24-wk-old NOD/LtSz-*scid* mouse. *C*, Low power magnification showing massively enlarged thymus consisting mainly of lymphoma cells. *D*, Higher power magnification of cortex showing lymphocytic lymphoma composed of basophilic cells with noncleaved nuclei.

(6.2%) and CD8$^+$ (2.4%) populations lacking CD3 expression observed in NOD/LtSz-*scid* mice ($p = 0.006$ and 0.004, respectively). In absolute cell numbers, there were significantly lower numbers of CD4$^+$ spleen cells in NOD/LtSz-*Rag1*^{null} mice compared with NOD/LtSz-*scid* mice ($4.1 \pm 0.9 \times 10^5$ vs $11.5 \pm 2 \times 10^5$, respectively; $p < 0.02$). Similarly, there were significantly lower numbers of CD8$^+$ spleen cells in NOD/LtSz-*Rag1*^{null} mice compared with NOD/LtSz-*scid* mice ($1.5 \pm 0.4 \times 10^5$ vs $4.5. \pm 0.7 \times 10^5$, respectively ($p < 0.01$)). Two-color analyses revealed that there was no double staining of CD4$^+$ or CD8$^+$ cells with CD3, confirming that these populations were not mature T cells (data not shown). NOD/LtSz-*Rag1*^{null} mice maintained a low percentage of B220$^+$ (13%) and lacked significant numbers of IgL,κ^+ (0.5%) spleen cells while maintaining elevated percentages of Mac-1$^+$ monomyeloid cells (61%) and Gr-1$^+$ granulocytes (28%). These observations are consistent with previous analyses of spleen cell populations in immunodeficient NOD/LtSz-*scid* mice (7). In NOD/LtSz-*Rag1*^{null} mice, 88.3% of cells expressed the MHC class I marker H-2D^b. This is a significant decrease from the 98.7% positive staining observed in the NOD/LtSz-*scid* mice ($p < 0.02$) and the 97.7% positive staining seen in the NOD/Lt +/+ mice ($p < 0.03$). In addition, only 16.5% of NOD/LtSz-*Rag1*^{null} spleen cells express MHC class II molecules compared with 35.9% in NOD+/+ mice.

Peripheral blood counts and differential counts

Total peripheral leukocyte and erythrocyte counts were determined in 10 pairs of NOD/LtSz-*Rag1*^{null} and NOD+/+ mice at 8–9 wk of age. NOD/LtSz-*Rag1*^{null} mice had a significant reduction ($p < 0.001$) in peripheral leukocyte numbers ($2.4 \pm 0.1 \times 10^6$ leukocytes/ml) as compared with NOD/Lt +/+ ($4.1 \pm 0.2 \times 10^6$ leukocytes/ml). There was no significant difference in numbers of RBC between NOD/LtSz-*Rag1*^{null} mice and NOD+/+ mice (data not shown). Peripheral blood samples collected from five pairs of 8-wk-old male NOD/LtSz-*Rag1*^{null} and NOD/Lt +/+ mice were analyzed by flow cytometry to determine percentages of granulocytes, monocytes, lymphocytes, and immature RBC. NOD/LtSz-*Rag1*^{null} mice lacked mature T cells and B cells in peripheral blood (data not shown). This result mirrored the results of flow cytometry analyses in the spleen. NOD/LtSz-*Rag1*^{null} mice had a significantly higher percentage of granulocytes (Gr-1$^+$ cells) in peripheral blood as compared with NOD/Lt +/+ mice ($58 \pm 1.9\%$ vs $21.6 \pm 1.9\%$, $p < 0.05$), and also expressed a significantly higher percentage of monocytes (Mac-1$^+$ Gr-1$^-$) in the peripheral blood compared with NOD/Lt +/+ mice ($33 \pm 1.1\%$ vs $13.5 \pm 0.4\%$, $p < 0.001$, respectively). There was no significant difference between NOD/LtSz-*Rag1*^{null} and NOD+/+ mice in numbers of immature (Ter-119$^+$) erythrocytes (data not shown). Differential cell counts of blood smears confirmed the flow cytometry results (data not shown).

Mouse serum Ig levels

Levels of serum Ig were assayed in 11 female and 12 male NOD/LtSz-*Rag1*^{null} mice between 104 and 268 days of age. There was no detectable Ig observed (<0.4 μg/ml) in any NOD/LtSz-*Rag1*^{null} mouse. Positive control sera from three NOD/Lt +/+ mice at 4 wk of age showed 754.7 ± 69 μg/ml of serum Ig.

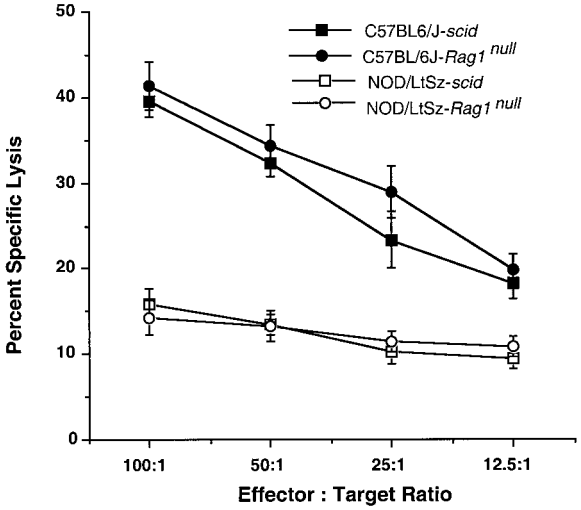

FIGURE 5. Poly(I:C) induced NK cell activity of spleen cells against YAC-1 target cells at various E:T ratios. A representative experiment is shown in which spleen cells from four to five males per strain at 6–8 wk of age were used as effector cells. NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* spleen cells show low NK cell activity, while C57BL/6-Rag1^{null} and C57BL/6-scid spleen cells exhibit high NK cell activity.

Poly(I:C)-induced NK cell activity

The ability of NOD/LtSz-*scid* mice to support high levels of human hemopoietic cell engraftment is associated with the relatively low NK cell activity of these mice (7). Analyses of poly(I:C)-stimulated NK cell activity in NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice showed that both of these immunodeficient NOD/Lt strains expressed low NK cell activity (Fig. 5). In contrast, C57BL/6J-*scid* mice and C57BL/6-*Rag1*^{null} mice expressed relatively high levels of NK cell activity. Two additional experiments confirmed the low level of NK cell activity in NOD/LtSz-*Rag1*^{null} mice. These striking results clearly indicate the significant role that strain background plays in the expression of NK cell activity.

