Alloimmune Injury and Rejection of Human Skin Grafts on Human Peripheral Blood Lymphocyte–Reconstituted Non-Obese Diabetic Severe Combined Immunodeficient β_2-Microglobulin-Null Mice

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Small animal models with the capacity to support engraftment of a functional human immune system are needed to facilitate studies of human alloimmunity. In the present investigation, non-obese diabetic (NOD) severe combined immunodeficient *(scid)* β_2-microglobulin-null (*B2m^{null}*) mice engrafted with human peripheral blood lymphocytes (hu-PBL-NOD-*scid B2m^{null}* mice) were used as *in vivo* models for studying human skin allograft rejection. Hu-PBL-NOD-*scid B2m^{null}* mice were established by injection of human spleen cells or PBLs and transplanted with full-thickness allogeneic human skin. Human cell engraftment was enhanced by injection of anti-mouse CD122 antibody. The respective contributions of human CD4$^+$ and CD8$^+$ cells in allograft rejection were determined using depleting antibodies. Human skin grafts on unmanipulated NOD-*scid B2m^{null}* mice uniformly survived but on chimeric hu-PBL-NOD-*scid B2m^{null}* mice exhibited severe immune-mediated injury that often progressed to complete rejection. The alloaggressive hu-PBLs did not require prior *in vitro* sensitization to elicit targeted effector cell activity. Extensive mononuclear cell infiltration directed towards human-origin endothelium was associated with thrombosis and fibrin necrosis. No evidence of graft-versus-host disease was detected. Either CD4$^+$ or CD8$^+$ T cells may mediate injury and alloimmune rejection of human skin grafts on hu-PBL-NOD-*scid B2m^{null}* mice. It is proposed that Hu-PBL-NOD-*scid B2m^{null}* mice engrafted with human skin will provide a useful model for analysis of interventions designed to modulate human allograft rejection. Exp Biol Med 228:1096–1104, 2003

Key words: hu-PBL-*scid*; skin allograft; graft rejection; transplantation

A major goal of transplantation immunology is to achieve allograft survival in the absence of chronic immunosuppression (1–3). The realization of this goal would lead to durable graft survival without the toxicity and complications of long-term immunosuppression. As knowledge of immune system activation and tolerance induction advances, a large number of immune-based therapeutic reagents are being developed for clinical investigation (2, 4–6). Study of these reagents invariably originates using small animal models, primarily mice and rats, that ultimately provide the underlying mechanistic rationale for developing human-targeted therapy. Translation of rodent results to humans is not always successful, however, and many approaches effective in small animal models fail in larger animal species and in humans (2, 5, 7). Importantly, reagents directed towards human immune cells and molecules, particularly anti-human lymphocyte antibodies like alemtuzumab (Campath-1H, Millennium Pharmaceuticals, Cambridge, MA) (8), often cannot be tested directly in rodent models due to species specificity of the reagent.

Because testing investigational therapeutics in humans is often constrained by technical and ethical considerations, most experimental reagents are evaluated in nonhuman primates prior to initiation of phase I clinical trials. However, high cost, limited primate availability, and genetic heterogeneity hamper such studies, in particular the rapid screen-

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ing of new reagents. Consequently, expensive small-scale studies are often marred by unacceptable variability, necessitating additional experimentation to achieve statistical validation. In addition, human immune-based therapeutics must have a high level of cross-reactivity with the nonhuman primate used in the analysis.

These limitations have generated a need for standardized small animal models that will facilitate the *in vivo* study of human immune responses without putting patients at risk and that are not restricted by limited animal availability and prohibitive cost. Our laboratories have developed mouse models that support human lymphocyte engraftment for *in vivo* studies of human immune responses and efficacy analyses of human-specific therapeutics (9, 10). We have based our approach on early observations that human fetal hematopoietic tissues (11) and adult peripheral blood mononuclear cells (PBLs) (12) can engraft in C.B-17 mice homozygous for the severe combined immunodeficient *Prkdc*^{scid} (*scid*) mutation (9). Unfortunately, low levels of engraftment and an inconsistent ability to generate functional human immune responses have compromised the utility of this model. The latter issue has remained problematic despite numerous reports suggesting that primary and secondary immune responses can be elicited in hu-PBL-*scid* mice (13–15).

Our approach to overcoming these limitations is based on our finding that genetic modification of the mouse recipient improves the engraftment and function of the human immune system. We backcrossed the *scid* mutation onto the non-obese diabetic (NOD)/Lt strain, which expresses multiple defects in innate immunity, and the resulting NOD/LtSz-*Prkdc*^{scid}/*Prkdc*^{scid} mice (hereafter referred to as NOD-*scid* mice) display much improved engraftment of human cells (9). NOD immunodeficient mice are now the strain of choice for most human xenograft studies (9, 10). More recently, we backcrossed the β_2-microglobulin-null (*B2m*^{null}) allele onto the NOD-*scid* strain and documented that NOD-*scid B2m*^{null} mice support even higher levels of human hematopoietic cell engraftment (16). β_2-Microglobulin is a light chain for three disparate groups of molecules. These include class I major histocompatibility complex (MHC) molecules, the protective Fc receptor (FcRn), and the HFE iron-binding molecule that is required for appropriate iron uptake and storage. β_2-Microglobulin is required for the transport of MHC class I molecules to the cell surface. Thus *B2m*^{null} mice lack MHC class I expression and have severely depressed natural killer (NK) cell activity. The FcRn molecule normally protects IgG from catabolism. Its absence in NOD-*scid B2m*^{null} mice results in shortened IgG half-life in the circulation as we have previously reported (16). Although the basis for the improved xenochimerism in NOD-*scid B2m*^{null} remains unclear, we have speculated that residual NK activity, which is lower than that detected in other *scid* strains, may play a key role (16, 17).

