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Research Articles

The SCID-hu Mouse: Murine Model for the Analysis of Human Hematolymphoid Differentiation and Function

J. M. McCUNE, R. NAMIKAWA, H. KANESHIMA, L. D. SHULTZ, M. LIEBERMAN, I. L. WEISSMAN

The study of human hematopoietic cells and the human immune system is hampered by the lack of a suitable experimental model. Experimental data are presented showing that human fetal liver hematopoietic cells, human fetal thymus, and human fetal lymph node support the differentiation of mature human T cells and B cells after engraftment into mice with genetically determined severe combined immunodeficiency. The resultant SCID-hu mice are found to have a transient wave of human CD4$^+$ and CD8$^+$ T cells and human IgG (immunoglobulin G) in the peripheral circulation. The functional status of the human immune system within this mouse model is not yet known.

TO APPROXIMATE THE EVALUATION OF DISEASE STATES IN man, biomedical research has relied heavily upon animal models. Of these, experiments with the laboratory mouse have contributed much to our understanding of the immune system, the cells involved, the products that they express, and their differentiation pathways. After immunization in vivo, murine splenic B cells can be immortalized as hybridoma lines making monoclonal antibodies (*1*), and functional, antigen-reactive murine T cells can easily be cloned (*2*). After adoptive transfer into lethally irradiated hosts, the murine pluripotent hematopoietic stem cell can be identified, purified, and studied (*3, 4*). These are important findings. They are not, however, easily or directly applicable to man; except in rare circumstances, humans cannot be subjected to experimental immunizations or to lethal irradiation and could not provide internal lymphoid organs.

The emergence of the acquired immunodeficiency syndrome (AIDS) underscores the need for direct and comprehensive analysis of the human immune system (*5*). With this epidemic, as well as with those associated with other human lymphotropic retroviruses, little is known about the course of infection in vivo. In the absence of a testable model, the accumulation of knowledge may be slow. Clinical trials represent the only available means of evaluating therapeutic or prophylactic modalities. Although similar animal retroviruses are associated with immunodeficiency states, in no case is the virus identical to human immunodeficiency virus or the disease identical to AIDS. Indeed, if any pertinent knowledge has been gained from the study of animal retroviruses, it is that retroviruses affecting man are best (and perhaps only) studied in the context of human, and not animal cells.

We have taken an alternative approach, one that might create an animal model precisely for the study of the human immune system, its physiology, and its pathophysiology. We now present a method by which the human T and B cell lineages and their hematopoietic precursors can be obtained, transplanted, and observed to differentiate within a mouse.

Several key concepts of immunology guide these experiments.

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First, during fetal life, tolerance to "self" is governed not only by genetic constraints, but also by the environment in which the developing immune system resides. In the experiments of Billingham, Brent, and Medawar (*6*), the introduction of A strain tissue into the developing embryos of CBA mice permitted the resultant offspring to accept A as well as CBA strain skin grafts. Second, self-nonself education of T cells occurs primarily in the thymus. Thus, the development of tolerance and major histocompatibility complex (MHC) restriction in the T cell lineage is dictated primarily by the thymic microenvironment in which T stem cells initially differentiate (*7, 8*). Finally, after T cells are mature, self-recognition is fixed; MHC-disparate cells are rejected.

We reasoned that human T cell differentiation might proceed in a physiologic fashion, provided that human hematopoietic stem cells and human thymus were introduced together into a mouse which itself was unable to reject them. We therefore sought to use a mouse stock that expresses a specific defect in lymphocyte maturation so that the absence of murine T and B cells might facilitate the engraftment of human hematopoietic and lymphoid tissues.

C.B-17 *scid/scid* mice (congenic partners of BALB/cAn) lack functional T cells and B cells (*9–11*). Homozygotes (hereafter designated as SCID mice) have severe combined immunodeficiency, an inability to mount an effective cellular or humoral immune response to foreign antigens. Usually, these mice die of opportunistic infection at an early age; often, the causative agent is *Pneumocystis carinii*. It is thought that the inherent deficiency is related to a defective recombinase function; that is, antigen receptor genes in differentiating T and B cells are not productively rearranged (*12*). SCID mice are otherwise replete with all other hematopoietic lineages, and in fact show a relative increase in the granulocytic series (*13, 14*). Their hematopoietic microenvironment, including the thymic stroma, is intact. When mice are engrafted with syngeneic bone marrow cells, reconstitution with functional T cells and B cells can occur. The absence of bone marrow engraftment results in acceptance of MHC-disparate allogeneic skin grafts. Indeed, xenogeneic hybridoma cells grow well without rejection when placed intraperitoneally within these mice (*15*).

In order to reconstitute SCID mice with human T cells, a source of human hematopoietic progenitors of thymocytes is required. From studies in murine and human systems, it is clear that such cells may be found in adult bone marrow. Careful subfractionation yielded 0.05 percent of murine bone marrow cells that represented a source of pluripotent hematopoietic stem cells. When these cells are given intravenously, 20 to 30 of them can reconstitute 50 percent of lethally irradiated mice (*4*). In humans, donor bone marrow depleted of mature T cells may be used to reconstitute immunodeficient patients; in the absence of such depletion, the donor T cells are responsible for a graft-versus-host immune reaction. An alternative source of hematopoietic stem cells is the human fetal liver. This organ, the major site of hematopoiesis in humans between 8 to 24 weeks of gestation, contains progenitors for all hematolymphoid progenitors, including the erythroid, the myelomonocytic, and the lymphoid lineages (*16*). Most, if not all, of the fetal liver T cell progenitors have not yet migrated to and through the human fetal thymus. Accordingly, they are not committed to a given pattern of self-nonself recognition and have proved to be a reliable source of cells for engraftment, even without the preliminary elimination of mature T cells (*17*).

Here, we describe the hematochimeric SCID-hu mouse, formed by the engraftment of human fetal liver, thymus, and lymph node into the SCID mouse, in such a manner that human stem cell differentiation may be observed to occur.

Construction of the SCID-hu mouse. Human fetal thymus, liver, lymph node, and spleen were obtained and prepared for introduction into SCID mice by surgical implantation or by intravenous injection (see legend to Fig. 1) (*18*). The resultant mice were identified by code, for example, X/Y/Z, wherein X refers to the donor code of the thymus implant, Y to the donor code of the fetal liver implant, and Z to the donor code of the fetal lymph node (or spleen) implant. The donor codes, in turn, were catalogued with information related to the gestational age of the fetus and the results of typing for common HLA class I antigens. With a bank of frozen tissue available, it becomes readily possible to construct SCID-hu mice with a variety of human lymphoid organs of defined (and, as desired, different) genetic origin.

