Immunohistology and Immunocytology of Human T-Cell Chimerism and Graft-Versus-Host Disease in SCID Mice

By Gertrud Hoffmann-Fezer, Claudius Gall, Ursula Zengerle, Bernd Kranz, and Stefan Thierfelder

Surprisingly little graft-versus-host disease (GVHD) has been observed in severe combined immunodeficient (SCID) mice injected intraperitoneally (IP) with human blood lymphocytes (hu-PBL–SCID), which raised the question as to whether GVHD in such a distant species is sporadic or suppressed because of immunologic reasons. After screening for blood T-cell chimerism, we hereby describe generalized lethal xenogeneic human GVHD in unconditioned SCID chimeras, which resembles GVHD in SCID mice injected with allogeneic lymphocytes. We adapted an immunocytochemical slide method for minute cell numbers, which allowed us to follow, by multimarker phenotyping of weekly mouse-tail bleeds, the chimeric status of 100 hu-PBL–SCID injected with 10^7 or 10^8 hu-PBL of Epstein-Barr virus$^-$ (EBV$^-$) donors. More than half of the mice showed no or less than 2% T cells. However, 13% to 21% developed substantial blood T-lymphocyte chimerism (10% to 80% human CD$^+$ cells) and high mortality. Immunohistology showed more human CD8$^+$ than CD4$^+$ T cells in the splenic white pulp. The cells developed HLA-DR activation markers and infiltrated the red pulp where human B cells also appeared. Expression of activation and proliferation markers increased within 5 to 6 weeks. Many human CD3$^+$ cells were also found in the portal triads of the liver and in the lung, pancreas, and kidney. The thymus also became heavily infiltrated. The intestines and skin of hu-PBL–SCID were less infiltrated by donor cells than in SCID with allogeneic GVHD. The tongue contained almost no human T cells. Our data show that a relatively low overall incidence of human xenogeneic GVHD, even when high numbers of human PBL are injected, is the consequence of a dichotomy between mice with no or transient T-cell chimerism and a minority of mice with high-blood T-lymphocyte chimerism and GVHD mortality.

INFORMATION on human blood lymphocyte (hu-PBL) chimerism in severe combined immunodeficient (SCID) mice is scarce, although it is evident that, for unknown reasons, it is usually disappointingly low (<2%), both in SCID-hu chimeras coimplanted with human fetal thymus and liver tissue[1,2] and in hu-PBL–SCID chimeras[3] injected with hu-PBL.[4] Likewise, it is unclear why human graft-versus-host disease (GVHD) is a rare, ill-defined entity in SCID mice. Of 800 hu-PBL–SCID reconstituted with 20 to 50 × 10^6 PBL observed, only eight injected with PBL from the same donor displayed clinical symptoms consistent with transient GVHD.[5] The question was raised as to whether more fundamental immunologic reasons inhibit GVHD between distant species like humans and mice. Huppes et al[6] recently reported an atypical "discordant human xenogeneic" GVHD in PBL-injected young preirradiated SCID and immunodeficient CBA/n mice, the inducibility of which they related to their lack of natural antibodies.

In the present study we wondered whether failure to develop xenogeneic GVHD in hu-PBL–SCID was related to low T-cell chimerism. To avoid having to kill individual hu-PBL–SCID for an immunophenotypic analysis, we adapted an immunocytochemical method that allowed us to type minute numbers of blood lymphocytes by fixing them electrostatically onto poly-L-lysine-coated slides. Thus, we could follow the chimeric status of 100 mice by weekly tail bleeds, and we noted a minority of unconditioned adult SCID mice with substantial T-cell chimerism and morbidity. Detailed immunohistochemical analysis of these mice showed GVHD-like donor cell infiltrates and other changes that we also found in GVHD of allogeneically reconstituted SCID, but not in congenic SCID chimeras.

MATERIALS AND METHODS

Mice. C.B-17 scid/scid mice were originally obtained from H. Wagner, University of Ulm, Germany, with the kind permission of M.J. Bosma. They were bred and maintained under pathogen-free conditions in our animal facility. They were housed in horizontal laminar-flow cabinets and were given autoclaved food (Altromin 1314 fortified, Lage, Germany). None of the SCID mice used showed substantial leakiness. Balb/c and C57Bl/6 (Thy-1.1) mice, originally obtained from Jackson Laboratories (Bar Harbor, ME), were also kept at the GSF animal facilities.

Transplantation of lymphoid cells. Usually 10^8 hu-PBL or 5 × 10^6 spleen cells from congenic Balb/c mice or from allogeneic C57BL/6 (Thy-1.1) were injected intraperitoneally into adult, 6- to 8-week-old unconditioned SCID mice.

Preparation of hu-PBL. Healthy human blood donors were serologically tested for their Epstein-Barr virus (EBV) status at the Munich University Hospital Großhadern. Isolation of sterile hu-PBL before intraperitoneal (IP) injection included (1) density-gradient centrifugation with Ficoll Hypaque (d = 1.077; Biochrom, Berlin, Germany), (2) assessment of viability by trypan blue exclusion, and (3) aliquoting of donor PBL in approximately 750 μL of cell-suspension containing 10^8 cells/SCID mouse.