We now report the use of the hu-PBL-NOD-*scid B2m*^{null} mouse to establish a model system for human skin allograft rejection. We demonstrate that engrafted human immune cells can reproducibly injure and in some cases ultimately reject full-thickness human skin allografts. Our data suggest that human lymphocytes can initiate allograft rejection in hu-PBL-NOD-*scid B2m*^{null} mice and that CD4$^+$ or CD8$^+$ cells can mediate the final destructive effector cell activity.

Methods and Materials

Animals. NOD/LtSz-*Prkdc*^{scid}/*Prkdc*^{scid} *B2m*^{null} (NOD-*scid B2m*^{null}) male and female mice 5–9 weeks old were obtained from colonies maintained by L.D.S. at The Jackson Laboratory (Bar Harbor, ME). All animals were certified to be free of Sendai virus, pneumonia virus of mice, murine hepatitis virus, minute virus of mice, ectromelia, lactate dehydrogenase–elevating virus, GD7 virus, Reo-3 virus, mouse adenovirus, lymphocytic choriomeningitis virus, polyoma, *Mycoplasma pulmonis* infection, and *Encephalitozoon cuniculi* infection. The mice were housed under specific pathogen-free conditions in microisolator cages and given *ad libitum* access to autoclaved food. All immunodeficient mice were maintained on an alternate schedule of autoclaved water and a suspension of sulfamethoxazole-trimethoprim (Goldline Laboratories, Ft. Lauderdale, FL) (16). Animals were maintained in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996) and the guidelines of the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

Antibodies. Directly conjugated anti-human CD3, CD4, CD8, CD20, CD33, CD45, and CD64 fluorochrome-conjugated monoclonal antibodies (mAbs) and anti-mouse CD45 fluorochrome-conjugated antibody used for flow cytometry were obtained from BD PharMingen (La Jolla, CA). Anti-mouse CD122 (interleukin-2 [IL-2] receptor β-chain, clone TM-β1, rat IgG2b) (18), the gift of T. Tanaka (Osaka University Medical Center), anti-human CD4 (clone OKT4, ATCC, Manassas, VA), and anti-human CD8 (clone OKT8, ATCC) were produced as ascites. Fibrin debris was removed from the collected ascites by filtration through nylon wool, and aliquots were frozen until used. Concentrations of mAb were quantified using anti-mouse IgG radial immunodiffusion plates (The Binding Site, San Diego, CA) (17) or by enzyme-linked immunosorbent assay for rat Ig. For *in vivo* depletion studies, the mAbs were diluted to a concentration of 1 mg/ml in phosphate-buffered saline (PBS) immediately prior to injection.

Skin Transplantation. Discarded full-thickness human foreskin, obtained with internal review board approval, was prepared and transplanted as described (19). Skin grafts were subjected to visual and tactile inspection three times weekly for evidence of rejection or graft injury. Graft injury was classified according to visible evidence of erythema,

thrombosis, and epithelial sloughing, affecting greater than 30% of the graft during the 36-day observation period.

Establishment of Hu-PBL-NOD-*scid B2m^{null}* Mice. Human xenochimeric mice were prepared using human spleen cells or human PBLs (both termed hu-PBL-NOD-*scid B2m^{null}* mice). Single-cell suspensions of spleen cells from human cadaveric donors were prepared as described (17). Briefly, spleen tissues were dissociated into single-cell suspensions and subjected to red blood cell lysis and three washes with RPMI-1640. Nucleated cell counts and viability were assayed by trypan blue exclusion using a hemocytometer and cell viability was greater than 85% in all cases. PBLs were prepared by Ficoll-Hypaque density gradient centrifugation. Using a dosage regimen described by Murray *et al.* (20), mice were injected intraperitoneally with 100–300 $\times$ 10^6 human cells coincident with skin grafting or 4 weeks after transplantation of skin grafts. Engraftment was facilitated by intraperitoneal injection of 1.0 mg rat anti-mouse IL-2 receptor β-chain (CD122) mAb (clone TM-β1) 12–24 hr prior to human cell engraftment. TM-β1 rat anti-mouse CD122 mAb recognizes the β-chain of the mouse IL-2R, is expressed on subpopulations of $CD8^+$ T cells and most $NK1.1^+$ cells, and has been shown to both attenuate NK activity and enhance human PBL xenochimerism in immunodeficient mice (13, 17, 21).