Radiosensitivity

Groups of 6-wk-old NOD/LtSz-*Rag1*^{null}, NOD/LtSz-*scid*, and NOD/Lt +/+ mice were irradiated with 400-1000 rad and monitored for 8 wk. As shown in Table II, NOD/LtSz-*scid* mice, as expected, were highly susceptible to effects of radiation (Table II). Whereas 60% of the NOD/LtSz-*scid* mice survived doses of 400 rad, none of these animals survived 600 rad. In contrast, 80% of the NOD/LtSz-*Rag1*^{null} mice and 100% of the NOD/Lt +/+ mice survived 900 rad. To follow the long-term survival of irradiated NOD/LtSz-*Rag1*^{null} mice, an additional group of eight NOD/LtSz-*Rag1*^{null} mice at 6 wk of age was irradiated with 750 rad and necropsied when they appeared moribund. These NOD/LtSz-*Rag1*^{null} mice survived to 200.3 ± 8.3 days of age. This represents a survival of ~22 wk postirradiation. Tissues from three of these irradiated NOD/LtSz-*Rag1*^{null} mice were examined histologically. Analyses of H&E-stained paraffin sections of these mice revealed FCC lymphomas. In addition, we examined the radiosensitivity of newborn NOD/LtSz-*Rag1*^{null} mice. In a preliminary experiment, one-half of the mice in one litter of NOD/LtSz-*Rag1*^{null} ($n = 8$) and one litter of NOD/LtSz-*scid* mice ($n = 10$) were irradiated with 100 rad at 1 day of age, and the remaining mice in each litter served as nonirradiated controls. Four of five of the irradiated NOD/LtSz-*scid* and all of the irradiated NOD/LtSz-*Rag1*^{null} mice survived throughout the observation period of 6 wk. At 6 wk of age, the mice were necropsied and the spleen cells of the irradiated and nonirradiated control mice were analyzed by flow cytometry. There were no mature T cells ($CD3^+CD4^+$ or $CD3^+CD8^+$) or mature B cells (IgL,κ^+) detected in any of the spleen cell suspensions from NOD/LtSz-*Rag1*^{null} mice. In contrast, two of four of the irradiated NOD/LtSz-*scid* mice had low levels (1–4%) of mature T cells in the spleen. None of the nonirradiated control mice of either genotype showed mature T cells. Histological analyses of thymuses from these mice showed early thymic lymphomas in four of four irradiated NOD/LtSz-*scid* mice. In contrast, none of the irradiated NOD/LtSz-*Rag1*^{null} mice developed thymic lymphomas. As expected, there were no lymphomas observed in the nonirradiated NOD/LtSz-*scid* or NOD/LtSz-*Rag1*^{null} mice at this age.

Table II. *Radiation sensitivity of NOD/LtSz*-Rag1^{null} *mice*[a]

Strain	Dose of X-Irradiation (rads)					
	400	600	700	800	900	1000
NOD/Lt +/+	ND	0	0	0	0	5
NOD/LtSz-*scid*	2	5	ND	ND	ND	ND
NOD/LtSz-*Rag1*^{null}	ND	0	0	0	1	5

[a] Data show numbers of mice which died out of five mice exposed to each dose of X-irradiation.

Because the adult NOD/LtSz-*Rag1*^{null} mice retained the NOD/Lt strain characteristic radioresistance and survived relatively high doses of irradiation, an additional litter of 15 one-day-old NOD/LtSz-*Rag1*^{null} mice was divided into two groups, and eight pups were irradiated with 550 rad, while seven pups were not irradiated. All of the nonirradiated and seven irradiated mice survived throughout a 7-wk observation period. The mice were necropsied and the spleen cell suspensions were analyzed by flow cytometry. There were no mature T or B cells in the spleens of these mice, as determined by flow cytometry analyses (data not shown).

Engraftment of human PBMC

The ability of NOD/LtSz-*Rag1*^{null} mice to support engraftment with human lymphoid cells was determined 4 wk after the i.p. injection of 20×10^6 human PBMC. In six independent experiments, there was no significant difference in the percentages of human PBMC engrafting in the spleen or peripheral blood of NOD/LtSz-*Rag1*^{null} mice compared with NOD/LtSz-*scid* mice. Results of a representative experiment are shown in Figs. 6 and 7. Human $CD45^+$ cells were detected in four of five NOD/LtSz-*Rag1*^{null} and five of five NOD/LtSz-*scid* mice. The percentages of human $CD45^+$ cells in the spleens of NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice were respectively 38.1 ± 5% and 34.5 ± 5%. Previous flow cytometry analyses of NOD/LtSz-*scid* mice injected with human PBMC have shown that the majority of engrafted human T cells were $CD8^+$ (1). The present results obtained with NOD/LtSz-*Rag1*^{null} mice are consistent with the data obtained with NOD/LtSz-*scid* mice. There was no significant difference in the percentages of $CD8^+$ or $CD4^+$ cells in the spleens of engrafted NOD/LtSz-*Rag1*^{null} mice compared with the spleens of engrafted NOD/LtSz-*scid* mice. NOD/LtSz-*Rag1*^{null} mice had 27 ± 4.3% $CD8^+$ cells in the spleen, while NOD/LtSz-*scid* mice had 21.9 ± 4% $CD8^+$ cells; $p > 0.05$ (Fig. 6). There was also no significant difference between NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice in the percentages of $CD4^+$ cells in the spleen. These percentages were 12.3 ± 0.9% and 10.6 ± 2.5%, respectively ($p > 0.05$). Analyses of human cell numbers in the peripheral blood of the engrafted mice showed similar levels of engraftment between NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice. There was no significant difference in the percentages of $CD8^+$ or $CD4^+$ cells