Antibodies. The antibodies used for immunohistochemistry and immunocytochemistry are listed in Table 1. We thank P.C.L. Beverly, London, UK, H.R. McDonald, Lausanne, Switzerland; and U. Kummer, K. Reinecke, and R. Schuh, Munich, Germany, for providing the antibodies. Mouse and rat normal sera were used as negative controls. Peroxidase-conjugated or alkaline phosphatase-conjugated mouse antirat IgG (H + L) and rat antimouse IgG (H + L), which were used as labeled secondary antibodies, were obtained commercially from Jackson Immunoresearch, West

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Table 1. Antibodies Used for Immunocytochemistry and Immunohistochemistry (Source in Parentheses)

	Target Cells	
Specificity	Human	Mouse
CD3	UCHT1 (mouse)*	17A2 (rat)†
CD4/L3T4	Anti–Leu-3a (mouse)‡	RmCD4-1‖
	38/II/8 (rat)§	
CD8/Ly-2	Anti–Leu-2a (mouse)‡	RmCD8-1‖
	38/I/10 (rat)§	
CD11c	Anti–Leu-M5 (mouse)‡	
Mac1		M1/70 (rat)¶
HLA-DR/Ia	IFH-Ia 7510 (mouse)#	M5/114 (rat)**
CD20	Coulter Clone B1 (mouse)††	
CD25	B1-49.9 (mouse)‡‡	AMT-13 (rat)§§
Nuclear Ki67	Ki67 (mouse)‖‖	
Ig	μ-chains (rabbit)‖‖	IgG (H + L) (rat)¶¶
	λ-Light chains (rabbit)‖‖	IgG (H + L) (goat)¶¶
	κ-Light chains (rabbit)‖‖	

* P.C.L. Beverly, London, UK.
† H.R. McDonald, Lausanne, Switzerland.
‡ Becton Dickinson, Mountain View, CA.
§ R. Schuh, München, Germany.
‖ K. Reinecke, München, Germany.
¶ Serotec, Oxford, Germany.
# U. Kummer, München, Germany.
** Boehringer, Mannheim, Germany.
†† Coulter Immunology, Hialeah, FL.
‡‡ Immunotech S.A., Marseille, France.
§§ GIBCO BRL, Gaithersburg, MD.
‖‖ Dakopatts, Glostrup, Denmark.
¶¶ Jackson Immunoresearch, West Grove, CA.

Grove, CA. Peroxidase-labeled avidin was obtained from Vector Lab, Burlingame, CA, and alkaline phosphatase-labeled avidin from Sigma, Deisenhofen, Germany.

Immunohistochemistry. Spleen, lymph nodes, thymus, bone marrow (BM), liver, kidney, skin, heart, and tongue were removed at weekly intervals up to 8 weeks, starting 1 week after transplantation. Additional moribund-transplanted mice were killed up to 12 weeks postinjection of hu-PBL. Organs were frozen in liquid nitrogen and 5-μm–thick cryostat sections were air-dried, fixed in acetone for 10 minutes, incubated with the antibodies for 1 hour, then washed in Tris-buffered saline. Peroxidase activity was shown with amino-ethyl-carbazol. The tissue sections were counterstained with hematoxylin.

Double-labeling experiments. Coexpression of T-cell antigens and major histocompatibility complex (MHC) class II antigens or interleukin-2 (IL-2) receptor (CD25) was shown by the following consecutive incubations: (1) mouse or rat antibodies against HLA-DR, human CD25, mouse Ia, or mouse T-cell antigens; (2) peroxidase- or alkaline phosphatase-labeled rat antimouse Ig or mouse antirat Ig; (3) mouse or rat normal serum for saturation of secondary antibodies; (4) biotin-labeled antihuman CD3, antimouse T-cell antigen, or antimouse CD25; (5) alkaline phosphatase- or peroxidase-labeled avidin.

Immunocytochemistry. Parallel studies were performed by analyzing 100-μL samples of tail blood of SCID-hu–chimeric mice at regular intervals. Briefly, tail-blood samples were collected in heparinized 50-μL capillary tubes, and diluted with minimum essential medium (MEM) (100 μL). Red blood cell (RBC) lysis was performed by incubating the blood-MEM suspension with lysis buffer (Becton Dickinson, Mountain View, CA), followed by a separation of damaged cells by Percoll-density-gradient-centrifugation (d = 1.069). Immunocytochemical staining was performed on cells bound to poly-L–lysine spots on multispot slides, according to a method described by Bross[7] and modified by Kranz et al.[8,9] Heart-blood samples were investigated immunocytochemically in hu-PBL–SCID mice killed for multimarker immunohistochemistry at weekly intervals up to 8 weeks after reconstitution. Untreated SCID mice were used for negative controls and positive controls were donor lymphocytes for immunocytochemistry and palatine tonsils for immunohistochemistry.

RESULTS

Incidence of human blood T-cell chimerism in hu-PBL–SCID. One hundred unconditioned SCID mice were injected intraperitoneally (IP) with PBL isolated from the blood of healthy donors. The mice were typed for human $CD3^+$ cells by weekly tail bleeds. Table 2 summarizes the incidence of T-cell chimerism in 84 hu-PBL–SCID mice. Although groups of usually 10 to 20 SCID were reconstituted at different days from different donors, a substantial proportion of SCID consistently failed to show $CD3^+$ chimerism. A comparable number of SCID remained low-chimeric (<2%). Unstained leukocytes were mostly murine granulocytes and a few Thy-1^+ cells. In Table 2, 13% to 21% developed more than 10% $CD3^+$ lymphocytes (10% to 80%). Young, 4-week-old mice showed comparable chimerism. Injection of only 10×10^6 PBL did not reduce the incidence of chimerism. Chimerism occurred independently of the type of donor lymphocytes, as shown in Table 3, where mice receiving PBL from the same donor developed variable or no T-cell chimerism at all.