In an initial experiment with 21 SCID mice, 10 were segregated to an untreated control group. During the next weeks, these animals showed signs of illness, including weight loss, ruffled fur, and a rapid respiratory rate. All were dead within 4 months. Such a syndrome in SCID mice is most often attributable to opportunistic infection, including that caused by *Pneumocystis carinii*. We subsequently observed that SCID colonies can be easily maintained on prophylactic antibiosis with trimethoprim-sulfamethoxasole (TMS) (see legend to Fig. 1) (*19–21*). No further outbreaks of disease have occurred; all mice now have the appearance of a healthy BALB/c mouse.

Eleven other SCID mice in this experiment were converted into SCID-hu mice; they were implanted with human fetal thymus and then given an intravenous or intrathymic injection of human fetal liver cells. Even in the absence of TMS prophylaxis, all of these mice remained healthy. Among this group, some were specifically killed for analysis; others are still alive 17 months later.

A reconstituted human immune system may have protected the SCID-hu mice against the acquisition of opportunistic infection. While exploring this possibility, we made the following observations.

Human fetal thymus growth and microscopic anatomy. The human fetal thymus undergoes considerable growth when implanted under the kidney capsule of a SCID mouse. A thymic graft (10 by 5 by 5 mm) is shown in Fig. 1; 13 weeks earlier, this tissue had been implanted as a thymic lobe (0.5 by 0.5 by 2 mm) from a donor in the ninth gestational week. Vasculature, of yet undetermined origin, is clearly evident. Our experience (with approximately 200 thymic implants of this type) indicates that growth potential is at least partially related to the gestational age of the donor, and optimal if used before the 20th gestational week.

The microscopic anatomy of the SCID-hu thymus can bear close resemblance to that of its normal human counterpart (*22*). The cortical and medullary compartments are well demarcated by immunohistochemical stains for the epithelial antigens CDR2 and MD1 (*23*) (Fig. 2, A and B). The cortex, but not the medulla, is densely populated with $CD1^+$ thymocytes (Fig. 2C); reciprocally, medullary cells are readily stained with antibodies to MHC class I antigens (as shown below). Immunohistochemical stains for human CD4 and CD8 reveal reactive cortical and medullary thymocytes. Double-color analysis by fluorescein-activated cell sorting (FACS) divides these reactive cells into the expected subpopulations: double-positive, double-negative, and single-positive (Fig. 3B). Quantitatively, the representation of each population is similar to that found in a normal, age-matched human fetal thymus (Fig. 3A). In this particular experiment, there were actually more cells with the characteristics of phenotypically mature human T cells, expressing either CD4 or CD8 but not both.

The only histologic difference that we have observed in the SCID-hu thymus is revealed by antibodies to MHC class II antigens. Both normal human fetal thymus and SCID-hu thymus show the expected pattern of human class II antigen distribution (Fig. 2D). The SCID-hu thymus, however, also contains murine class II–positive

cells (Fig. 2E), which have a dendritic morphology and are detectable in the medulla and the mid- and deep cortex. The migratory mechanisms required to produce such microchimerism are apparently not promoted reciprocally. Thus, human T cells and dendritic cells can be observed to move from human fetal liver preparations into the human thymic implant of the SCID-hu mouse, as discussed later, but no human cells are detectable in the host (mouse) thymus with antibodies to the human markers CD2, CD3, CD4, CD8, MHC class I, and MHC class II.

Human T cells in the SCID-hu peripheral circulation. SCID-hu mice with human fetal thymus implants were injected with human fetal liver cells, intravenously or intrathymically. The fate of these cells was then monitored by serial analysis of peripheral blood (Fig. 4). On day 0, four SCID-hu mice with thymic implants (from donor A) were injected intravenously with 10^7 human fetal liver cells (from donor D). Group A/D peripheral blood cells were obtained at intervals of 27, 42, 49, and 64 days thereafter, stained with a mixture of murine monoclonal antibodies to human CD2 (MAb OKT11), CD3 (OKT3), CD4 (OKT4), and CD8 (OKT8), and quantified by FACS. No cells with a human phenotype were observed at day 27 (Fig. 4A). Later, however, such cells were present, representing 17 percent (day 42) and 5 percent (day 49) of total mononuclear cells. By day 64, this population was no longer detectable. Peripheral blood cells from unmanipulated SCID mice were not reactive at any time with this set of antibodies to human T cell markers (Fig. 4B).

Several characteristics are associated with the generation of human T cells in the SCID-hu peripheral circulation. (i) The phenomenon is only observed when the mice have received human thymic implants and human fetal liver cells. The latter, given alone (eight separate experiments to date), do not give rise to phenotypically mature human T cells later. (ii) The timing is predictable (Fig. 5). Whether the fetal liver cells are given intravenously or intrathymically, peripheral human cells are not easily detectable for 3 to 4 weeks. Thereafter, as many as 40 percent of total mononuclear cells in the SCID-hu peripheral circulation may be reactive with antibodies to human T cell markers. In most cases, the circulating human T cells are no longer detectable after 10 to 12 weeks. Variations in kinetics and extent of reconstitution are present. We suspect that this may be dictated by factors related to supply (for example, of stem cells or growth factors or both), to distribution (into body compartments different from peripheral blood), or to changes in human T cell longevity, as, for example, a result of rapid destruction by nonlymphoid murine effector cells.

When the phenotypes were examined immunologically for subset distribution, the T cell subsets in the SCID-hu circulation were similar to those in normal human peripheral blood. In this analysis, peripheral blood cells from SCID-hu mice were stained first with fluorescein isothiocyanate (FITC)–conjugated antibody to HLe-1 (the common human leukocyte antigen, CD45), counterstained with either Texas red–conjugated antibody to CD4 or Texas red–conjugated antibody to CD8, and then quantified with a dual laser FACS. The ratio of CD4 to CD8 (calculated as the percentage of $CD4^+$, HLe-1^+ cells divided by the percentage of $CD8^+$, HLe-1^+ cells) was about 3.8 (standard deviation = 2.0, n = 23), a value not dissimilar to that found in normal human cord blood (mean = 4.0 to 5.0) (*24*). No SCID-hu mice have yet been observed to have ratios of CD4 to CD8, as determined above, of less than one.

Staining patterns in the above experiments are concordant with monoclonal antibodies to different epitopes on the same human T cell marker (for example, OKT4 compared to Leu3a), regardless of whether they are used in two-stage assays (as in Fig. 4) or are directly conjugated. The cells did not stain above background with isotype-matched controls. Finally, within the same mouse, the human T cell wave is both time-dependent and tissue-restricted. For instance, even when human cells are readily detected in the peripheral blood by FACS analysis, few are found in the mouse spleen and none in the mouse lymph node or thymus (not shown); this might be predicted from the known species-specificity of the lymphocyte homing receptor (*25*).