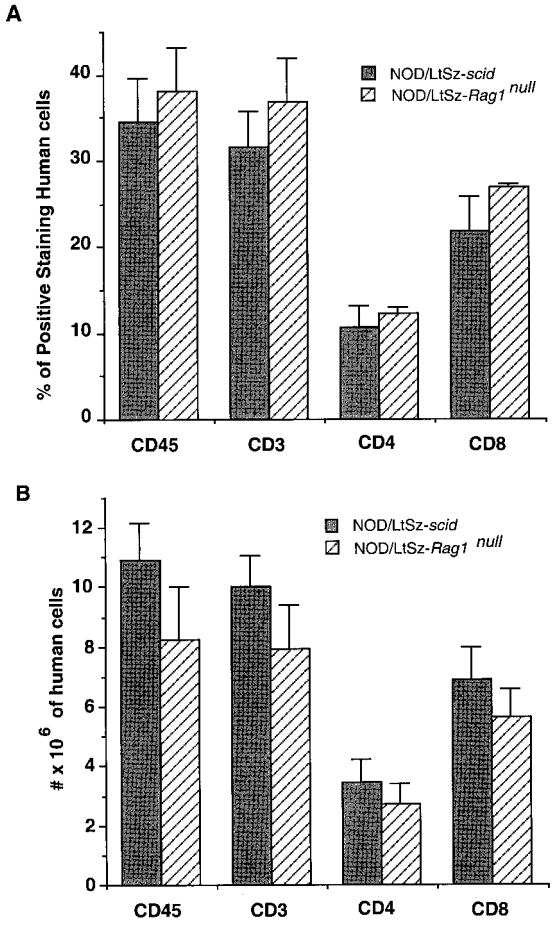

FIGURE 6. Flow cytometry analysis of spleen cells from mice engrafted with human PBMCs at 4 wk postengraftment. Cells were stained with mouse anti-human mAbs. Shown are representative data from one of six human engraftment experiments. *A*, Percentage of positive staining human cells in the spleen. *B*, Number of positive staining human cells ($\times 10^6$) in the spleen.

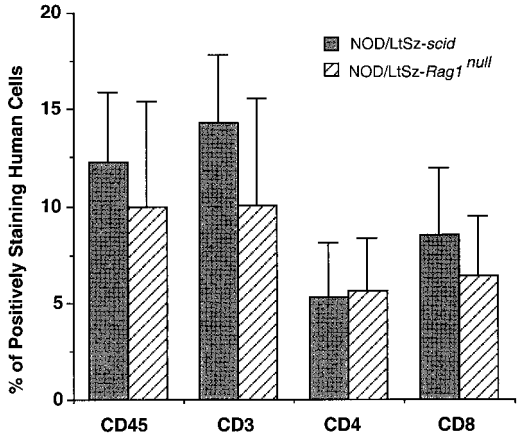

FIGURE 7. Flow cytometry analysis of peripheral blood leukocytes from mice engrafted with human PBMCs at 4 wk postengraftment. Cells were stained with mouse anti-human mAbs. Shown are representative data from one of six human engraftment experiments. Data are expressed as percentage of positive staining human cells in the peripheral blood.

in the peripheral blood of engrafted NOD/LtSz-*Rag1*^{null} mice compared with the peripheral blood of engrafted NOD/LtSz-*scid* mice (Figs. 6 and 7).

Human Ig levels in sera of PBMC-engrafted mice

Levels of human IgG and IgM were determined in the sera of eight NOD/LtSz-*Rag1*^{null} and seven NOD/LtSz-*scid* recipients of human PBMC 4 wk after engraftment. There was no significant difference in human IgG levels between NOD/LtSz-*Rag1*^{null} mice (1428 $\pm$ 108 μg/ml) and NOD/LtSz-*scid* mice (1012 $\pm$ 210 μg/ml; $p > 0.09$) Likewise, there was no significant difference in human IgM levels in sera from NOD/LtSz-*Rag1*^{null} mice (320 $\pm$ 73 μg/ml) as compared with serum from NOD/LtSz-*scid* mice (158 $\pm$ 39 μg/ml; $p > 0.06$).

HIV-1 infection of human PBMC-engrafted NOD/LtSz-Rag1^{null} *mice*

We next determined the susceptibility of human cells engrafted in NOD/LtSz-*Rag1*^{null} mice to infection with HIV-1. There was no significant difference between the frequency of HIV-1 infection in NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice. Decreased percentages of human cells, particularly $CD3^+$ and $CD4^+$ T cells, were apparent at 8 wk after HIV-1 infection in both groups of mice (Fig. 8*A*). The proportion of mice with evidence of HIV-1 viral RNA from plasma or viral DNA from blood or spleen cells did not differ significantly between the two groups of mice at any time point (Fig. 8, *B* and *C*). Virus levels were highest at 8 wk after infection, a time point corresponding with the loss of the human cells from the peripheral circulation. Quantitative recovery of HIV-1 from spleen cells was also highest at 8 wk after infection, and was not significantly different between NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* mice (Fig. 8*D*).

Engraftment of human umbilical cord blood cells

NOD/LtSz-*Rag1*^{null} mice were irradiated with varying doses of irradiation (750–850 rad) and injected i.v. with unfractionated cord blood ($1–10 \times 10^6$) cells or with $CD34^+$ cord blood (0.2×10^6) cells. As shown in Fig. 9, injection of 10×10^6 cord blood mononuclear cells resulted in moderate levels of engraftment in the bone marrow (0.5–12.8% human cells). Similarly, injection of enriched $CD34^+$ cells resulted in 0.6 to 6.9% of human cell engraftment in the bone marrow of NOD/LtSz-*Rag1*^{null} mice. Multilineage hemopoiesis, including lymphoid $CD45^+$ $CD19^+$ pre-B cells (Fig. 9, *B* and *C*) and myeloid as well as erythroid CFU (data not shown), demonstrates that these mice were engrafted with primitive human SCID-repopulating cells (SRC). SRC are primitive $CD34^+/CXCR4^{-/low}$ $CXCR4^+$ stem cells that are capable of homing to the bone marrow of immunodeficient mice and repopulating it with multilineage human lymphoid and myeloid progenitor cells (18).

Adoptive transfer of diabetes to NOD/LtSz-Rag1^{null} *mice following injection with diabetogenic NOD spleen cells*

NOD/LtSz-*Rag1*^{null} mice do not spontaneously develop diabetes and have normal islets with no evidence of mononuclear cell infiltration throughout their life span (Fig. 10*A*). In contrast, insulitis is prevalent in islets from 3–4-mo-old NOD/Lt +/+ mice (Fig. 10*B*). In two independent experiments, seven NOD/LtSz-*Rag1*^{null} and four NOD/LtSz-*scid* females were injected i.v. with 2×10^7 spleen cells from diabetic NOD/Lt +/+ female donors. All of the recipients developed diabetes 1–2 mo later. There was no significant difference in the time of onset or in the levels of hyperglycemia (734 $\pm$ 53 mg/dl in NOD/LtSz-*Rag1*^{null} vs 597 $\pm$ 51 mg/dl in NOD/LtSz-*scid* mice). Representative islets of diabetic NOD/LtSz-*Rag1*^{null} and NOD/LtSz-*scid* adoptive recipients are shown in Fig. 9, *C* and *D*.