Follow-up phenotyping of individual SCID injected with PBL from EBV^- donors. First appearance of human $CD3^+$ cells in the peripheral blood (PB) was noted within 2 to 3 weeks postinjection. The percentage was generally low (<10%) and morphologic evaluation showed only small lymphocytic HLA/DR^- cells. Most mice showed no signs of weight loss or other clinical symptoms. In the following weeks, two patterns became obvious. In about half of all mice, human T-cell chimerism disappeared (Fig 1). These mice showed no clinical symptoms suggestive of GVHD. A minority of hu-PBL–SCID developed an increase in T-cell

Table 2. Human $CD3^+$ T-Cell Chimerism in the Blood of Unconditioned hu-PBL–SCID Mice

	Cells Injected		
T-Cell Chimerism*	10^8 PBL IP†	10^8 PBL IP‡	10^7 PBL IP†
Negative	24/44 = 54.5%	5/12 = 41.7%	8/28 = 28.6%
≤2%	12/44 = 27.3%	5/12 = 41.7%	10/28 = 35.7%
3%-10%	2/44 = 4.6%	0	4/28 = 14.3%
>10%	6/44 = 13.6%	2/12 = 16.6%	6/28 = 21.4%
Total	20/44 = 45.5%	7/12 = 58.3%	20/28 = 71.4%

* Weekly tail bleeds of individual hu-PBL–SCID were immunocytochemically typed for human $CD3^+$ lymphocytes.
† PBL separated from blood of healthy volunteers were injected IP in 8- to 12-week-old SCID mice.
‡ PBL were injected in 4-week-old SCID mice.

Table 3. Human T Lymphocytes in the Blood of SCID Mice IP Injected With 10^7 PBL From Two Donors (in %)

		Week After Injection of Human PBL											
Mouse	Donor	1	2	3	4	5	6	7	8	9	10	11	12
A	a			0		≤2	0		0		/		
B	a			0		≤2	≤2		5		/		
C	a			0		≤2	≤2		3		9		18
D	a			0		0	0		0		0		0
E	a			0		≤2	0		/				
F	a			0		0	0		0		≤2		0
G	a			0		≤2	≤2		≤2		4		8
H	a			0		0	3		13		12		40
I	a			0		≤2	≤2		3		8		6
K	b				0	≤2				5		7	
L	b				0	0				0		0	
M	b				0	0				32			
N	b				≤2	≤2				36			

Each mouse was injected with 10^7 human PBL IP. Mouse A to I received PBL from donor a; mouse K to N received PBL from donor b.

Abbreviation: /, mouse had died.

values of more than 10% with maximal variance between 18% and 85% of all PBLs, murine cells included, 7 to 8 weeks after injection. $CD4^+/CD8^+$ cell ratios varied between 0.34 and 3.94. $CD3^+$ cells became activated with coexpression of HLA-DR (up to 85% $CD3^+$ and 68% $HLA\text{-}DR^+$ cells) and blast-cell morphology of increased cell size and typical heterochromatic structure. Concurrent with these findings, the mice developed clinical symptoms, starting with loss of activity, abnormal posture, intermittent diarrhea, anemia, and weight loss of more than 20% during the advanced stage. The fatal outcome was indicated by general kachexia and respiratory disturbances. Neither B cells, natural killer (NK) cells, nor monocytes were detected in the test blood.

Immunohistochemistry. Immunohistologic examination showed three different patterns: mice without human cells, mice with low chimerism, and mice with low chimerism in the early phase switching to substantial T-cell chimerism in the later phase (3 to 4 weeks post injection).

Early findings in spleen and lymph nodes. Up to about 3 weeks postinjection of PBL, few or several human $CD3^+$ cells were found in the white pulp region. The red pulp rarely contained human $CD3^+$ lymphocytes (Fig 2A). Most were $CD8^+$. $CD4^+$ and Ig^+ human B lymphocytes were rare. One week after PBL injection, the human cells were $HLA\text{-}DR^-$. After 2 to 3 weeks, some of the human cells in the spleen were $HLA\text{-}DR^+$. The white pulp regions of the SCID mice were narrow and contained single mouse $L3T4^+$ cells peripheral to the human cells. Mouse $Ly\text{-}2^+$ cells were even more rare. Many cells in the red pulp were mouse Ia^+. Lymph nodes contained human cells of a similar phenotype. They were scattered throughout the SCID lymph node, in which no clear distinction between cortex and paracortex was possible.

Early findings in other organs. All other organs examined, including thymus and BM, did not contain human cells, except for occasional intravascular human $CD3^+$ lymphocytes in the lumen of blood vessels.

Later findings in spleen and lymph nodes. From about 4 weeks post injection onward, severe alterations were found in the spleen. Human $CD3^+$ cells were scattered throughout the whole splenic white and red pulps. In the red pulp, they prevailed in regions adjacent to the splenic capsule and to splenic trabeculae. Two of 25 SCID mice injected with human PBL 5 weeks before or earlier displayed a white pulp that was almost atrophic and devoid of human cells. Most human cells were $CD3^+$ (Fig 2B), and, in the red pulp, of the $CD8^+$ type (Fig 2C). The majority of $CD3^+$ cells in the white pulp were weakly $CD4^+$ (Fig 2D). A high percentage of the human cells were $HLA\text{-}DR^+$ (Fig 3A), and many were proliferating cells expressing Ki67. Double-labeling experiments showed that between 30% and 80% of the total $CD3^+$ and $HLA\text{-}DR^+$ cells were $CD3^+HLA\text{-}DR^+$ double-positive, whereas a smaller percentage was $CD3^+CD25^+$ double-positive. Varying numbers of human polyclonal Ig^+ lymphoid cells had also settled in the white pulp, showing either a broadly stained rim or plasma cell-like appearance with intensely stained cytoplasm (Fig 3B). Several $CD11c^+$ cells with a lymphocytic appearance were distributed in the white and red pulps.

Mouse $L3T4^+$ or $Lyt2^+$ cells were found less frequently in the splenic white pulp during this later stage of spreading human T-cell infiltration, compared with hu-PBL–SCID that had not become chimeric. Several SCID mice showed enhanced hematopoietic activity in the red pulp.