To confirm further that the SCID-hu peripheral cells were of human origin, we prepared total cellular DNA from peripheral blood cells and hybridized it with a probe for the human Alu sequence, BLUR 8 (*26*). Human DNA elicits a strong signal; SCID DNA does not (Fig. 6A). Mice given only human fetal liver cells on day 0 (−/E) show background hybridization by day 35. Littermates given human fetal thymus and human fetal liver cells (A/D) show a strong signal of Alu-hybridizing DNA by that time. The semiquantitative titration suggests that the amount of human DNA in the SCID-hu (A/D) population is about 1 percent of that found in human peripheral blood. Since the number of total mononuclear cells in the SCID peripheral blood is lower than normal, this figure is concordant with the FACS-generated statistic that 10 percent of the mononuclear cells in the A/D group are human. When SCID-hu peripheral blood cells are directly visualized by in situ hybridization with an RNA probe to BLUR 8, cells of human origin are found in the midst of unreactive (murine) cells (Fig. 6B). A further correlation with the immunofluorescence data is noted when DNA is analyzed from the peripheral blood of the group F/H/I. This group of mice had received a fetal thymus implant 38 days earlier, fetal liver cells 18 days earlier, and fetal lymph node tissue 11 days earlier. By FACS analysis, the human T cell wave had not yet appeared; by BLUR 8 probing, the human DNA signal is at or only slightly above background.

Human hematopoietic precursors move to and through the SCID-hu thymus. Apparently, the intravenous injection of human fetal liver cells permits T cell progenitors therein to home to and differentiate through the engrafted human fetal thymus. To test this inference directly, fetal liver cells were injected intravenously into a SCID-hu mouse with an MHC-disparate fetal thymus implant (Fig. 7). The fetal liver cells were HLA-$A2^+$, HLA-$B40^-$; in contrast, the thymic implant was HLA-$A2^-$, HLA-$B40^+$. Ten weeks after the injection of fetal liver cells, thymic sections were stained with MB40.2, an antibody to HLA-B40, or with MA2.1, an antibody to HLA-A2. As expected (*27*), MB40.2 was reactive with epithelial cells in the cortex and scattered medullary dendritic cells. No thymocytes were reactive; instead, the densely packed thymocytes in the medulla were HLA-$A2^+$. Since cortical thymocytes normally express low levels of class I antigens, definition of their origin by this assay is not possible. However, HLA-$A2^+$ dendritic cells are visible in the thymic cortex (Fig. 7B). Thus, progenitor cells to this population must also be capable of movement into the fetal thymus implant.

The subsequent movement of these cells into the peripheral circulation was shown by FACS analysis. Ten weeks after the injection of HLA-$A2^+$, HLA-$B40^-$ fetal liver cells into a SCID-hu mouse with an HLA-$A2^-$, HLA-$B40^+$ fetal thymus, cells in the peripheral circulation were double-stained with antibodies to HLe-1 and various class I determinants. No cells in the peripheral circulation of a SCID mouse were reactive with antibodies to HLe-1 or with W6/32, a monoclonal antibody to a monomorphic human class I determinant (Fig. 8A). In the SCID-hu peripheral blood, cells positive for each are detectable (Fig. 8B). Of these, none stain with MB40.2, an antibody to HLA-B40 (Fig. 8C); all stain with MA2.1, a monoclonal antibody to the fetal liver cell type, HLA-A2 (Fig. 8D).

Experiments of a similar nature have been carried out in four groups of SCID-hu mice. In two of these groups, homing of cells to

and through the thymus can be demonstrated; homing in the other two groups was not detectable. The apparent complexity of this biology may be related to the relative developmental stages of the thymus or fetal liver cell implants, or to some restriction on the survival of the transplanted cells. It is clear, for instance, that the avian and murine thymic rudiments pass through discrete stages of receptivity for T cell progenitors (*3, 28, 29*). Whether the human thymus may pass through similar phases during ontogeny remains to be determined.

The problem of T cell function and the presence of human plasma cells. The foregoing studies demonstrate that providing normal human fetal thymus and fetal liver cells to a SCID mouse results in the generation of phenotypically mature human T cells in the peripheral circulation. Dendritic cells of both murine and human origin are also observed to move into the engrafted human fetal thymus. The functional status of these cells is unknown. Put briefly, we observe the following. First, SCID-hu mice do not manifest signs of graft-versus-host disease. Second, they appear to be protected against opportunistic infection. We infer that the human immune system, if functional, has adapted to interact with (or be tolerant of) murine elements. Possibly, recognition of both murine and human MHC antigens in the thymus has promoted tolerance and restriction to each. This inference requires direct validation.

If human T cells differentiate from human fetal liver cells within the SCID-hu mouse, human B cell progenitors may follow suit. If so, and if the T and B cells were functionally competent, immunoglobulin (Ig) class-switched human plasma cells might be present. The selective binding of protein A to murine and human IgG was used to purify reactive proteins from the plasma of various groups of SCID-hu mice. These proteins were analyzed by SDS-PAGE (Fig. 9A). Human plasma (Hu) contains significant amounts of IgG heavy and light chains; SCID plasma, on the other hand, does not.

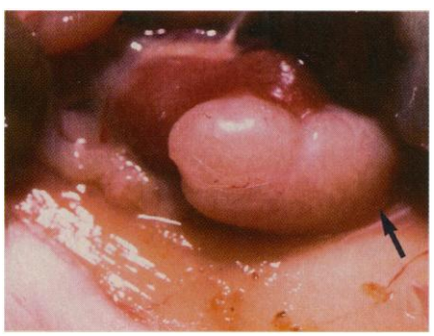

Fig. 1. Gross anatomy of the SCID-hu fetal thymus implant. An autopsy was performed on a SCID-hu mouse, 13 weeks after an implantation of a human fetal thymus under the left kidney capsule; simultaneously, the mouse had received 10^7 fetal liver cells intravenously. Exposed for visualization (cephalad direction to the right) in the left upper quadrant of the SCID-hu abdomen is the SCID left kidney and, growing on it, a human fetal thymus (marked with an arrow). At the time of implantation, the latter represented one lobe of a 9-gestational-week thymus and was less than 0.5 by 0.5 by 2 mm in size. At autopsy, it measured approximately 10 by 5 by 5 mm. The SCID mice were maintained in bonneted isolator cages. They received TMS in suspension through the drinking water for 3 days of each week (40 mg of trimethoprim and 200 mg of sulfamethoxazole per 5 ml of suspension; 0.125 ml of suspension for every 4 ml of drinking water per mouse per day). The drinking water bottle was turned daily while the drug was being administered; standard drinking water was substituted for the remaining 4 days of each week. The gestational age of human fetal tissue was determined (*38*). Those organs used in the construction of SCID-hu mice were placed in RPMI 1640 medium with 10 percent fetal calf serum (FCS); the remaining tissue was fixed in Formalin. The fetal organs were cut into pieces (2 by 2 by 4 mm). For single cell suspensions, the thymus was pressed between the frosted ends of two glass slides, thereby releasing a suspension of thymocytes. The fetal liver was minced, and cells that remained suspended in the medium were separated into a mononuclear fraction by Ficoll-Hypaque centrifugation; the interface cells were then washed three times in RPMI 1640 with 10 percent FCS and resuspended at a concentration of 10^8 cells per milliliter for intravenous administration. For future use, organ fragments or dissociated cells were frozen in 10 percent dimethyl sulfoxide and 40 percent FCS. For histotyping, thymocytes were incubated first with a panel of monoclonal antibodies to human class I antigens (*27*), including MA2.1 (IgG_1:A2, B17), PA2.1 (IgG_1:A2, A28), BB7.1 (IgG_1:B7, Bw42), MB40.2 (IgG_1:B7, B40), GAP A3 (IgG_{2a}:A3), MEI (IgG_1:B7, B27, Bw22), and W6/32 (IgG_{2a}:monomorphic), and then with FITC-conjugated goat antiserum to IgG or IgM. The tissues were given a letter code and catalogued with the derived HLA type, gestational age of the fetus, and the presence or absence of fetal anomalies (where known). For implantation of tissues, mice were first anesthetized with halothane. A 1-cm flank incision was made to expose the right or left kidney. The same technique was used to introduce fragments of tissue subcutaneously and into the SCID spleen. Sutures were placed to approximate successive peritoneal and fascial layers, and metal clips were secured over the wound to ensure healing. Suspended fetal liver cells were injected intravenously by a retro-orbital approach, with a 30-gauge needle. [Photograph by F. A. Dusel III]