For analysis of the effects of *in vivo* anti-human CD4 and CD8 antibody treatments on human lymphocyte engraftment and the ability of human T cells to reject human skin allografts, NOD-*scid B2m^{null}* mice were injected intraperitoneally with anti-human CD4 or anti-human CD8 mAb (17). In these experiments 1 mg of anti-human CD8 mAb was injected immediately prior to cell transfer. However, depletion monotherapy against $CD4^+$ cells was delayed for 2 weeks based on our previous observation that human PBLs fail to engraft in NOD-*scid* mice if anti-CD4 mAb therapy is initiated at the time of lymphocyte injection (17, 22). One milligram of anti-human CD4 mAb was administered 2 weeks after human spleen cell injection.

Flow Cytometry. Red blood cells were removed from single-cell suspensions of spleens recovered from hu-PBL-NOD-*scid B2m^{null}* mice by hypotonic lysis, and the leukocytes were suspended in PBS plus 5% fetal bovine serum and prepared for two-color flow cytometry analysis as described (17). Multiparameter flow cytometry analysis used fluorescein isothiocyanate and phycoerythrin conjugated anti-human CD3, CD4, CD8, CD20, CD33, CD45, or CD64 mAb plus anti-mouse CD45 (Ly5). Following labeling, cells were fixed in PBS containing 1% paraformaldehyde and 0.02% NaN_3 and at least 10,000 events were acquired on Becton Dickinson FACS equipment (Becton Dickinson, San Jose, CA). The data were analyzed using WinList software (Verity Software House, Inc., Topsham, ME). For all analyses, mouse $CD45^+$ cells were concurrently analyzed and were excluded from calculated percentages of human cells. Matching isotype antibodies were used as negative controls and the reported values have been corrected using these isotype control values.

Histology and Immunohistochemistry. For immunohistochemistry, human skin grafts including adjacent margins of host mouse skin were recovered 30–36 days after skin transplantation, fixed in 10% buffered formalin, and embedded in paraffin. Sections (4 μm) were cut and stained with hematoxylin and eosin or used for immunohistochemistry. Immunohistochemical staining was performed with mAb specific for human CD45 (Fisher Scientific, Savannah, GA) or the UEA-1 lectin (Vector, Inc., Burlingame, CA) using a standard avidin-biotin complex methodology as described (23). Controls included omission of primary antibodies and the use of species-matched nonbinding control antibodies. The sections were developed using 3-diaminobenzidine and counterstained with hematoxylin for visualization by light microscopy. To monitor animals for evidence of graft-versus-host disease (GVHD), ear, tongue, intestine, liver, and pancreas were recovered from host animals for conventional histological examination and stained with hematoxylin and eosin. A qualified pathologist who was unaware of the treatment of the tissue donor evaluated all tissues.

Results

Establishment of Human Skin Grafts on NOD-*scid B2m^{null}* Mice. Human full-thickness foreskin successfully grafted on 13 of 13 NOD-*scid B2m^{null}* mice. Within 2 weeks, skin graft sites were uniformly healed in all animals and representative histological features are shown in Figure 1A–D. The graft integrity remained intact throughout the 36-day experimental observation period without any grossly visible evidence of rejection. Microscopic evaluation of these grafts failed to identify any inflammatory lesions and revealed normal epidermis and dermis architecture and keratinocyte maturation (Fig. 1A–D). The majority of blood vessels in the dermis of the human skin graft were of human origin as demonstrated by binding the human endothelium-specific UEA-lectin (Fig. 1D) (23, 24). Erythrocytes were observed within these vessels without any evidence of thrombosis or exudation.

Human Cell Engraftment in NOD-*scid B2m^{null}* Mice Treated with Anti-Mouse CD122 Monoclonal Antibody. We (17) and others (13) reported enhanced human mononuclear cell engraftment in *scid* mice preconditioned by administration of anti-mouse CD122 mAb (anti-mouse IL-2 receptor β-chain). The observed increase in xenochimerism presumably was related to antibody-induced loss of endogenous NK cell activity. NOD-*scid B2m^{null}* mice were pre-treated with anti-mouse CD122 mAb, engrafted with 100–300 $\times$ 10^6 human spleen or PBL cells, and given full-thickness human skin grafts. Skin graft recipients were evaluated for evidence of skin graft injury or rejection and analyzed at 36 days for levels of human lymphohematopoietic cell engraftment. Eight of 11 mice engrafted with human skin and pre-treated with anti-CD122 mAb before