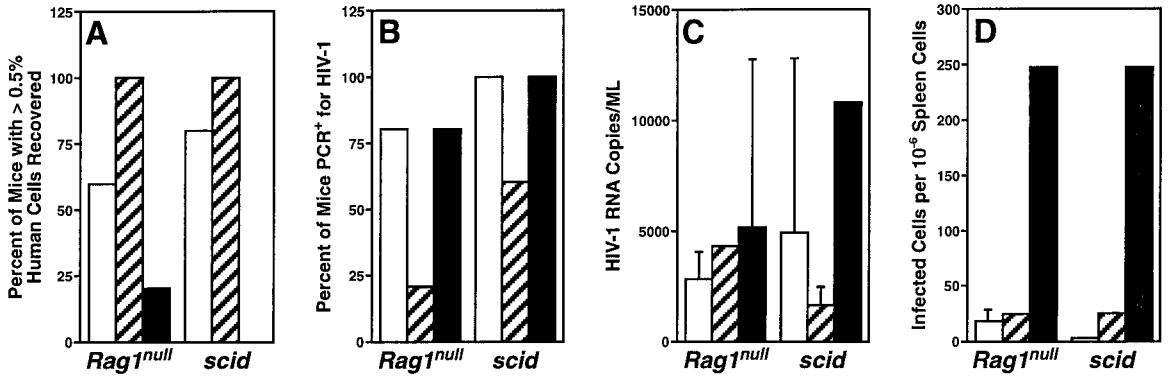

FIGURE 8. Recovery of human cells and HIV-1 from NOD/LtSz-*Rag1*^{null} and NOD/LtSz-scid mice engrafted with human PBMC. Mice were engrafted i.p. with 2×10^7 human PBMC from a single donor and infected i.p. 3 wk later with 100 AID_{50} of HIV-1. Mice were killed 3 (□), 4 (▨), and 8 (■) wk after HIV-1 infection for flow cytometry and virus recovery from spleen and blood. Data represent five mice per group. *A*, Percentage of mice in each group that had recoverable human $CD3^+$ T cells (>0.5% of total cells examined). *B*, Percentage of mice positive for HIV-1 by PCR of plasma, blood cells, or spleen cells. *C*, Viral load in mice shown as numbers of copies of HIV-1 RNA per ml of plasma. *D*, Quantitation of recovered HIV-1 DNA from the spleens of mice. Virus recovery is shown as the inverse of the cell number required for detection of viral DNA.

Discussion

In the present study, we have generated NOD/LtSz-*Rag1*^{null} mice to test the hypothesis that strain background-modifying genes are critical in determining the ability of immunodeficient *scid* or *Rag1*^{null} mice to support human hematolymphoid cell engraftment. Mice homozygous for the *scid* mutation have been used to develop several important models for the study of human hematolymphoid cells (3–5). The Hu-PBL-SCID model was established by i.p. injection of PBMC into *scid* recipients (3). The Hu-SRC-SCID model was established by i.v. injection of hemopoietic stem cells into irradiated *scid* recipients (4). In both models, NOD/LtSz-*scid* mice supported higher levels of human hematolymphoid cell engraftment than C.B-17-*scid* (1, 9). We now show that NOD/LtSz-*Rag1*^{null} mice, similar to NOD/LtSz-*scid* mice, function as excellent recipients of human PBMC and hemopoietic stem cells.

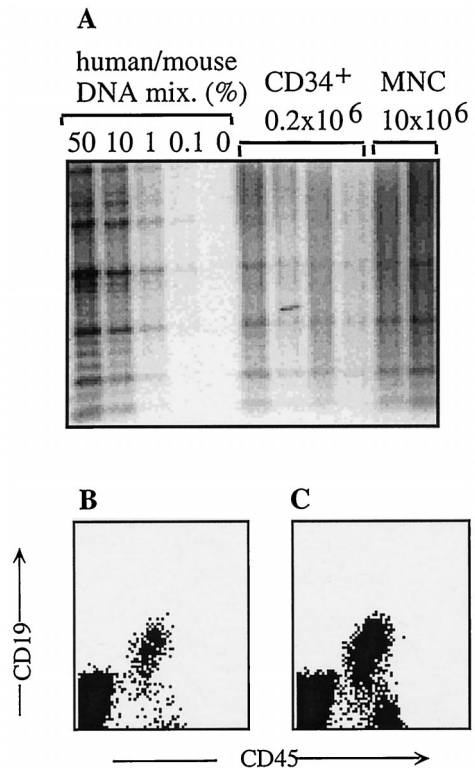

FIGURE 9. Quantitative analyses of human hemopoietic stem cells after engraftment of NOD/LtSz-*Rag1*^{null} mice with human umbilical cord blood cells. *A*, Representative Southern blot of bone marrow DNA from individual mice transplanted with 0.2×10^6 $CD34^+$ human cord blood cells or with 10×10^6 unfractionated mononuclear cord blood cells (MNC). DNA was extracted from the bone marrow of engrafted mice 1 mo posttransplantation and hybridized with a human chromosome 17-specific α-satellite probe. The level of human engraftment was determined by comparing the bands with those of human/mouse DNA mixtures. The marrow of six individual mice transplanted with $CD34^+$ cord blood cells or unfractionated MNC is shown. *B*, Flow cytometry of bone marrow of a mouse engrafted with $CD34^+$ cells. *C*, Flow cytometry of bone marrow of a mouse engrafted with unfractionated cord blood cells.

Previous attempts to utilize *Rag2*^{null} mice as hosts for human hematolymphoid engraftment have been unsuccessful (22, 23). The *Rag2*^{null} allele was on a segregating strain background in those studies. The poor engraftment of these *Rag2*^{null} mice maintained on segregating backgrounds may have been due to effects of background-modifying genes on levels of innate immunity. Our previous studies have demonstrated significant strain background effects in the ability of *scid* mice to support human hematolymphoid engraftment (7, 8, 11, 12). *Rag1*^{null} mice on segregating backgrounds express elevated NK cell activity (L. D. S., unpublished observations). The heightened NK cell activity in C57BL/6-*Rag1*^{null} mice as compared with NOD/LtSz-*Rag1*^{null} mice confirms the critical effects of background-modifying genes on NK cell development. We observed that NOD/LtSz-*Rag1*^{null} mice express these defects in innate immunity associated with NOD/Lt background-modifying genes and support levels of human lymphoid cell engraftment that are comparable with those observed in NOD/LtSz-*scid* mice. These observations support our overall hypothesis that manipulation of the host strain background will enhance human hematolymphoid cell engraftment in *scid* and *Rag1*^{null} recipients of human hematolymphoid cells.