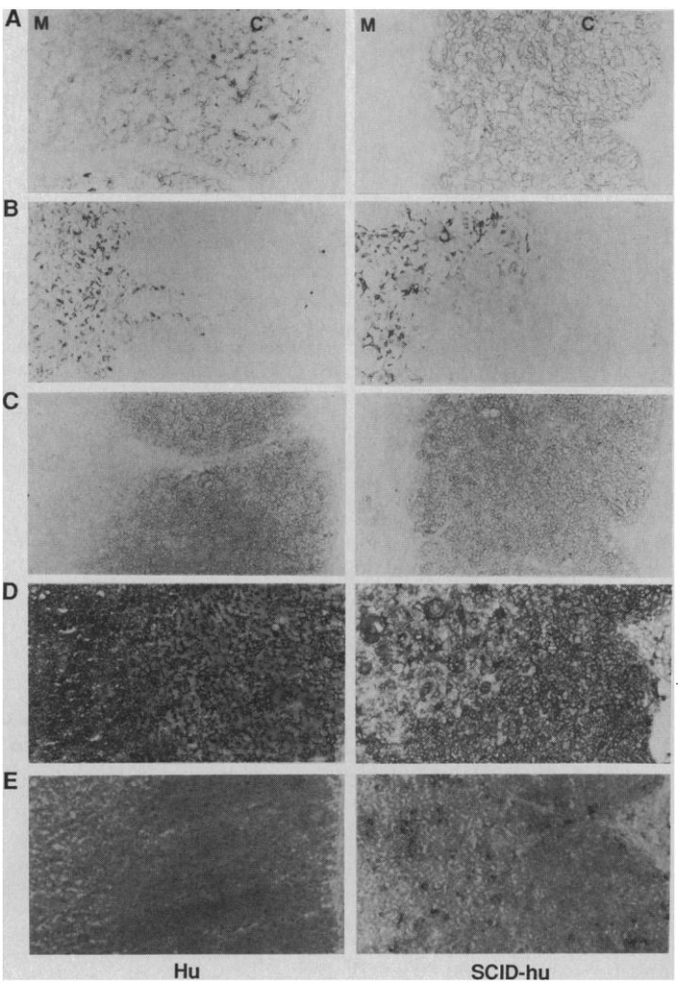

Fig. 2. Microscopic anatomy of the SCID-hu fetal thymus implant. Four weeks after implantation of a 20-gestational week human fetal thymus into SCID-hu group F/H/I (right) (Fig. 9), serial thin sections were prepared. By comparison, sections were also obtained from a normal, age-matched fetal thymus (left). All sections were aligned with the medulla (M) on the left and the cortex (C) on the right. The antibodies used for staining were (**A**) CDR2, an antibody to human cortical epithelial cells; (**B**) MD1, an antibody to epithelial cells in the human medulla, in the subcapsular region, and in Hassal's corpuscles; (**C**) OKT6 (CD1), against cortical human thymocytes; (**D**) L243, an antibody to a monomorphic determinant on human class II antigens; (**E**) M5/114.15.2, an antibody to a monomorphic determinant on murine class II antigens. Fresh tissue was dissected into pieces, 3 by 3 by 3 mm, embedded in OCT (Ames, Elkhart, Indiana), quickly frozen in liquid nitrogen, and stored at −70°C. Frozen sections (6 μm) were fixed with acetone for 1 minute at room temperature and then stained by the avidin-biotin-peroxidase complex (ABC) method with the ABC kit (Vector, Burlingame, California) for mouse monoclonal antibodies. For MD1 and M5/114.15.2 (rat monoclonal antibodies), peroxidase-labeled rabbit antibody to rat Ig (Dako, Santa Barbara, California) was used as the secondary antibody. Peroxidase activity was developed by 3,3′-diaminobenzidine.

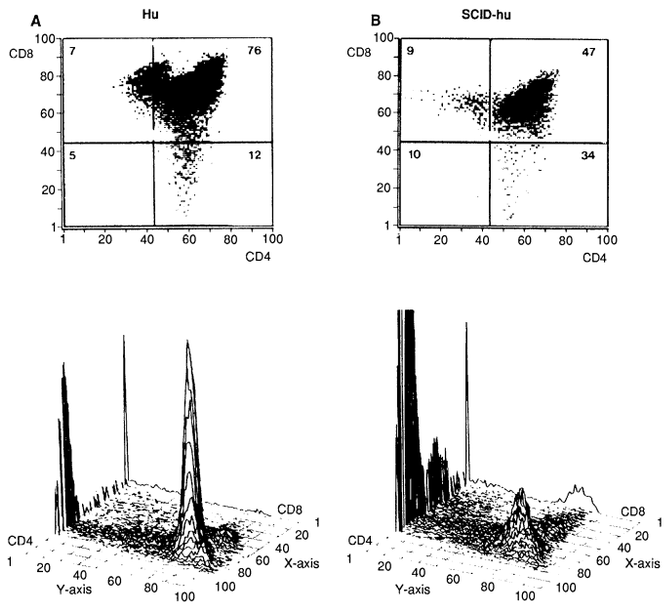

Fig. 3. FACS analysis of SCID-hu thymocytes. The thymic specimens shown in Fig. 2 were prepared as a single-cell suspension, stained with FITC-Leu 3a (CD4) and phycoerythrin (PE)-Leu 2a (CD8), and analyzed by FACS. Two-color profiles of age-matched human thymus are shown on the left (**A**); those of the SCID-hu thymus are on the right (**B**). Subpopulations that are double-negative, single-positive, and double-positive for CD4 and CD8 (and their respective percentage of total thymocytes) are shown in the corresponding quadrants of the top graphs. FITC-Leu 3a and PE-Leu 2a were used as described by the supplier (Becton Dickinson, Mountain View, California).