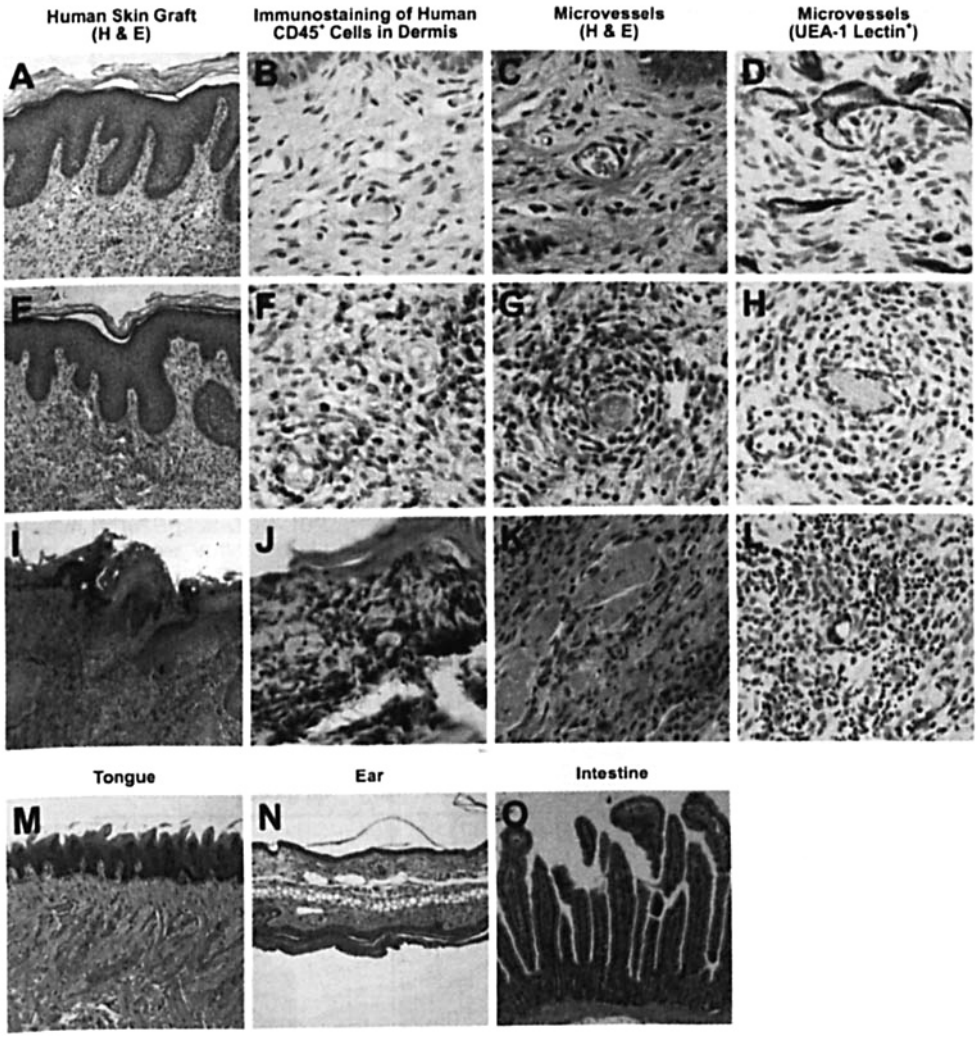

Figure 1. Histology of human skin allografts and mouse tissues in hu-PBL-NOD-*scid* $B2m^{null}$ mice. Histological analysis of full-thickness human foreskin grafts on untreated NOD-*scid*-$B2m^{null}$ mice or mice injected with human spleen cells within 24 hr of skin transplantation. All tissues were recovered 36 days after skin grafting for analysis, and representative histological data are shown. In untreated NOD-*scid* $B2m^{null}$ mice, fully healed and vascularized skin grafts are apparent (A–D). Human skin grafts on mice injected with anti-CD122 and engrafted with allogeneic human spleen cells at levels >1% exhibit extensive human mononuclear cell infiltration and evidence of graft injury (E–L). A destructive mononuclear cell inflammation within the dermis and vascular thrombosis are shown in panels E–H. In some grafted hu-PBL-NOD-*scid* $B2m^{null}$ mice, complete graft rejection characterized by epithelial sloughing and vasculature necrosis was seen (I–L). Normal morphology without mononuclear cell inflammation was observed in host mouse tissues (tongue, ear, intestine) that typically hallmark evidence of GVHD (M–O). Original magnification ×100 (A, E, I, M, N, O) and ×250 (B, C, D, F, G, H, J, K, L).

human lymphocyte injection demonstrated splenic $CD45^+$ cell engraftment (>1% human $CD45^+$ cells in the spleen; Fig. 2A). Overall, 19 of the 22 mice in all three treatment groups bearing human skin grafts were confirmed by flow cytometry to be engrafted with human lymphocytes (Fig. 2A).

Injury and Rejection of Human Skin Grafts on NOD-*scid* $B2m^{null}$ Mice Engrafted with Human Allogeneic Spleen Cells. In the 19 human lymphocyte–engrafted mice bearing human skin grafts, all (100%) exhibited partial to complete graft injury and destruction. As shown in Figure 2B, 11 mice were given human skin and human PBL or spleen cells and treated within 24 hr of grafting with anti-CD122 mAb. Of these 11 mice, the eight mice with confirmed human lymphocyte engraftment also demonstrated histological evidence of graft injury. All mice receiving anti-CD122 mAb treatment in combination with either depleting anti-human CD4 (six of six) or anti-human CD8 (five of five) mAb were engrafted with human lymphocytes and all demonstrated graft-specific pathology. Injury was defined by lesions involving greater than 30% of the grossly visible graft surface and was characterized by erythema, thrombosis, and epithelial sloughing. Microscopic representations of these lesions are shown in Figure 1E–L.