Development of NOD/LtSz-*Rag1*^{null} mice now provides an immunodeficient model that circumvents the generation of low levels of functional T and B lymphyocytes (leakiness) observed in occasional NOD-*scid* mice as they age (7). Both *Rag1* and *Rag2* gene expression are required for development of functional lymphoid cells (20, 21, 39). *Rag1*^{null} or *Rag2*^{null} mice cannot express and receive signals through the pre-TCR (20, 21). As a result, thymocyte development in these mice terminates at the $CD4^-/8^-$, IL-$2R\alpha^+$ stage and precludes any potential development of mature T cells due to their inability to initiate V(D)J recombination (40).

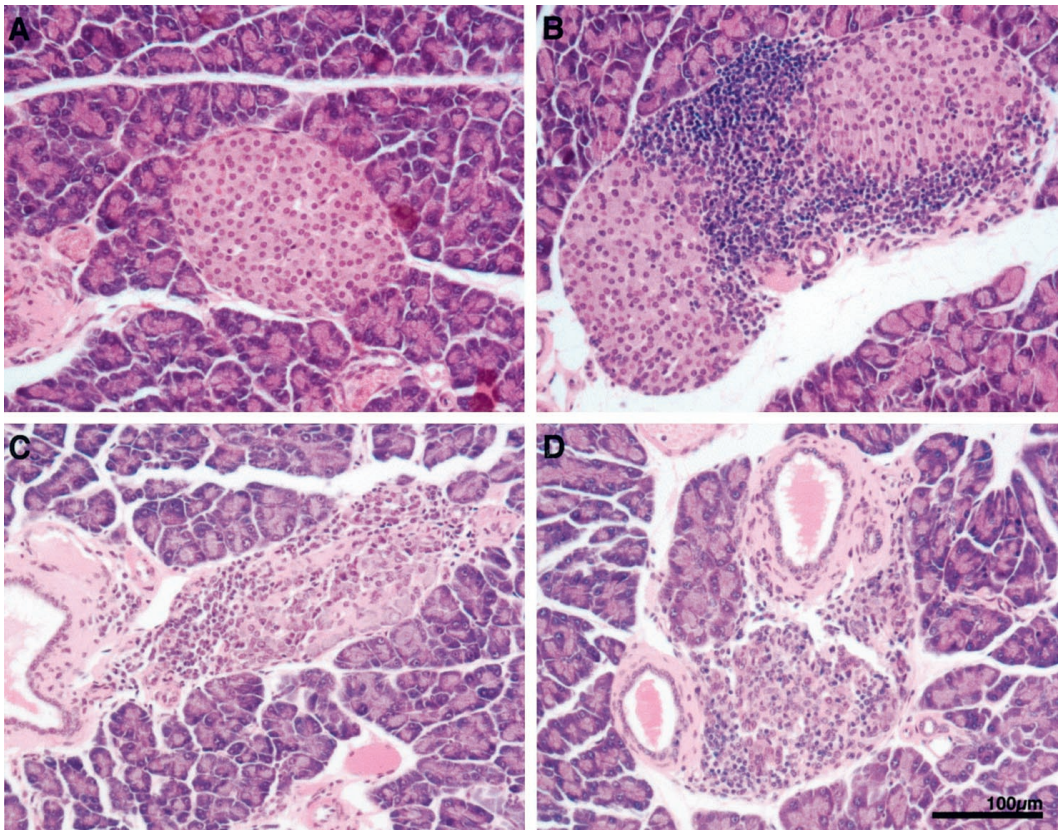

FIGURE 10. Pancreatic islets. *A*, Lack of inflammatory cells in islets from an 8-wk-old NOD/LtSz-*Rag1*^{null} female. *B*, Marked accumulation of lymphocytes in an islet from a 8-wk-old NOD/Lt +/+ female. *C* and *D*, End stage insulitis in an islet from an 11-wk-old NOD/LtSz-*Rag1*^{null} female (*C*) and an 11-wk-old NOD/LtSz-*scid* female (*D*) 4 wk following injection with 2×10^7 spleen cells from a NOD/Lt +/+ female diabetic donor (H&E).

Likewise, neither *Rag1*^{null} nor *Rag2*^{null} mice can produce functional B cells. As expected, based on the effects of this mutation on lymphocyte development, no serum Ig or TCR$^+$ cells were detected in aged NOD/LtSz-*Rag1*^{null} mice. Increased sensitivity to γ-irradiation is also characteristic of *scid* mice. The wild-type allele at the *Prkdc*^{scid} locus has been shown to function as a DNA repair gene, and the *scid* mutation confers a marked defect in ability to repair dsDNA damage (24, 25, 41–43). Our studies demonstrate that irradiated NOD/LtSz-*Rag1*^{null} mice provide an excellent environment for human stem cell engraftment and permit longer-term studies compared with NOD/LtSz-*scid* mice.

Because NOD/LtSz-*Rag1*^{null} mice survive much higher doses of irradiation compared with NOD/LtSz-*scid* mice, they might be particularly useful for studies of immune responses. Previous investigations found that irradiation of C.B-17-*scid* mice before engraftment with human PBMC was required to generate a primary human immune response (44). Although immunization of these mice led to specific Ab production by 16 to 18 days, the mice could not repair the irradiation-induced DNA damage and invariably died by 4 wk after engraftment. Because NOD/LtSz-*Rag1*^{null} mice are quite radioresistant, as are standard NOD mice, and engraft at high levels with human PBMC, experiments are underway in our laboratories to determine whether irradiated NOD/LtSz-*Rag1*^{null} mice that are engrafted with human PBMC are able to generate a primary immune response.