At the later stage, lymph nodes were also densely populated by human cells. Here again, most of the $CD3^+$ cells were $CD8^+$, whereas $CD4^+$ cells were more loosely distributed. Many T cells were also $HLA\text{-}DR^+$ and $Ki67^+$. Numerous polyclonal Ig^+ cells were intermingled. Mouse T cells were very rare.

Later findings in BM and thymus. Both contained small or larger aggregates of human $CD3^+$ cells, mostly $CD8^+$ $HLA\text{-}DR^+$ and also $Ki67^+$. Sometimes single Ig^+ cells were also found.

SCID mouse thymi had a weakly stained mouse Ia^+ framework populated with $Thy\text{-}1^+$ and mouse cells showing cytoplasmic staining for CD3. Mouse $L3T4^+$ and mouse $Ly\text{-}2^+$ cells were rarely found as single cells or in small clusters. Thymus glands with heavy human-cell infiltrates contained fewer or none of the above-mentioned mouse thymocytes.

Later findings in nonlymphoid organs. Later-stage chimerism in SCID mice also contained many human cells in nonlymphoid organs. Severe alterations were found in the liver where an intense accumulation of mononuclear cells in portal triads had occurred (Fig 3C). Dense infiltrates consisted of a mixture of $CD3^+$, $CD8^+$, $HLA\text{-}DR^+$ cells; of varying amounts of $CD4^+$, human Ig^+, $Ki67^+$ cells; and a few mouse $Ly\text{-}2^+$, mouse $L3T4^+$, and mouse $Mac1^+$ cells. Similar infiltrates were observed in the kidney between the tubules and under the pelvic epithelium. Kidney glomerula contained polyclonal human Ig. The epidermal and subepidermal lamina propria of the intestines contained some human $CD3^+$ and $HLA\text{-}DR^+$ cells, more $CD8^+$ than $CD4^+$. In contrast, the epidermal layers of the tongue were almost devoid of human cells. Two of 25 hu-PBL–SCID showed

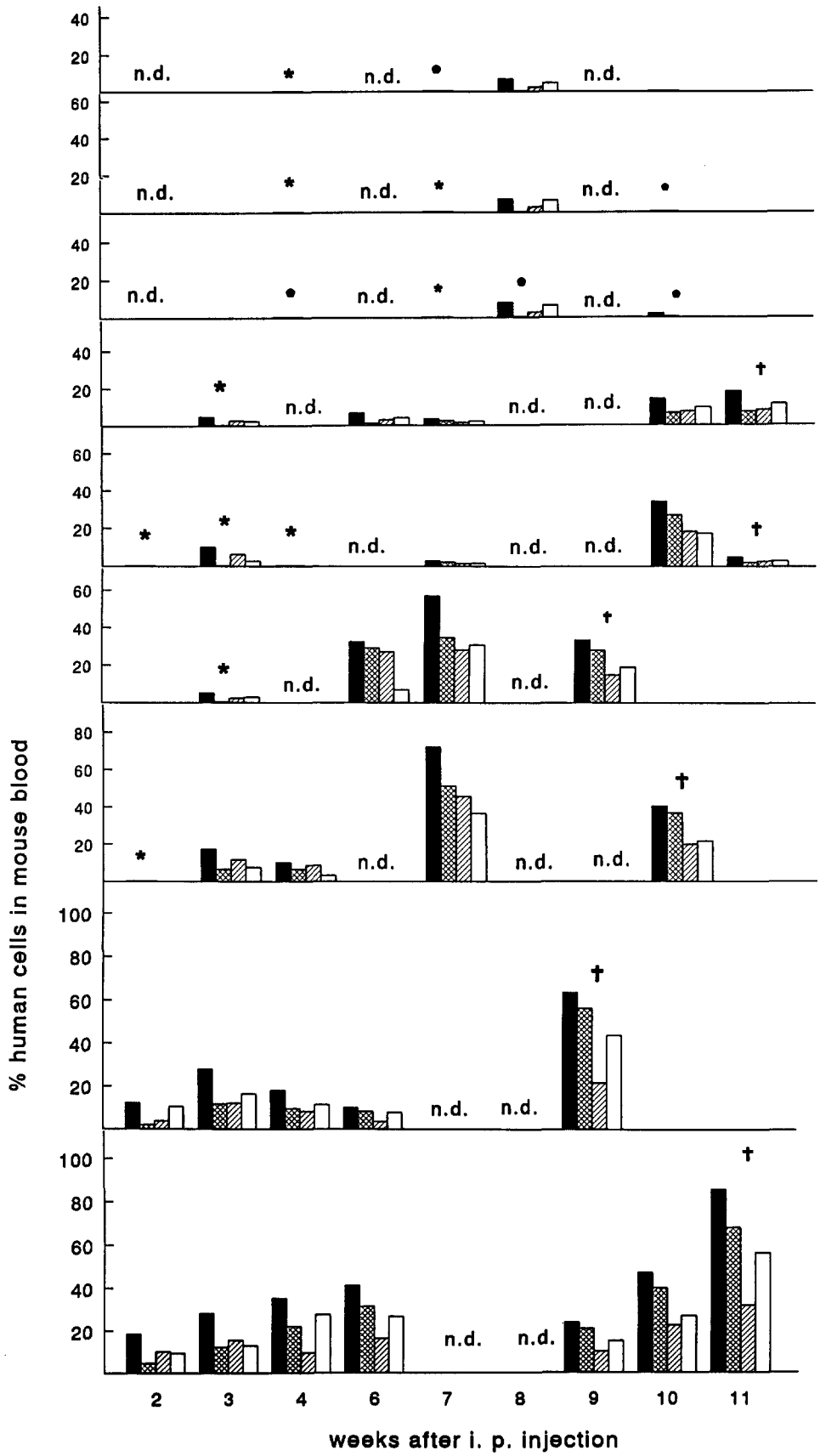
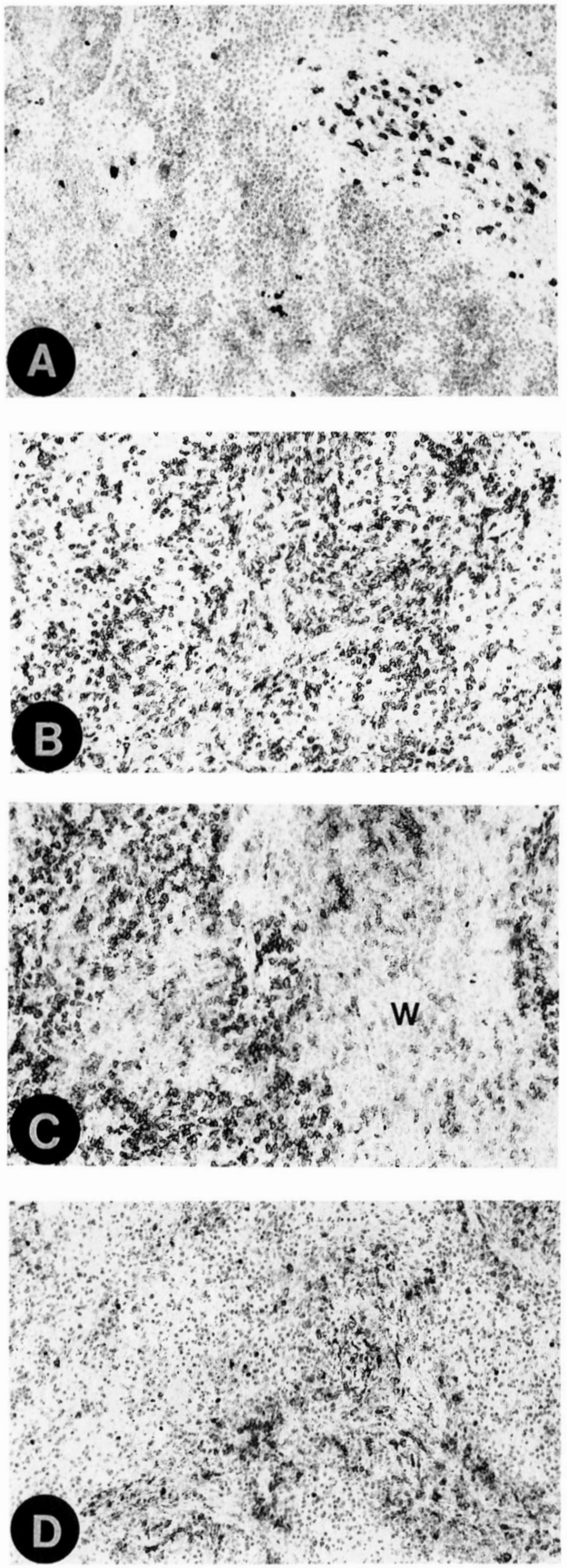