No IgG was seen in the case of the SCID-hu group −/E, which had received human fetal liver cells but not human fetal thymus. However, all other groups of SCID-hu mice (most notably, K/K and F/H/I) were found to make IgG.

The origin of the plasma immunoglobulins was determined by Ouchterlony analysis (Fig. 9B). In conjunction with the data in Fig. 9A, two points are raised:

1) Many of the SCID-hu mice (for instance, K/K, well No. 8) were making readily detectable levels of mouse IgG. SCID mice (unmanipulated littermates) showed low levels of serum IgM, detectable by Ouchterlony and by silver-stained SDS-PAGE; they did not demonstrate detectable levels of IgG. Thus, the provision of human tissues to SCID mice exposes the "leakiness" of the SCID strain (*9, 30*) and might induce the differentiation of B cells to plasma cells or promote their survival. The progenitors of some of these plasma cells may in turn class switch to IgG.

2) One group of SCID-hu mice, F/H/I, produced human IgG. Using an enzyme-linked immunoabsorbent assay, the amount of IgG in this set of mice was 1 mg/ml, about 10 percent of that found in normal human serum. This set of mice was distinctive in another respect: it was the only group that had been engrafted with human fetal liver cells, human fetal thymus, and also human fetal lymph node. Biopsy specimens derived from that lymph node were prepared for immunohistochemical staining. Its morphology was similar to that of a normal adult lymph node, but cortical and medullary areas were not as well defined (Fig. 10A). The central area of the node was found to contain numerous lymphocytes which bear the CD4 or CD8 markers. By Giemsa stain, plasma cells were seen in the periphery of the node (Fig. 10B). When stained with antibodies to human IgM (Fig. 10C) or human IgG (Fig. 10D), these cells were

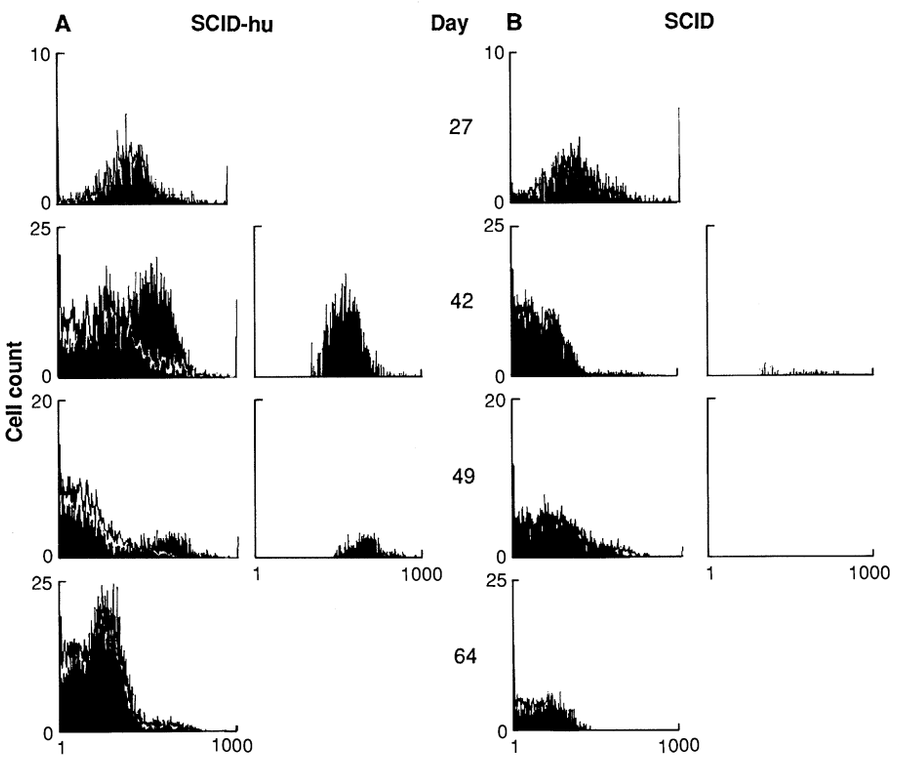

Fig. 4. Detection of human T cells in the peripheral circulation of SCID-hu mice. SCID-hu mice (group A/D) with a fetal human thymic implant (A) were injected intravenously with 10^7 human fetal liver cells (D) on day 0. At intervals thereafter, peripheral blood cells were stained with a mixture of monoclonal antibodies to human T cell markers (CD3, CD4, CD8, CD2) and analyzed by FACS (**A**, left panels). For comparison, peripheral blood from an unmanipulated SCID mouse was analyzed in parallel (**B**, right panels). Background staining was assessed with a monoclonal control antibody and is represented by the light curve. Specific staining is shown in the darkened curve. On those days when specific staining was apparent above background in the SCID-hu mice (days 42 and 49), channel per channel subtraction of the two curves was performed to generate the additional plot. After subtraction, 17 percent of the cells stained specifically in the SCID-hu on day 42; 5 percent stained specifically on day 49. Approximately 100 μl of whole blood was obtained by tail vein incision and mixed with equal volumes of phosphate-buffered saline (PBS) containing EDTA at 3 mg/ml and of PBS containing 2 percent dextran T-500. Under these conditions, most erythrocytes sediment after 30 minutes at 37°C. Nucleated peripheral blood cells were removed, washed once in RPMI 1640 medium with 10 percent FCS, and then incubated either with a mixture of monoclonal antibodies (OKT3:CD3, OKT4:CD4, OKT8:CD8, OKT11:CD2) or with X63 (an IgG monoclonal antibody to actin). The antibodies to human T cell were used at dilutions previously shown to be optimal for staining; X63 was used at an equivalent protein concentration (1 to 5 μg/ml). After 30 to 60 minutes at room temperature, the samples were resuspended, underlayered with undiluted newborn calf serum, centrifuged, and then stained with FITC-antibody to mouse IgG or M (Tago, Inc. Burlingame, California) for 30 to 60 minutes at room temperature. After a final washing, the cells were fixed in 1 percent Formalin and then incubated with propidium iodide (PI) (1 μg/ml). This step permitted FACS analysis to proceed quantitatively in the presence of variable numbers of contaminating erythrocytes. Thus, all (Formalin-fixed) nucleated cells were first located by PI staining; mononuclear cells within this population were then analyzed for FITC staining.