As observed in NOD/LtSz-*scid* mice (7), NOD/LtSz-*Rag1*^{null} mice have low levels of NK cell activity. The deficit in NK cell activity is an effect of NOD/Lt background genes. NK cells are known to be primary mediators of hematolymphoid graft rejection (45, 46), and as predicted, NK cell activity appears to correlate inversely with levels of engraftment in NOD/LtSz-*scid* (7, 11, 12) and NOD/LtSz-*Rag1*^{null} mice. Of particular interest was our unexpected observation that NOD/LtSz-*Rag1*^{null} mice develop spontaneous lymphomas. NOD-*scid* mice develop thymic lymphomas at high rates as they age, resulting in a short life span (7, 19). The development of thymic lymphomas is accelerated in NOD/LtSz-*scid B2m*^{null} mice, presumably due to their extremely low NK cell activity (11). The lymphomas that develop at later time points in NOD/LtSz-*Rag1*^{null} mice include FCC pre-B cell lymphomas that metastasize to the spinal cord as well as thymic lymphomas. These FCC lymphomas express the B220 B cell marker and are CD4 and CD8 negative (L. D. S., unpublished). The tissue site and metastasis of the FCC tumors were different from that observed with thymic lymphomas, as metastases of the FCC lymphomas appeared to target preferentially the spinal cord. Xenotransplantation of a human Burkitt lymphoma (Daudi) into C.B-17-*scid* mice was reported to result in metastasis of this B cell tumor to the spinal cord resulting in hind limb paralysis (47) similar to our observation of pre-B cell lymphomas in NOD/LtSz-*Rag1*^{null} mice. In a stock of NOD/LtSz-*scid* mice in which the *emv30* proviral gene was removed, development of thymic lymphomas was retarded, but not eliminated (48). The genetic basis for the development and metastasis patterns of FCC lymphomas in NOD/LtSz-*Rag1*^{null} mice is unknown.

Based on the relative radioresistance, absence of any leakiness with age, and the longer life span, it was important to assess the functional characteristics of NOD/LtSz-*Rag1*^{null} mice as recipients of human PBMC and hemopoietic progenitor cells. The human cell engraftment levels obtained in NOD/LtSz-*Rag1*^{null} mice were comparable with those in NOD/LtSz-*scid* mice using the same pool of PBMC. Moreover, the ability to infect the engrafted human PBMC with HIV-1 was similar in NOD/LtSz-*Rag1*^{null} and NOD/

LtSz-*scid* mice. Viral RNA and DNA can be readily recovered from infected NOD/LtSz-*Rag1*^{null} mice, and numbers of engrafted human cells decrease in infected mice at the same rate as seen in infected NOD/LtSz-*scid* mice.

Because NOD/LtSz-*Rag1*^{null} mice do not develop leakiness with age and have a decreased incidence of thymic lymphomas, they may have more utility for long-term HIV-1 infection studies than NOD/LtSz-*scid* mice. It is interesting that the NOD-*Rag2*^{null} strain of mice was first reported in 1996 (49), but demonstration of high levels of human PBMC engraftment in these mice has not been reported. This may be due to the observation that NOD mice from different colonies have highly variable levels of NK cell activity (50) and differ significantly in their expression of autoimmune diabetes (51). It will be important to determine whether the previously described NOD-*Rag2*^{null} strain of mice has low levels of NK cell activity or supports human PBMC engraftment at levels comparable with the NOD/LtSz-*Rag1*^{null} strain of mice described in this study.

It has been also reported that spleen cells from diabetic NOD mice adoptively transferred diabetes to the NOD-*Rag2*^{null} strain of mice (49), and we have now confirmed this characteristic in our NOD/LtSz-*Rag1*^{null} strain of mice. This recipient, due to its total inability to functionally rearrange T and B cell receptors and generate mature lymphocytes, is now the strain of choice for these adoptive transfer studies. It has also been reported (52, 53) that environmental perturbation of *scid* mice will enhance the expression of leakiness, making discrimination between the endogenous and adoptively transferred cells in this system difficult. Although NOD/Lt-*Thy-1*^{a} congenic mice have been utilized as a source of diabetogenic donor T cells to address this issue (38), the NOD/LtSz-*Rag1*^{null} strain of mice now eliminates this confounding variable, particularly in the B lymphocyte lineage that could not be discriminated based on donor vs host Thy-1 allele. Discriminating between donor and host B cells has become increasingly important due to the recently recognized role of B lymphocytes in diabetes pathogenesis (54, 55).

Based on the data reported in this study and previously published data, we are currently developing a number of additional NOD/LtSz-*Rag1*^{null} strains of mice to improve further human PBMC engraftment and function in immunodeficient hosts. For example, the CD4:CD8 ratio of human T cells following PBMC engraftment in NOD/LtSz-*Rag1*^{null} mice is ~0.5:1, whereas in NOD/LtSz-*scid B2m*^{null} mice that ratio is ~2:1, a ratio more comparable with that observed in human peripheral blood (11). To determine whether this is due solely to the *B2m*^{null} allele in NOD-*scid* mice, we are currently backcrossing the *B2m*^{null} allele onto the NOD/LtSz-*Rag1*^{null} genetic background.

In summary, the present study describes the development and characterization of a new immunodeficient host for human hematolymphoid cell engraftment, HIV-1 studies, and for analysis of NOD diabetes pathogenesis. The data further support our hypothesis that manipulation of the background of the immunodeficient host will permit improvement and utility of this model.

Acknowledgments

We thank David Serreze and Vitaly Ablamunits for critically reading the manuscript. We thank Priscilla Jewett, Greg Martin, and Isabelle Schweitzer for expert technical assistance. We are grateful to Rod Bronson and John Sundberg for helpful advice on histopathology.