Fig 1. Follow-up immunophenotyping of tail blood of nine hu-PBL–SCID. Percent of human CD3$^+$ (■), HLA-DR$^+$ (▩), CD4$^+$ (▨), and CD8$^+$ (□) cells in tail blood of SCID mice injected with human PBL. n.d., not done; blank, 0%; *, below 0.5%; †, killed when in bad condition.



moderate focal or isolated human CD3$^+$ HLA-DR$^+$ (mostly CD8$^+$) infiltrations of skin basal epidermal and subepidermal areas, especially in and around hair follicles (Fig 3D). The number of mouse Ia$^+$ cells in the basal layers of epidermis was higher than in hu-PBL–SCID that had no skin infiltrations.

Immunohistology of allogeneic and congenic SCID chimeras. To delineate xenogeneic GVHD in hu-PBL–SCID from the chimeric cell patterns of mouse-into-SCID-mouse chimeras, we compared immunohistochemical changes, which we observed after intraperitoneal reconstitution with allogeneic or congenic lymphocytes. For discrimination between donor and recipient T cells in allogeneic GVHD, 5×10^6 C57BL/6-Th-1.1 spleen and lymph node cells were injected in Thy-1.2$^+$ SCID. In contrast to the delayed T-cell reconstitution observed in hu-PBL–SCID, all SCID mice injected with allogeneic lymphocytes showed donor-cell accumulations in SCID peripheral lymphoid tissues, already from day 5 postinjection onward. In congruence to hu-PBL–SCID mice, donor Thy-1.1$^+$ (Fig 4A), CD3$^+$ mouse cells were scattered throughout the whole spleen. Ly-2$^+$ cells were present in the white and red pulps (Fig 4B). The majority of donor T cells in the white pulp were L3T4$^+$. For comparison, the pattern of Ly-2$^+$ and L3T4$^+$ cells in the spleen of SCID mice injected with congenic Balb/c lymphocytes is shown in Fig 4D and 4E. In allogeneic SCID chimeras, many allogeneic lymphocytes in the red as well as in the white pulp expressed CD25 and mouse Ia$^+$ activation markers. Double-labeling experiments showed that many of the CD3$^+$ and also many of the Ly-2$^+$ mouse T cells were CD3$^+$CD25$^+$ or Ly-2$^+$CD25$^+$ double-positive, respectively, whereas only a few of the L3T4$^+$ cells were L3T4$^+$CD25$^+$ double-positive. About 20% to 90% of the total Ia$^+$ and CD3$^+$ or Ly-2$^+$ cells were Ia$^+$CD3$^+$ or Ia$^+$Ly-2$^+$ double-positive.