Histological examination of the human skin grafts revealed the presence of intense human $CD45^+$ mononuclear cell infiltrates diffusely interspersed throughout the dermis (Fig. 1F and J). UEA-1^+ human vascular endothelial cells detected were markedly reduced in the infiltrated grafts and surviving UEA-1^+ microvessels were thrombosed and necrotic (Fig. 1H and L). Epidermal damage varied from localized inflammation and mononuclear infiltration subjacent to the basal layer of keratinocytes, to selected cases progressing to a complete erosion of the epithelium and sloughing of the entire skin allograft. Representative examples of these severe graft rejection lesions are shown in Figure 1I–L.

Absence of Graft-Versus-Host Disease. The infiltration and rejection of human skin grafts could be due to allogeneic rejection by the engrafted human spleen cells or to a nonspecific GVHD that induced bystander destruction of the human allograft. To discriminate between these possibilities, we examined tissues that are commonly infiltrated with mononuclear cells during GVHD. We observed no

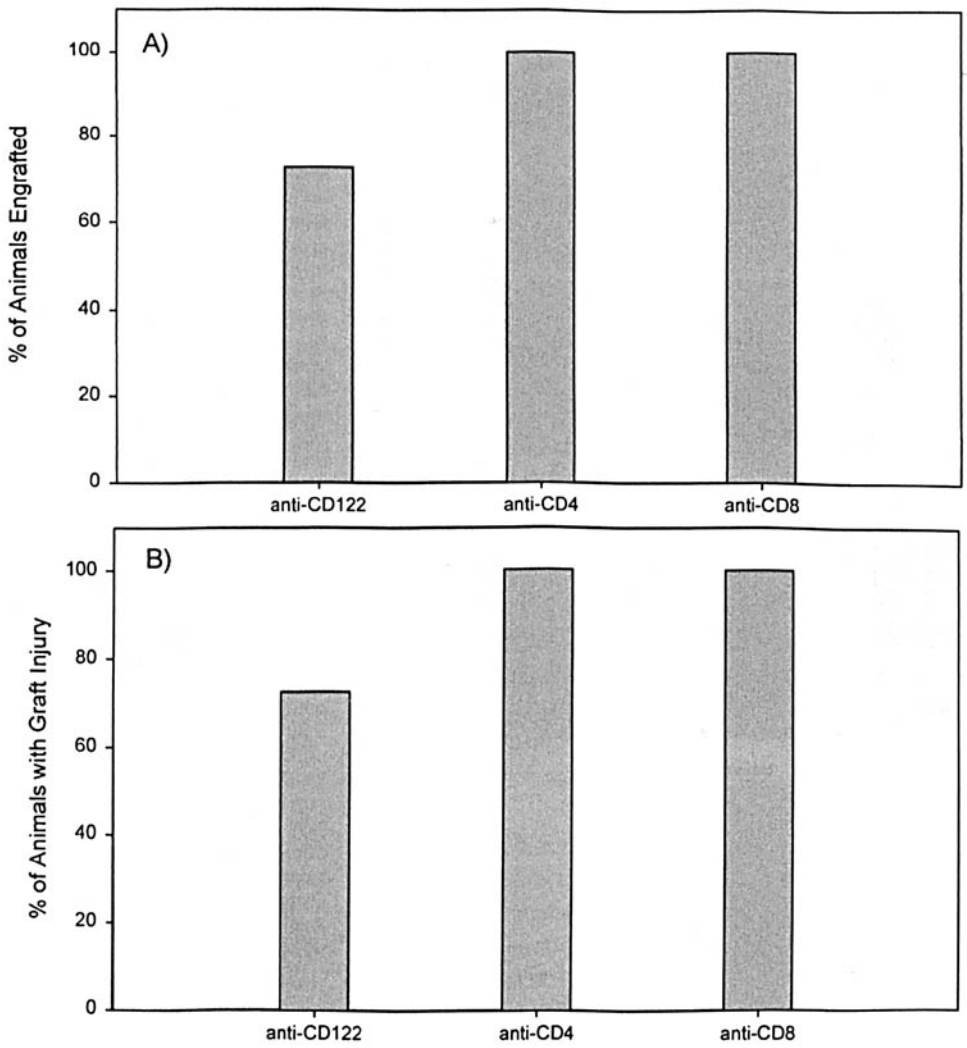

Figure 2. Human cell engraftment and skin allograft injury in hu-PBL-NOD-*scid-B2m*^{null} mice. (A) Human-origin cells in the spleens of NOD-*scid B2m*^{null} mice were determined 36 days after human cell injection and placement of full-thickness human foreskin grafts. The percentage of animals engrafted defined as >1% human CD45$^+$ cells in the spleen of hu-PBL-NOD-*scid-B2m*^{null} mice is shown. Mice were given anti-CD122 mAb (n = 11), anti-CD122 mAb plus anti-human CD4 (n = 6), or anti-CD122 mAb plus anti-human CD8 mAb (n = 5). (B) Percentage of hu-PBL-NOD-*scid B2m*^{null} mice displaying evidence of human skin graft injury (affecting >30% of entire graft) or complete rejection in each experimental group.

human (or mouse) mononuclear cell infiltration into tongue, skin, or intestine (Fig. 1M–O). Little to no human mononuclear cell infiltration was observed in nonhuman mouse tissues, even in mouse skin adjacent to the human skin allograft undergoing rejection (data not shown).