References

1. Hesselton, R. M., D. L. Greiner, J. P. Mordes, T. V. Rajan, J. L. Sullivan, and L. D. Shultz. 1995. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to HIV-1 infection in NOD/LtSz-*scid/scid* mice. *J. Infect. Dis. 172:774.*
2. McCune, J., H. Kaneshima, J. Krowka, R. Namikawa, H. Outzen, B. Peault, L. Rabin, C. Shih, E. Lee, M. Lieberman, et al. 1991. The SCID-hu mouse: a small animal model for HIV infection and pathogenesis. *Annu. Rev. Immunol. 9:399.*
3. Mosier, D. E., R. J. Gulizia, S. M. Baird, and D. B. Wilson. 1988. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature 335:256.*
4. Lapidot, T., F. Pflumio, M. Deodens, B. Murdoch, D. E. Williams, and J. E. Dick. 1992. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. *Science 255:1137.*
5. McCune, J. M., R. Namikawa, H. Kaneshima, L. D. Shultz, M. Lieberman, and I. L. Weissman. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science 241:1632.*
6. Greiner, D. L., and L. D. Shultz. 1998. The use of NOD/LtSz-*scid/scid* mice in biomedical research. In *NOD Mice and Related Strains: Research Applications in Diabetes, AIDS, Cancer and Other Diseases*. E. Leiter and M. Atkinson, eds. Landes Bioscience, Austin. *173–203.*
7. Shultz, L. D., P. A. Schweitzer, S. W. Christianson, B. Gott, I. B. Schweitzer, B. Tennent, S. McKenna, L. Mobraaten, T. V. Rajan, D. L. Greiner, et al. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J. Immunol. 154:180.*
8. Greiner, D. L., L. D. Shultz, M. C. Appel, G. Perdizet, R. M. Hesselton, I. Schweitzer, W. G. Beamer, K. L. Shultz, S. C. Pelsue, J. H. Leif, and T. V. Rajan. 1995. Improved engraftment of human spleen cells in NODSz-scid/scid mice as compared with C.B-17-scid/scid mice. *Am. J. Pathol. 146:888.*
9. Greiner, D. L., R. A. Hesselton, and L. D. Shultz. 1998. SCID mouse models of human stem cell engraftment. *Stem Cells 16:166.*
10. Lowry, P. A., L. D. Shultz, D. L. Greiner, R. M. Hesselton, E. L. Kittler, C. Y. Tiarks, S. S. Rao, J. Reilly, J. H. Leif, H. Ramshaw, et al. 1996. Improved engraftment of human cord blood stem cells in NOD/LtSz-scid/scid mice after irradiation or multiple-day injections into unirradiated recipients. *Biol. Blood Marrow Transplant. 2:15.*
11. Christianson, S. W., D. L. Greiner, R. A. Hesselton, J. H. Leif, E. J. Wagar, I. B. Schweitzer, T. V. Rajan, B. Gott, D. C. Roopenian, and L. D. Shultz. 1997. Enhanced human CD4^{+} T cell engraftment in β_2-microglobulin-deficient NOD-scid mice. *J. Immunol. 158:3578.*
12. Christianson, S. W., D. L. Greiner, I. B. Schweitzer, B. Gott, G. L. Beamer, P. A. Schweitzer, R. A. Hesselton, and L. D. Shultz. 1996. Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-*scid* mice and in C57BL/6J-*scid bg* mice. *Cell. Immunol. 171:186.*
13. Larochelle, A., J. Vormoor, T. Lapidot, G. Sher, T. Furukawa, Q. Li, L. D. Shultz, N. F. Olivieri, G. Stamatoyannopoulos, and J. E. Dick. 1995. Engraftment of immune-deficient mice with primitive hematopoietic cells from β-thalassemia and sickle cell anemia patients: implications for evaluating human gene therapy protocols. *Hum. Mol. Genet. 4:163.*
14. Pflumio, F., B. Izac, A. Katz, L. D. Shultz, W. Vainchenker, and L. Coulombel. 1996. Phenotype and function of human hematopoietic cells engrafting immune-deficient CB17-severe combined immunodeficiency mice and nonobese diabetic-severe combined immunodeficiency mice after transplantation of human cord blood mononuclear cells. *Blood 88:3731.*
15. Hogan, C. J., E. J. Shpall, O. McNulty, I. McNiece, J. E. Dick, L. D. Shultz, and G. Keller. 1997. Engraftment and development of human CD34^{+}-enriched cells from umbilical cord blood in NOD/LtSz-scid/scid mice. *Blood 90:85.*
16. Cashman, J. D., T. Lapidot, J. C. Wang, M. Doedens, L. D. Shultz, P. Lansdorp, J. E. Dick, and C. J. Eaves. 1997. Kinetic evidence of the regeneration of multilineage hematopoiesis from primitive cells in normal human bone marrow transplanted into immunodeficient mice. *Blood 89:4307.*
17. Ramirez, M., G. A. Rottman, L. D. Shultz, and C. I. Civin. 1998. Mature human hematopoietic cells in donor bone marrow complicate interpretation of stem/progenitor cell assays in xenogeneic hematopoietic chimeras. *Exp. Hematol. 26:332.*
18. Peled, A., I. Petit, O. Kollet, M. Magid, T. Ponomaryov, T. Byk, A. Nagler, H. Ben-Hur, A. Many, L. Shultz, et al. 1999. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science 283:845.*
19. Prochazka, M., H. R. Gaskins, L. D. Shultz, and E. H. Leiter. 1992. The NOD-*scid* mouse: a model for spontaneous thymomagenesis associated with immunodeficiency. *Proc. Natl. Acad. Sci. USA 89:3290.*
20. Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell 68:869.*
21. Shinkai, Y., G. Rathbun, K. P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell 68:855.*
22. Martin, A., M. Valentine, P. Unger, S. W. Yeung, L. D. Shultz, and T. F. Davies. 1994. Engraftment of human lymphocytes and thyroid tissue into scid and rag2-deficient mice: absent progression of lymphocytic infiltration. *J. Clin. Endocrinol. Metab. 79:716.*
23. Steinsvik, T. E., P. I. Gaarder, I. S. Aaberge, and M. Lovik. 1995. Engraftment and humoral immunity in SCID and RAG-2-deficient mice transplanted with human peripheral blood lymphocytes. *Scand. J. Immunol. 42:607.*
24. Disney, J. E., A. L. Barth, and L. D. Shultz. 1992. Defective repair of radiation-induced chromosomal damage in scid/scid mice. *Cytogenet. Cell Genet. 59:39.*