Mouse Ig$^+$ lymphoid cells, as well as large cells with a broad intensely stained (Ig$^+$) cytoplasmic rim, occurred in the white and the red pulp. The numbers of such Ig$^+$ cells were increased around the white pulp areas.

Lymph nodes were interspersed with many Ly-2$^+$ and fewer L3T4$^+$ donor-type lymphocytes. Many cells were mouse-Ia$^+$. The thymus contained more Thy-1.1$^+$ donor cells than host Thy-1.2$^+$ thymic cells. Small and scattered aggregates of Ly-2$^+$ and L3T4$^+$ cells were present. The general SCID thymus structure was unchanged, in contrast to SCID mice transplanted with syngeneic cells where thymic cortical areas developed. In the intestinal epithelium, Ly-2$^+$ cells were more frequent than L3T4$^+$ lymphocytes, in con-

Fig 2. T-cell colonization in the spleen of hu-PBL–SCID chimeras. (A) Early colonization of SCID mouse white pulp with human CD3$^+$ lymphoid cells 1 week after IP injection of human PBL. The red pulp contains only single human T cells. (B through D) Later period of spleen colonization 7 weeks after IP injection; (B) many human CD3$^+$ cells are distributed throughout the red and white pulp; (C) human CD8$^+$ cells predominate in the red pulp and are more infrequent in the white pulp (w); (D) human CD4$^+$ cells predominate in the white pulp. Original magnification × 110.

trast to SCID mice transplanted with congenic Balb/c spleen and lymph node cells, where L3T4$^+$ lymphocytes were more numerous than Ly-2$^+$ cells. Liver and kidney showed an infiltration of portal triads or intertubular tissue, respectively, with donor lymphocytes, most of which were Ly-2$^+$. Tongue and skin differed from those in hu-PBL–SCID insofar as the epithelium was always infiltrated by donor Ly-2$^+$ cells. Three to five layers of epithelial cells above the basal membrane consisted mostly of mouse-Ia$^+$ cells, whereas congenic SCID chimeras contained only isolated Ia$^+$ cells that were dendritic in appearance.

DISCUSSION

The present study documents the immunohistologic picture of generalized xenogeneic human GVHD in adult unconditioned SCID mice that is accompanied with substantial mortality. How is its observation in hu-PBL–SCID mice compatible with the experience of other investigators who find it very rare if not almost absent?[5] Our immunocytochemical screening for substantial blood T-lymphocyte chimerism in individual SCID mice may have helped to single out candidates with ongoing GVHD. It may be more specific than flow cytometry of organ cells or pooled blood of hu-PBL–SCID mice on predetermined dates. On the other hand, our typing of larger numbers of hu-PBL–SCID also showed a majority of mice without chimerism or with loss of chimerism before GVHD could develop. About 15% to 20% of all hu-PBL–SCID exhibited a chimerism of more than 10% human PBL. All those mice showed immunohistologic signs of GVHD. Even conditioning SCID mice with preirradiation, growth hormone, or antibodies reacting with NK cells could so far not eliminate the unpredictability of xenogeneic T-cell chimerism and GVHD (U. Zengerle, unpublished observation, September 1992). Variability of chimerism also remained among SCID mice injected with PBL of the same donor (Table 1). Clearly, variability of human-cell chimerism in SCID mice renders any evaluation of conditioning methods difficult. This variability remains a challenge for the transfer of a functional human immune system to SCID mice.

Late-phase findings in our hu-PBL–SCID with GVHD resembled GVHD in SCID chimeras that we had injected IP with allogeneic lymphocytes a few days before (Table 4). The reasons why allogeneic cells produce GVHD faster than xenogeneic cells could be that (1) they can migrate better, (2) their growth is better sustained, and (3) activation by