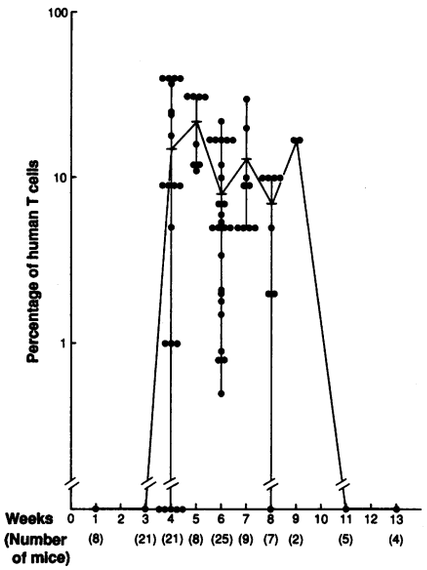

Fig. 5. Timing and reproducibility of the human T cell wave in the SCID-hu peripheral circulation. The log percentage of human T cells among total mononuclear cells in the peripheral circulation is shown as a function of time after the intravenous or intrathymic administration of 10^7 human fetal liver cells. The number of mice analyzed at each point is indicated in parentheses. The curve is drawn through the mean of experimental points. These data represent our cumulative experience after 10 months of experimentation. Some SCID-hu mice with human T cells in the periphery for periods as long as 20 weeks have since been noted; the exact conditions which permit such long-term reconstitution are unknown and under evaluation.

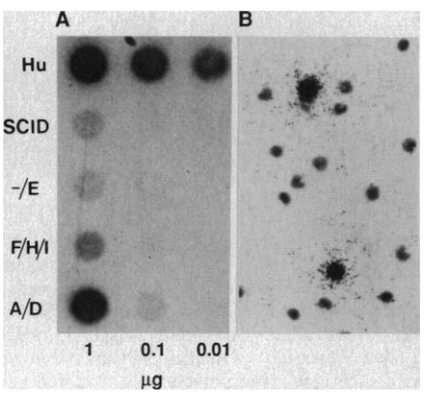

Fig. 6. Hybridization of SCID-hu peripheral blood cells with the human-specific Alu probe, BLUR 8. (**A**) Total cellular DNA was prepared from the peripheral blood of human (Hu), SCID, and three separate groups of SCID-hu mice: −/E, received fetal liver cells 35 days earlier (day −35), but no fetal thymus; F/H/I, received fetal thymus (day −38), fetal liver cells (day −18), and fetal lymph node (day −11); and A/D, received fetal thymus (day −61) and fetal liver cells (day −35). (The first two groups of SCID-hu mice showed no staining above background when analyzed on FACS with monoclonal antibodies OKT3, OKT4, OKT8, OKT11; in group A/D, 10 percent of cells were positive.) The DNA was diluted (log-orders), applied to nitrocellulose, and probed with the ^{32}P-labeled Alu probe, BLUR 8. Nucleated cells were prepared from whole blood (as described in Fig. 4) and lysed in a buffer containing 0.5 percent SDS, 10 m*M* EDTA, 10 m*M* tris-HCl (*p*H 7.4), 10 m*M* NaCl, and proteinase K at 100 μg/ml. After 12 hours at 37°C, the lysate was extracted three times with a mixture of phenol, chloroform, and isoamyl alcohol (24:24:1). Total cellular DNA was precipitated from the aqueous phase and diluted in log order (starting at 1 μg/50 μl). To each sample was added 30 μl 2*M* NaOH and 100 μl 20× SSC (1× SSC = 0.15*M* NaCl, 0.015*M* sodium citrate); after 10 minutes at 80°C, the sample was neutralized with 320 μl 1*M* tris-HCl, *p*H 7.4, and applied to a nitrocellulose filter in a dot-blotting manifold (Schleicher & Schuell). The filter was baked at reduced pressure for 2 hours, hybridized with the ^{32}P-labeled Alu probe BLUR 8 under conditions as described (*39*), and then processed for autoradiography (*40*). (**B**) Peripheral blood cells from A/D were prepared for in situ hybridization with a ^{35}S-labeled RNA probe to BLUR 8. Cells with grains in the autoradiograph are presumably of human origin. Peripheral blood cells were subjected to in situ hybridization as described (*41*). The RNA probe for BLUR 8 was prepared by subcloning the 0.3-kb Bam HI insert of (plasmid) BLUR 8 into the Bam HI site of pGEM2, to create pGB-8; transcription then proceeded via the T7 promoter in the presence of ^{35}S-labeled UTP. Autoradiography proceeded for 7 days. Under the conditions used, the probe shows no hybridization to murine spleen cells.

shown to be of human origin. No plasma cells in this lymph node were found to stain with reagents specific for mouse IgG or IgM.

These data indicate that human B cells can differentiate to IgG-secreting plasma cells in the context of a SCID-hu mouse given human fetal thymus, fetal liver cells, and fetal lymph node. Such a differentiative process is normally one in which functional T cells, functional B cells, and functional antigen-presenting cells must interact within a peripheral lymphoid organ; we infer that functional networks are operative, possibly in the lymph node. It is not clear whether T cells derived from the human fetal liver (H) are driving B cell differentiation to plasma cells in the fetal lymph node. (I). Certainly, resident T and B cells in the lymph node at the time of implantation might do the same.

Human hematopoietic differentiation in the SCID-hu mouse. The SCID-hu mouse represents a murine model system in which human hematopoietic cells of the T and B cell lineages can be observed to differentiate. Differentiation of T cells requires a hematopoietic stem cell source (human fetal liver) and a human fetal thymus. The process appears to require active species-specific homing of intravenously administered cells through the thymus. In a time-dependent and time-limited fashion, phenotypically mature human T cells are then found in the peripheral circulation of the mouse. The rules for human B cell and plasma cell differentiation in the SCID-hu are less certain; the engraftment of intact human lymphoid organs (such as lymph node) appears to be necessary, and perhaps sufficient.

Xenogeneic bone marrow transplantation has been attempted in the past, most notably in the setting of "radiation chimeras" (*31–34*). Usually, the immune system of the host is quantitatively suppressed by lethal irradiation and allo- or xenoreactive cells in the donor bone marrow are depleted with antibodies to T cells and complement. The results have been variable and, in general, less than encouraging.

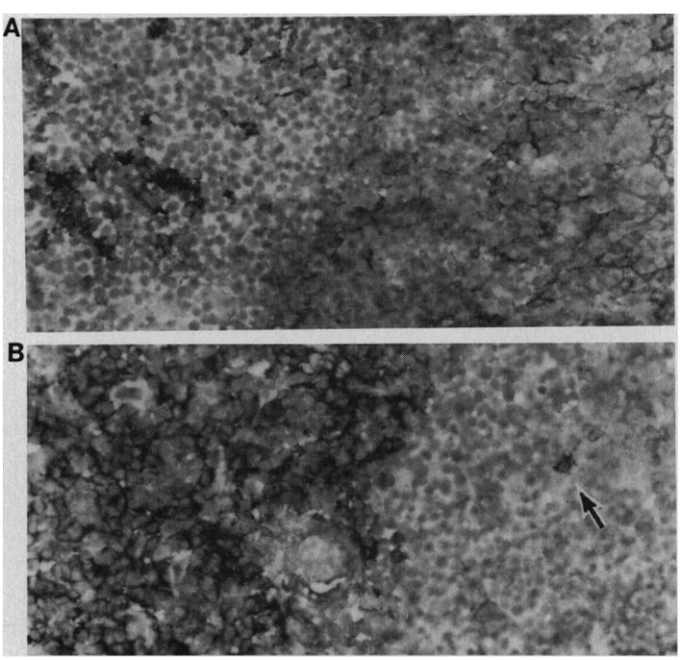

Fig. 7. Movement of human hematopoietic cells from fetal liver preparations to the SCID-hu thymus. A SCID-hu mouse was engrafted with a human fetal thymus (L: HLA-A2$^-$, HLA-B40$^+$) and injected intravenously with 10^7 fetal liver cells (N: HLA-A2$^+$, HLA-B40$^-$). Ten weeks later, a biopsy of the thymic implant was sectioned and stained with (**A**) MB40.2, antibody to HLA-B40, or (**B**) MA2.1, antibody to HLA-A2. The sections are aligned with the medulla on the left and the cortex on the right. The arrow (in B) points to an HLA-A2$^+$ dendritic cell in the host (HLA-A2$^-$) thymic cortex. Methods were as described in Fig. 2.