Engraftment and Rejection of Human Skin Allografts in Hu-PBL-NOD-*scid B2m*^{null} Mice Given Anti-Human CD4 or Anti-Human CD8 mAb. We next determined the effect of depletion monotherapy with anti-human CD4 or anti-human CD8 mAb on the engraftment and function of human spleen cells injected into hu-PBL-NOD-*scid B2m*^{null} mice. Previously, we demonstrated that anti-human CD4 mAb treatment initiated at the time of human PBL injection prevents engraftment of all human lymphocytes (17, 22). Therefore, injection of anti-human CD4 mAb was delayed until 2 weeks after human spleen cell injection to permit human lymphoid cell engraftment. Surprisingly, this delay in monotherapy administration only produced incomplete CD4$^+$ cell depletion. We observed that the overall frequency of human CD45$^+$ cell–engrafted mice was unaltered by administration of anti-human CD8 mAb (five of five, 100%) at the time of human cell injection or by delayed administration of anti-human CD4 (six of six, 100%; Fig. 2A). The percentage of human CD3$^+$, CD4$^+$, and CD8$^+$ cells of the total spleen cells of human skin–grafted mice is shown in Figure 3A. Human CD20$^+$ (B cells), CD33$^+$ (myeloid precursor), and CD64$^+$ (macrophage/monocyte) were not detected (<1%) in the spleens of recipients. The short-term survival of these hematopoietic cells following human PBL transfer was consistent with an earlier report (17).

Anti-human CD8 mAb injection of hu-PBL-NOD-*scid B2m*^{null} mice was effective in reducing human CD8$^+$ cell levels to less than 2% of the total spleen cell population (Fig. 3C). However, as noted above, the 2-week delayed injection of anti-human CD4 mAb only partially depleted human CD4$^+$ cells. Approximately 40% of the human CD45$^+$ population detected at 30–36 days in the anti-CD4-treated recipients expressed CD4 (Fig. 3B).

Surprisingly, no differences were encountered in the frequency of skin allograft injury or rejection in hu-PBL-NOD-*scid B2m*^{null} mice treated only with anti-CD122 as compared with mice additionally treated with either anti-human CD4 or anti-human CD8 mAb. Evidence of graft