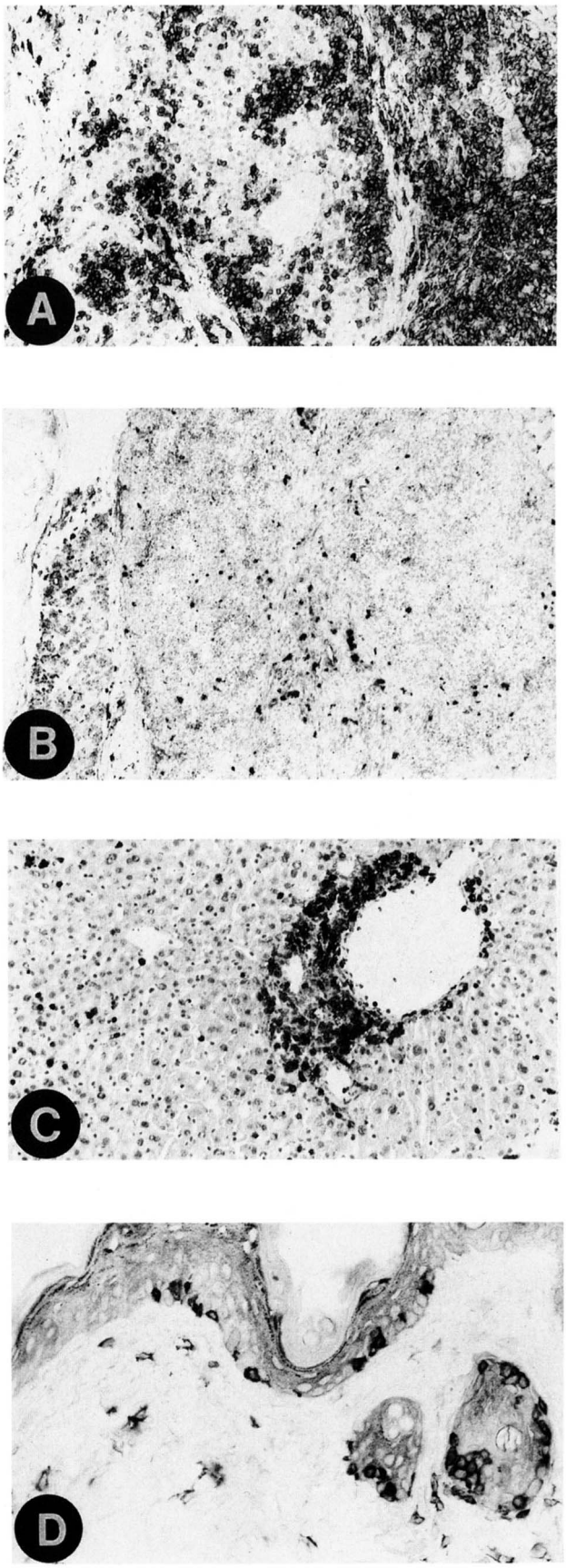


Fig 3. Donor-cell infiltration in hu-PBL–SCID chimeras. (A) Most human cells in the SCID mouse spleen are activated HLA-DR$^+$ cells; (B) human Ig$^+$ cells with surface or cytoplasmic staining occur in the white as well as in the red pulp; (C) moderate infiltration of liver portal triads with CD3$^+$ (mostly also HLA-DR$^+$) human lymphoid cells (A through C), 7 weeks after injection of human PBL, original magnification × 110; (D) low infiltration of basal epidermal layers of skin and hair follicles with CD3$^+$ human cells, 12 weeks after injection of human PBL, original magnification × 280.

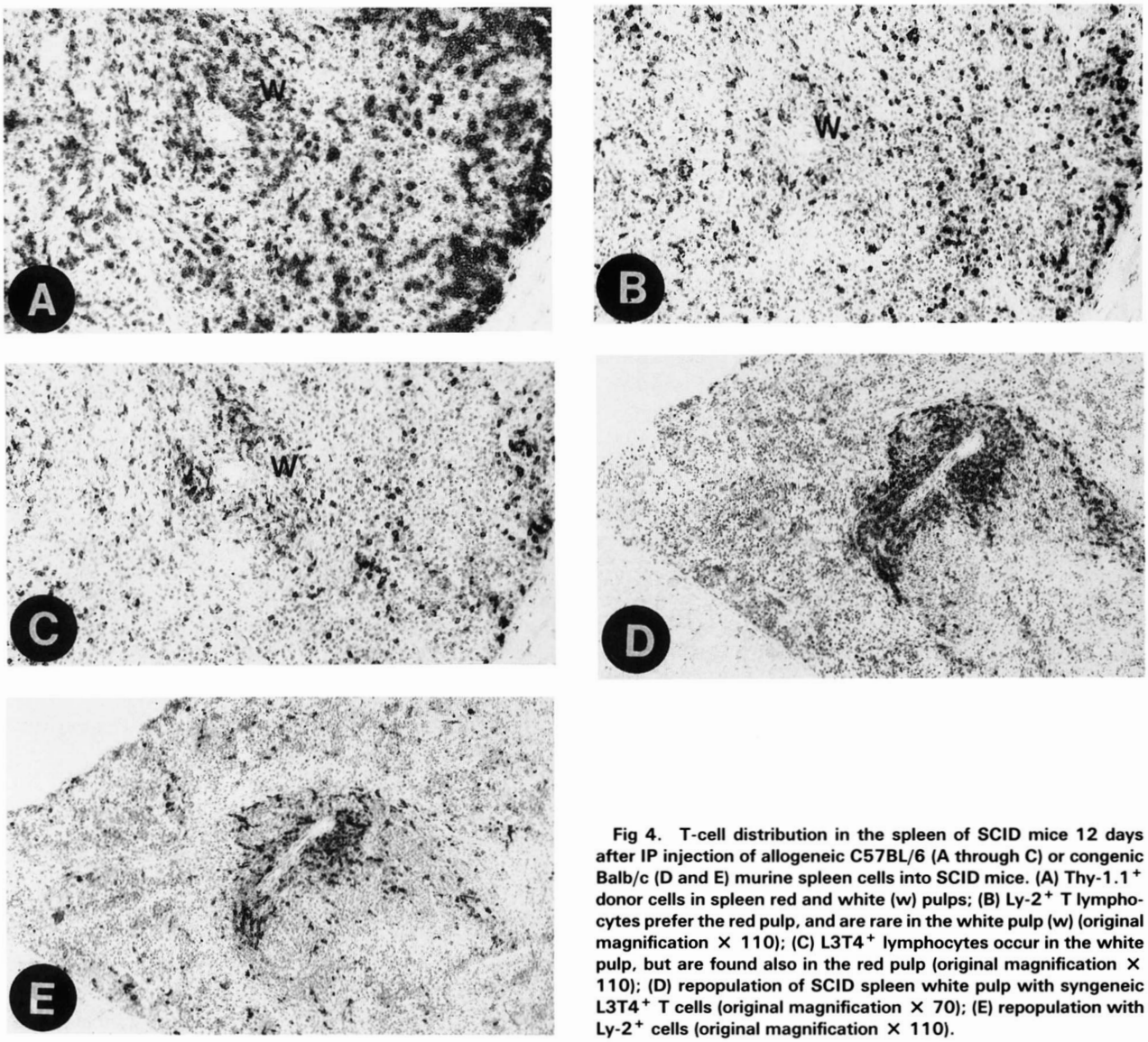


Fig 4. T-cell distribution in the spleen of SCID mice 12 days after IP injection of allogeneic C57BL/6 (A through C) or congenic Balb/c (D and E) murine spleen cells into SCID mice. (A) Thy-1.1$^+$ donor cells in spleen red and white (w) pulps; (B) Ly-2$^+$ T lymphocytes prefer the red pulp, and are rare in the white pulp (w) (original magnification × 110); (C) L3T4$^+$ lymphocytes occur in the white pulp, but are found also in the red pulp (original magnification × 110); (D) repopulation of SCID spleen white pulp with syngeneic L3T4$^+$ T cells (original magnification × 70); (E) repopulation with Ly-2$^+$ cells (original magnification × 110).