A wasting syndrome (secondary disease) often results and is characterized by varying degrees of immunoincompetence (*35, 36*). Better results have been obtained with the technique of "mixed chimerism," in which lethally irradiated mice are reconstituted with a mixture of syngeneic and allo- or xenogeneic bone marrow cells depleted of mature T cells (*33, 37*). This approach has been used recently with success in human bone marrow transplantation. To our knowledge, no reports have appeared in which human → mouse radiation chimeras have been successfully prepared, by any technique.

Our system offers several advantages over the radiation chimera. First, the SCID mouse stock has a selective and total defect in lymphoid cells and does not require preparative irradiation. Breeding colonies are easily maintained with antibiotic (TMS) prophylaxis. Xenografts are implanted without rejection. Second, the use of human fetal tissue obviates the need for T cell depletion. The human T cell progenitor may differentiate to recognize both mouse and human MHC as self. Finally, after engraftment of human tissues, it appears that the SCID-hu mouse is more immunocompetent than its SCID counterpart, not less.

The limitations of the model are obvious. There is variability in the extent and duration of peripheral human T cell reconstitution; at best, it is partial and transient. Further, it is not now clear that the human lymphoid cells are physiologically functional.

Even so, as it stands, the SCID-hu mouse presents new opportunities for the direct analysis of human physiology and pathophysiology. It is reasonable to expect, for example, that the circulating human T cells will present ready targets for infection by human T lymphotropic viruses. The same is likely to be true of $CD4^+$ T cells and myelomonocytic cells in the human lymph node and thymus implants. Possibly, the SCID-hu mouse can serve as a small animal model system for the analysis of diseases caused by human retroviruses. This will most certainly be true for the acute phases of infection, about which we now know the least. Therapeutic agents that might prevent new infection or suppress ongoing infection might likewise be evaluated in vivo.

The mature human T cells in the peripheral circulation of the SCID-hu mouse were derived from human fetal liver stem cells. Our evidence suggests that fetal thymus is a necessary intermediate organ. As such, it represents a readout assay for human hematopoi-

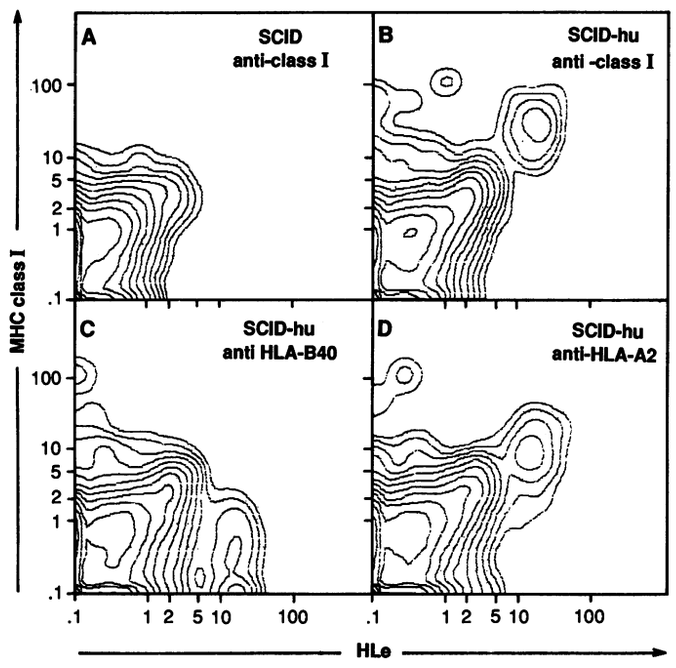

Fig. 8. Movement of human hematopoietic cells from fetal liver cell preparations to the SCID-hu peripheral circulation. Ten weeks after the injection of HLA-$A2^+$ fetal liver cells into a SCID-hu mouse with an HLA-$B40^+$ thymus (group L/N, see Fig. 7), peripheral blood cells were stained with FITC-labeled antibody to HLe-1 (CD45), a common marker on all human leukocytes. They were counterstained with (**B**) W6/32, which recognizes a monomorphic determinant on human class I antigens; (**C**) MB40.2 antibody to HLA-B40; (**D**) MA2.1, antibody to HLA-A2. These secondary reagents were visualized with the fluorochrome Texas red. As a control, peripheral blood cells from a SCID mouse were reacted with FITC-conjugated antibody to HLe-1 and Texas red W6/32 in (**A**). Staining procedures were carried out as described (*42*) with subsequent analysis on a highly modified dual-laser FACS (*43*).