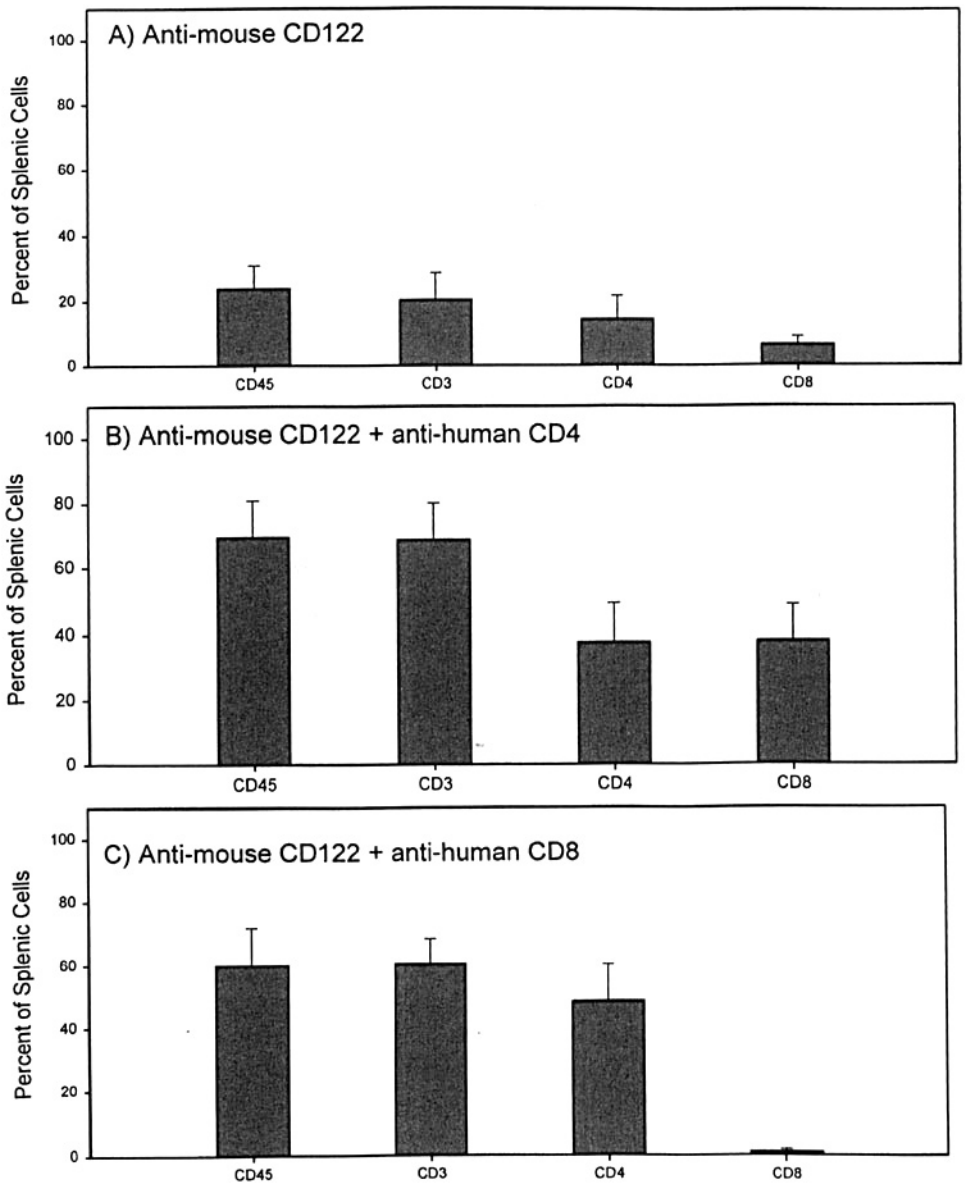

Figure 3. Engraftment of human lymphocytes in the spleen of NOD-*scid B2m*^{null} mice. The percentages of total human lymphocytes (CD45$^+$), T lymphocytes (CD3$^+$), and T-lymphocyte subsets (CD4$^+$ and CD8$^+$) in the spleen of hu-PBL-NOD-*scid B2m*^{null} mice 36 days after human spleen cell injection are shown. Hu-PBL-NOD-*scid B2m*^{null} mice were treated with anti-mouse CD122 mAb (A, n = 11), anti-mouse CD122 mAb plus anti-human CD4 mAb (B, n = 6), or anti-mouse CD122 mAb plus anti-human CD8 mAb (C, n = 5). Error bars represent the mean value ± SEM.

injury was apparent in six of six (100%) and five of five (100%) of recipient mice treated with anti-human CD4 or CD8 mAb, respectively (Fig. 2B). The severity of graft rejection lesions was comparable between hu-PBL-NOD-*scid B2m*^{null} mice given supplemental depleting monotherapy and those given skin allografts and anti-CD122 alone (data not shown).

Human PBLs Mediate Graft Injury in Hu-PBL-NOD-*scid B2m*^{null} Mice with Established Human Skin Allografts. We next tested the ability of human PBLs to mediate skin allograft injury in NOD-*scid B2m*^{null} mice with established human skin grafts. Recipient mice (n = 3) were given full-thickness human skin, and the skin grafts were permitted to heal in for 4 weeks (Fig. 4A and B). Animals were then treated with anti-mouse CD122 mAb 12 hr prior to an intraperitoneal injection with 100×10^6 human PBLs. Recipient mice were xenochimeric as demonstrated by a mean human CD45$^+$ percentage of 31.9% (range, 15.6%–58.2%) in the recipient spleen 13 days later. Skin allograft injury affecting 30%–60% of the total graft was observed in all three mice. Histological analysis of these skin allografts revealed abnormalities indistinguishable from those described above (Fig. 4C and D).

Discussion

We have demonstrated that hu-PBL-NOD-*scid B2m*^{null} mice engraft at high levels with human lymphocytes capable of mediating skin allograft injury and rejection. These data suggest a new, potentially powerful approach for evaluating the human allograft rejection model in hu-PBL-NOD-*scid B2m*^{null} mice.

In the hu-PBL-NOD-*scid B2m*^{null} mice given human skin allografts, immune injury was evident even in the nearly complete absence of human CD8$^+$ cells. The immune response in hu-PBL-NOD-*scid B2m*^{null} mice given human

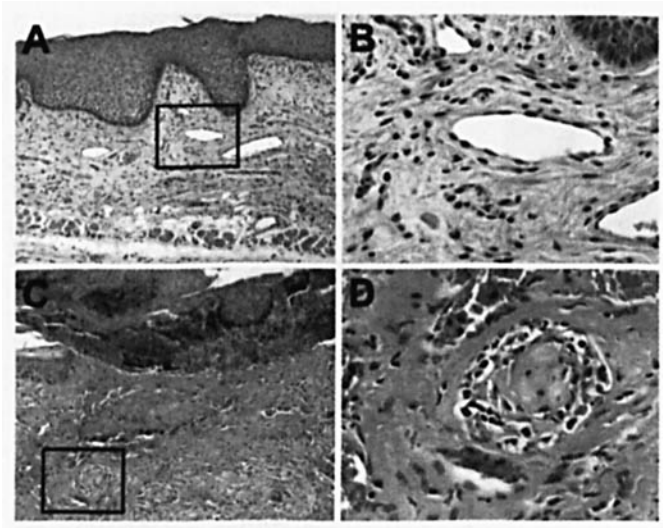

Figure 4. Skin allograft injury in NOD-*scid B2m*^{null} mice engrafted with human PBLs. Human skin xenografts were allowed to fully heal in NOD-*scid B2m*^{null} mice (A and B). Three mice with fully healed skin xenografts were then given 100 × 10^6 human PBLs (C and D). In contrast to control animals (A and B), destructive mononuclear cell inflammation is uniformly apparent at 13 days within the dermis, resulting in necrosis of the supporting vasculature (C and D). Extensive sloughing of the epidermis is also seen (C). Higher magnification shows typical vasculitis and fibrin necrosis of a microvessel in the dermis (D) that is absent in controls (B). Original magnification panels A and C = ×100, panels B and D = ×250. Hematoxylin and eosin staining.