antigen-presenting cells is more matched in the allogeneic host than in the xenogeneic host. The findings were analogous in lymphatic and hematopoietic organs and in liver, intestines, kidney, and lung, and in a few animals also in the skin. Donor- and host-cell phenotypes with activation marker as well as localization were quite similar. Particularly, the abundance of activated T cells of the CD8$^+$ or Ly-2$^+$ subtype in the red pulp was highly specific, as well as the moderate prevalence of weakly CD4$^+$ or L3T4$^+$ cells in the white pulp region. Activated human T cells have been described in hu-PBL–SCID. Although they even reacted against mouse cells in vitro, no GVHD was observed.[4,10] A quickly deteriorating "discordant xenogeneic GVHD," after injection of high numbers (3×10^8) of EBV-untested human PBL into young SCID mice conditioned with high doses (400 mg) of cyclophosphamide, was found to have little resemblance to acute allogeneic GVHD but in later stages resembled a malignant process. Its massively invading human lymphocytes were observed in other immunodeficient mouse strains as well, and attributed to the lack of natural antibodies in these mice.[6] We found similar tumorous infiltrations, only in hu-PBL–SCID that were injected with PBL from EBV$^+$ donors (data not shown). Cellular infiltrates in the periportal space, consistent with microscopic GVHD, were reported in SCID mice injected with PBL of a laboratory worker with a history of mouse exposure, raising the issue of sensitization as a relevant factor for development of xenogeneic GVHD.[11]

T-cell infiltration in the tongue was not found in "discordant xenogeneic GVHD,"[6] nor in the present study, except for very rare single T cells. The reason for this difference between xenogeneic and allogeneic GVHD is not clear. It may be caused by differential distribution or presentation of xenogeneic and allogeneic target antigens in the host. Also, host cells responsible for lymphocytic infiltration may not be activating the xenogeneic lymphocytes. In fact, mouse Ia antigens in the tongue were not activated in xenogeneic GVHD, but found in increased amounts in our SCID mice with allogeneic GVHD. It is a known phenomenon in human, rat, and mouse GVHD.[12-14]

Table 4. Comparison of Immunohistochemical Findings in Xenogeneic and Allogeneic GVH Reactions in SCID Mice

Organ	Infiltrated*	Xenogeneic	Allogeneic
Spleen red pulp	$CD3^+$, $CD8^+$, $MHCII^+$ ($CD25^+$) (Ig^+ plasma cell-like cells)	+	+
white pulp	$CD3^+$ CD4 ($^+$) $MHCII^+$ ($CD8^+$)	+	+
Lymph node	$CD3^+$, $CD8^+$, $MHCII^+$ ($CD4^+$)	+	+
Thymus focal BM focal	$CD3^+$, $CD8^+$, $MHCII^+$ ($CD4^+$)	+	+
Liver periportal	$CD3^+$, $CD8^+$, $MHCII^+$ ($CD4^+$), ($CD25^+$)	+	+
Kidney, between tubules and under pelvic epithelium	$CD3^+$, $CD8^+$, $MHCII^+$	+	+
Intestines	$CD3^+$, $MHCII^+$, $CD8^+$ ($CD4^+$)	+	+
Tongue, between and under epithelial cells	$CD3^+$, $CD8^+$	−	+
Skin, focal in basal epidermal and subepidermal areas	$CD3^+$, $MHCII^+$, $CD8^+$	−/+ (2 of 25)	+
Onset of GVHD signs		From about 4 wk on	From d 5 on

* By predominant cell types, in parentheses: less frequent cell types.

An interesting histologic finding of mixed graft-versus-host and host-versus-graft reactivity, which did not appear to be outwardly detrimental, was described in hu-PBL–SCID with liver lesions and a dramatic increase in splenic murine hematopoiesis.[10] In our series, the latter was only found in a few chimeras, but was seen consistently in bovine-SCID mice that we had injected with bovine lymph node cells (data not shown).

In summary, we described the anatomical distribution of human lymphocyte subpopulations in unconditioned hu-PBL–SCID that, after settling in the lymphoid organs of the host, developed severe infiltrates of activated T and B cells in many organs of individual SCID mice. These changes were associated with a moribund condition of the mice before they were killed for immunohistologic analysis. The immunohistochemical changes were qualitatively very similar to those found in our control mice injected IP with allogeneic mouse PBL, but quantitatively less in the skin of the xenogeneic host and almost absent in the tongue. Morbidity with xenogeneic GVHD in hu-PBL–SCID started 5 weeks after injection of PBL compared with GVHD morbidity starting 1 week after IP injection of allogeneic lymphocytes in SCID mice.

Immunocytochemical follow-up of hu-PBL–SCID showed a majority of mice without chimerism or with transient low blood cell chimerism and 10% to 20% with substantial chimerism that can develop into full-fledged xenogeneic GVHD. We assume that the difficulty of inducing human xenogeneic GVHD in all SCID mice reflects a threshold condition rather than a fundamental barrier, although we do not expect that it can be overcome by increasing the PBL inoculum of 10^8 even more. Early events of cell engraftment and proliferation in the peritoneal cavity may be more important for the development of phylogenetically distant xenogeneic lymphopoiesis as well as for the triggering of xenogeneic GVHD by early lymphocyte activation.[15]

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