25. Fulop, G. M., and R. A. Phillips. 1990. The scid mutation in mice causes a general defect in DNA repair. *Nature 347:479.*
26. Biedermann, K. A., J. R. Sun, A. J. Giaccia, L. M. Tosto, and J. M. Brown. 1991. Scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA 88:1394.*
27. Hendrickson, E. A., X. Q. Qin, E. A. Bump, D. G. Schatz, M. Oettinger, and D. T. Weaver. 1991. A link between double-strand break-related repair and V(D)J recombination: the scid mutation. *Proc. Natl. Acad. Sci. USA 88:4061.*
28. Noben-Trauth, N., L. D. Shultz, F. Brombacher, J. Urban, H. Gu, and W. E. Paul. 1997. An IL-4-independent pathway for $CD4^+$ T cell IL-4 production is revealed in interleukin-4 receptor deficient mice. *Proc. Natl. Acad. Sci. USA 90:10838.*
29. Ledbetter, J., and L. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev. 47:63.*
30. Coffman, R. L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol. Rev. 69:5.*
31. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens. III. Hybridona antibodies reacting to antigens of the $H\text{-}2^b$ haplotype reveal genetic control of isotope expression. *J. Immunol. 126:317.*
32. Oi, V. T., P. P. Jones, J. W. Goding, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol. 81:115.*
33. Lillie, R. D. 1954. *Histopathology Technique and Practical Histochemistry*. Blakiston Press, New York.
34. Ikuta, K., T. Kina, I. MacNeil, N. Uchida, B. Peault, Y. H. Chien, and I. L. Weissman. 1990. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell 62:863.*
35. Kollet, O., R. Aviram, J. Chebath, H. ben-Hur, A. Nagler, L. Shultz, M. Revel, and T. Lapidot. 1999. The soluble interleukin-6 (IL-6) receptor/IL-6 fusion protein enhances in vitro maintenance and proliferation of human $CD34^{(+)}CD38^{(-/low)}$ cells capable of repopulating severe combined immunodeficiency mice. *Blood 94:923.*
36. Hesselton, R. M., R. A. Koup, M. A. Cromwell, B. S. Graham, M. Johns, and J. L. Sullivan. 1993. Human peripheral blood xenografts in the SCID mouse: characterization of immunological reconstitution. *J. Infect. Dis. 168:630.*
37. Koup, R. A., R. M. Hesselton, J. T. Safrit, M. Somasundaran, and J. L. Sullivan. 1994. Quantitative assessment of human immunodeficiency virus type 1 replication in human xenografts of acutely infected Hu-PBL-SCID mice. *AIDS Res. Hum. Retroviruses 10:279.*
38. Christianson, S. W., L. D. Shultz, and E. H. Leiter. 1993. Adoptive transfer of diabetes into immunodeficient NOD-*scid*/*scid* mice: relative contributions of $CD4^+$ and $CD8^+$ T lymphocytes from diabetic versus prediabetic NOD.NON-$Thy\text{-}1^a$ donors. *Diabetes 42:44.*
39. Oettinger, M. A., D. G. Schatz, C. Gorka, and D. Baltimore. 1990. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science 248:1517.*
40. Willerford, D. M., W. Swat, and F. W. Alt. 1996. Developmental regulation of V(D)J recombination and lymphocyte differentiation. *Curr. Opin. Genet. Dev. 6:603.*
41. Kirchgessner, C. U., C. K. Patil, J. W. Evans, C. A. Cuomo, L. M. Fried, T. Carter, M. A. Oettinger, and J. M. Brown. 1995. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science 267:1178.*
42. Jeggo, P. A., S. P. Jackson, and G. E. Taccioli. 1996. Identification of the catalytic subunit of DNA dependent protein kinase as the product of the mouse scid gene. *Curr. Top. Microbiol. Immunol. 217:79.*
43. Miller, R. D., J. Hogg, J. H. Ozaki, D. Gell, S. P. Jackson, and R. Riblet. 1995. Gene for the catalytic subunit of mouse DNA-dependent protein kinase maps to the scid locus. *Proc. Natl. Acad. Sci. USA 92:10792.*
44. Sandhu, J., B. Shpitz, S. Gallinger, and N. Hozumi. 1994. Human primary immune response in SCID mice engrafted with human peripheral blood lymphocytes. *J. Immunol. 152:3806.*
45. Murphy, W. J., V. Kumar, and M. Bennett. 1989. Immunobiology of bone marrow transplantation: studies using scid mice. *Curr. Top. Microbiol. Immunol. 152:251.*
46. Murphy, W. J., V. Kumar, J. C. Cope, and M. Bennett. 1990. An absence of T cells in murine bone marrow allografts leads to an increased susceptibility to rejection by natural killer cells and T cells. *J. Immunol. 144:3305.*
47. Ghetie, M. A., J. Richardson, T. Tucker, D. Jones, J. W. Uhr, and E. S. Vitetta. 1990. Disseminated or localized growth of a human B-cell tumor (Daudi) in SCID mice. *Int. J. Cancer 45:481.*
48. Serreze, D. V., E. H. Leiter, M. W. Hanson, S. W. Christianson, L. D. Shultz, R. M. Hesselton, and D. L. Greiner. 1995. *Emv30^{null}* NOD-*scid* mice: an improved host for adoptive transfer of autoimmune diabetes and growth of human lymphohematopoietic cells. *Diabetes 44:1392.*
49. Soderstrom, I., M. L. Bergman, F. Colucci, K. Lejon, I. Bergqvist, and D. Holmberg. 1996. Establishment and characterization of RAG-2 deficient non-obese diabetic mice. *Scand. J. Immunol. 43:525.*
50. Koyanagi, Y., Y. Tanaka, R. Tanaka, N. Misawa, Y. Kawano, T. Tanaka, M. Miyasaka, M. Ito, Y. Ueyama, and N. Yamamoto. 1997. High levels of viremia in hu-PBL-NOD-scid mice with HIV-1 infection. *Leukemia 3:109.*
51. Leiter, E. H. 1998. NOD mice and related strains: origin, husbandry, and biology. In *NOD Mice and Related Strains: Research Applications in Diabetes, AIDS, and Cancer*. E. H. Leiter and M. A. Atkinson, eds. Landes, Austin. pp. 1–3S.
52. Bosma, M. J., and A. M. Carroll. 1991. The *scid* mouse mutant: definition, characterization, and potential uses. *Annu. Rev. Immunol. 9:323.*
53. Bosma, G. C., M. Fried, R. P. Custer, A. Carroll, D. M. Gibson, and M. J. Bosma. 1988. Evidence of functional lymphocytes in some (leaky) *scid* mice. *J. Exp. Med. 167:1016.*
54. Serreze, D. V., S. A. Fleming, D. S. Chapman, S. D. Richard, E. H. Leiter, and R. M. Tisch. 1998. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated diabetes in nonobese diabetic mice. *J. Immunol. 161:3912.*
55. Serreze, D. V., H. D. Chapman, D. S. Varnum, M. S. Hanson, P. C. Reifsnyder, S. D. Richard, S. A. Fleming, E. H. Leiter, and L. D. Shultz. 1996. B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu *null* mice. *J. Exp. Med. 184:2049.*