skin grafts is characterized by extensive mononuclear cell infiltration of the graft that appears to be targeted to human-origin endothelium and produces vascular occlusion with coagulation necrosis. Complete sloughing (rejection) of the graft was observed in some cases. The destructive infiltration into the allografts was not due to collateral xenoreactive GVHD, as no evidence of mononuclear infiltration was observed in host tissues typically affected during GVHD. Finally, nonspecific inflammatory cytokines related to granulation tissue in the graft bed did not appear to play a role in the observed graft injury, because an acute vasculitic reaction was observed in animals bearing healed-in skin grafts given human PBLs. These data validate the hu-PBL-NOD-*scid B2m*^{null} mouse grafted with human skin as a model for the study of human allograft rejection.

It has been difficult to demonstrate human allograft rejection in hu-PBL-*scid* mice. Various manipulations have been employed, including injection of human T cells from HLA-sensitized donors (25), irradiation of the recipient (26), or injection of large numbers of human splenic cells (27). Preactivation (priming) of human cells *in vitro* prior to injection and other manipulations have also been used in attempts to induce allograft or xenograft rejection in hu-PBL-*scid* mice (28–33). In many of these studies, severe GVHD ensued, making it difficult to distinguish between graft injuries resulting from allospecific versus nonspecific inflammation. A promising series of studies has been described for skin allograft damage in hu-PBL-*scid* mice (20, 23). The authors demonstrated morphological characteristics at the graft site (24, 34, 35) that closely resembled human allograft rejection (34). Damage to microvessels of human but not mouse-origin blood vessels was detected. Skin allograft damage was inhibited by immunosuppression using a combination of cyclosporine and rapamycin (20) or by administration of murine antibodies or fusion proteins that blocked human CD2/LFA-3 interactions (24). However, the allograft damage was restricted to the microvasculature and gross rejection (scabbing and epidermal sloughing) was not described. The murine host used in these studies was the C.B-17-*scid* mouse, and anti-mouse CD122 mAb was not used to facilitate human cell engraftment. Our studies using the NOD-*scid B2m*^{null} mouse with concurrent administration of anti-mouse CD122 mAb led to increased engraftment of human lymphocytes and, in some cases, full rejection of the skin allografts. Importantly, alloaggression by the human PBLs did not require *in vitro* sensitization or activation prior to injection. The role of NK cells in modulating human lymphocyte engraftment in immunodeficient mice has been suggested by the sequentially improved human cell engraftment in NOD-*scid* and NOD-*scid B2m*^{null} mice over that of C.B-17-*scid* mice (10, 16). In each recipient, human lymphocyte engraftment correlated inversely with levels of NK cell activity. Additionally, human CD4$^+$ cell engraftment increased as NK cell activity decreased (16). Depletion monotherapy with anti-mouse CD122 mAb further attenuates mouse NK cell activity, resulting in enhanced human CD4$^+$ cell engraftment (17). The data suggest that our combination of improved recipient strain characteristics with anti-mouse CD122 mAb optimizes the hu-PBL-SCID model for both human cell engraftment and allograft rejection.

Surprisingly, we observed that human CD8$^+$ cells did not appear to be required for human skin allograft rejection in our model system. Although CD8$^+$ cells are postulated to be important mediators of allograft rejection, it has been reported that skin allograft rejection occurs in mice genetically deficient in CD8$^+$ cells (36, 37). In addition, mice genetically deficient in β_2-microglobulin, a molecule required for MHC class I expression and CD8$^+$ T-cell development, also exhibit allograft rejection, albeit it delayed (38). Conversely, we could not determine whether CD8$^+$ cells alone could mediate skin allograft rejection in the absence of CD4$^+$ cells. Our use of depleting anti-human CD4 mAb initiated 2 weeks after human cell injection did not fully ablate human CD4$^+$ cells. This result was not expected based on our previous studies demonstrating that a similar dose of anti-human CD4 mAb given to NOD-*scid* mice at the time of human cell engraftment prevented the engraftment not only of human CD4$^+$ cells but also of human CD8$^+$ cells (17, 22). We are currently investigating whether the immunoglobulin half-life is shorter in NOD-*scid B2m*^{null} mice as compared with NOD-*scid* mice. The failure of anti-human CD4 mAb to deplete human CD4$^+$ cells in hu-PBL-NOD-*scid B2m*^{null} mice may conceivably be due to their

lack of a protective Fc receptor important for the IgG salvage pathway (16).

In summary, the data document the utility of the hu-PBL-NOD-*scid* $B2m^{null}$ mouse to support human lymphocyte and skin engraftment. Engrafted lymphocytes are capable of allograft rejection as demonstrated by their ability to mediate injury, and in some cases, complete rejection of human skin grafts. Studies of human allograft injury in this model system should permit a controlled analysis of the efficacy of human-specific therapeutic reagents prior to translation to preclinical and clinical trials.

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