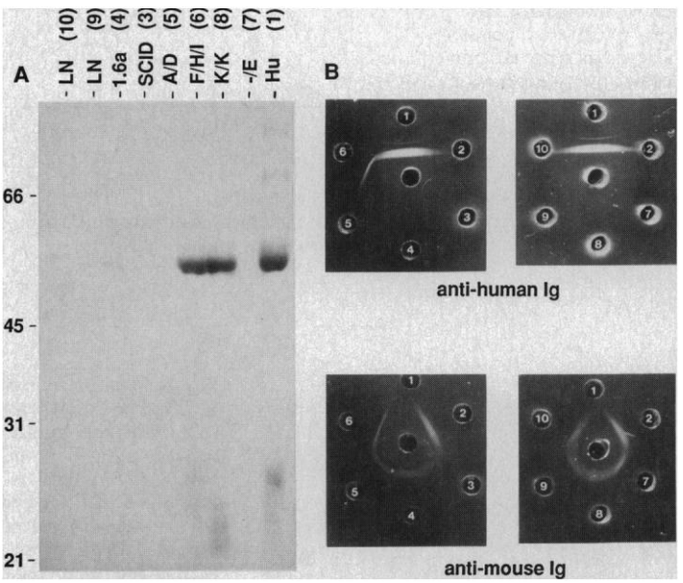

Fig. 9. Protein A-binding proteins in the plasma of SCID-hu mice. (**A**) Plasma specimens derived from human (Hu), SCID, and seven different groups of SCID-hu mice were incubated with protein A–Sepharose beads. Bound proteins were reduced and alkylated in SDS and resolved by SDS–polyacrylamide gel electrophoresis (PAGE). The gel was stained with Coomassie brilliant blue. The relative mobility of marker proteins is shown on the left. The numbers above each lane correspond to the Ouchterlony wells of (B). Whole blood was collected into an equal volume of PBS containing EDTA (3 mg/ml) and centrifuged at 2000 rpm for 20 minutes to yield plasma. Portions (30 μl in the case of human, 300 μl in the case of all other specimens) were incubated with 30 μl of 50 percent protein A–Sepharose (Pharmacia) for 2 hours at room temperature. The beads were washed, resuspended in an SDS-PAGE loading buffer containing 50 m*M* dithiothreitol, and prepared for SDS-PAGE (on a 10 percent Laemmli-type gel) (*44*), as described (*40*). The gel was subsequently stained in 0.125 percent Coomassie brilliant blue. (**B**) The same plasma specimens were placed on equal portions around central wells of Ouchterlony plates containing antibody to human Ig (top) or antibody to mouse Ig (bottom). The description of each SCID-hu (except 1.6a) is given as follows: X/Y/Z (X: gestational age of human fetal thymus at time of implantation; number of days since implantation. Y: gestational age of whole human fetal liver or fetal liver cell suspension; number of days since implantation. Z: gestational age of human fetal lymph node at time of implantation; number of days since implantation). (1) Human plasma; (2) BALB/c plasma; (3) SCID plasma; (4) SCID-hu plasma, 1.6a (received a serial transplant of fetal thymus from the thymus shown in Fig. 2, 170 days before assay; and fetal liver cells intravenously 140 days prior to assay); (5) SCID-hu plasma, A/D (A: 22 weeks, 96 days since implant/D; 22 weeks; 90 days since implant); (6) SCID-hu plasma, F/H/I (F: 20 weeks; 68 days since implant/H: 22 weeks; 48 days since implant/I: 15 weeks; 41 days since implant); (7) SCID-hu plasma, −/E (−: no fetal thymus/E: 19 weeks; 90 days since implant); (8) SCID-hu plasma, K/K (K: 20 weeks; 30 days since implant/K: 20 weeks; 30 days since implant); (9) SCID-hu plasma, L/N (L: 19 weeks; 20 days since implant/N: liver, 19 weeks; 20 days since implant); (10) SCID-hu plasma, L//N (L: 19 weeks; 20 days since implant/N: bone marrow, 19 weeks; 20 days since implant).

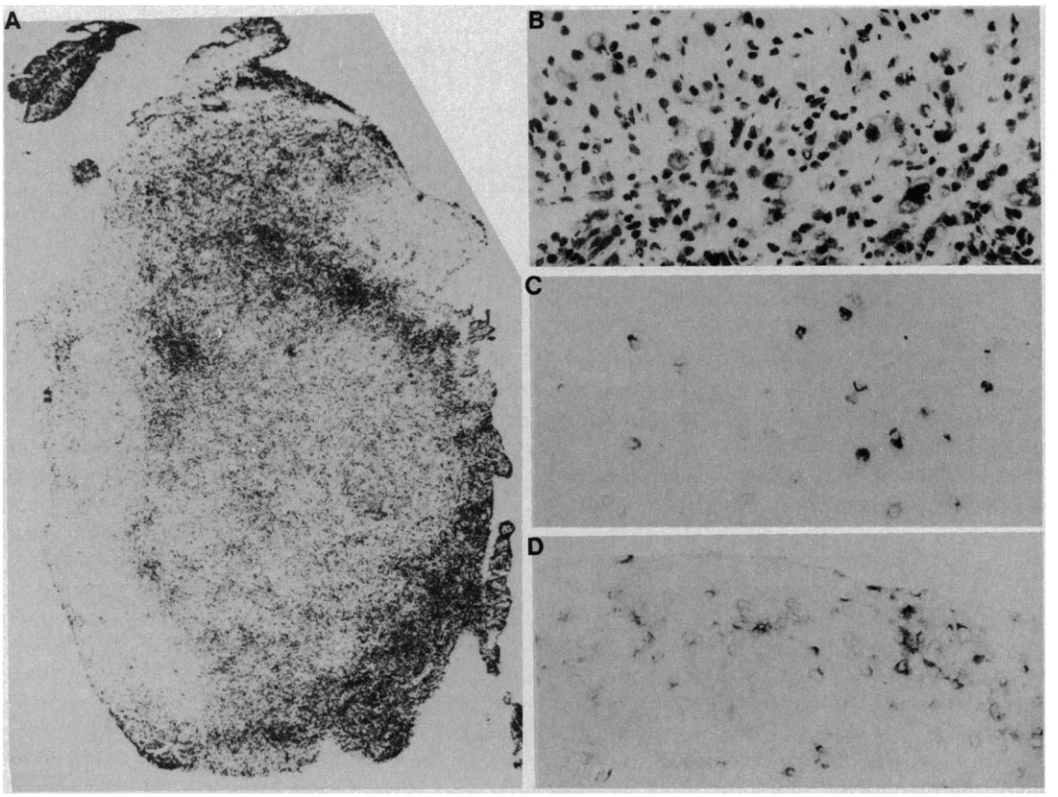

Fig. 10. Human plasma cells in the fetal lymph node of a SCID-hu mouse. Serial thin sections were prepared from a SCID-hu fetal lymph node 7 weeks after implantation of a 15-gestational-week human fetal lymph node into group F/H/I. The gross morphology is shown by Giemsa stain in frame (**A**); under higher magnification, cells with the morphology of plasma cells are seen (**B**). Lymph node cells are found to be reactive with antibodies against human IgM (**C**) and human IgG (**D**). No reactive cells are seen with antibodies to murine immunoglobulins. Peroxidase-conjugated primary antibodies were used in this experiment, as described in Fig. 2. The samples in (A) and (B) were Formalin-fixed; the frozen sections in (C) and (D) were acetone-fixed.

etic stem cells. A number of markers may be used to trace the movement of certain human cells into and through the thymus, for example, MHC types (as in these experiments), Y chromosomal markers, and chromosomal abnormalities. These markers, combined with selected monoclonal antibodies and FACS, should prove to make the SCID-hu a useful model for the description and isolation of the human pluripotent hematopoietic stem cell, as well as lineage-restricted progenitors.

When fetal liver cells, fetal thymus, and fetal lymph node are implanted in SCID mice, B cell differentiation to IgG-secreting plasma cells occurs. Much more needs to be learned about this set of events. It is possible that these developing cells might be specifically sensitized in vivo. If so, the SCID-hu mouse may thereby constitute a vehicle for the generation of human monoclonal antibodies.

Finally, the SCID mouse appears to present no barrier to transplantation of tissues from single or multiple human donors. Pathologic human tissues might be transplanted also, and thus be available for the direct study of human physiology and pathology in an experimental animal.

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