Human/Severe Combined Immunodeficient Mouse Chimeras

An Experimental In Vivo Model System to Study the Regulation of Human Endothelial Cell–Leukocyte Adhesion Molecules

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Abstract

The ability of circulating white blood cells to enter inflamed tissues is mediated by specific cell adhesion molecules thought to be expressed in a programmed and sequential manner to form an "adhesion cascade." Because of the complexity of this process, it is becoming increasingly important to develop in vivo models. Two major problems have limited the utility of current animal models. The first is the inability of many of the antibodies developed against cell adhesion molecules in human cell culture models to cross-react in animals. The second is the uncertainty in extrapolating animal (particularly rodent) findings to humans.

To circumvent these problems, full thickness human skin grafts were transplanted onto immunodeficient (severe combined immunodeficient) mice. After 4–6 wk, the transplanted skin grafts closely resembled normal skin histologically and maintained their human vasculature as determined by immunohistochemical staining with human-specific endothelial cell markers. Intradermal injection of tumor necrosis factor-α resulted in the reversible upregulation of the leukocyte-endothelial adhesion molecules E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1, and in an active inflammatory reaction with migration of murine leukocytes into cytokine-injected areas. These results indicate that the severe combined immunodeficient mouse/human skin transplant model provides a useful in vivo system in which to study human endothelium during the process of inflammation. (*J. Clin. Invest.* 1993. 91:986–996.) Key words: integrins • inflammation • selectins • severe combined immunodeficient mouse • animal models • endothelium • cell adhesion

Introduction

The ability of circulating white blood cells to enter inflamed tissues is initially controlled by interaction of cell surface adhesion molecules on leukocytes with vascular endothelium. It has been hypothesized that the sequential and programmed expression of specific cell adhesion molecules and chemoattractants comprise a so-called "leukocyte-endothelial cell cascade" that is required for successful white blood cell adhesion and diapedesis (1–4). Although a great deal of information about this postulated adhesion cascade has been obtained from cell culture and in vitro experiments, the complexity of the inflammatory response requires that in vivo models be developed to test its validity (5).

Two major problems have limited the utility of current animal models in studying leukocyte–endothelial cell interactions. First, many of the polyclonal and monoclonal antibodies that have been isolated to study cell adhesion molecules have been developed in human in vitro systems. The inability of many of these antibodies to cross-react in nonhuman systems restricts their experimental usefulness. Second, even when antibodies that react in animal model systems exist, the many recognized differences between the biology of animal (particularly rodent) and human physiology (see 6, 7) make extrapolation to human systems uncertain.

The ideal in vivo model in which to study endothelial cell–leukocyte interactions would therefore involve experiments conducted directly in humans. Investigators have, in fact, performed a few such studies by induction of an inflammatory reaction in human skin followed by an immunohistochemical analysis of the dermal vessels (8–16). Human skin in organ culture has also been examined (12, 17). Another approach has been to study the biology of endothelium in nonhuman primates (18–20). Although useful, each of these models has limitations. Obviously, only very limited experimentation can be conducted in human subjects. Organ culture, although one step closer to the in vivo situation than tissue culture, exposes cells to nonphysiological experimental conditions and does not allow the study of interactions between endothelium with blood. Experiments with primates are expensive, raise ethical concerns, require large amounts of reagents, and can still suffer from problems with lack of antibody cross-reactivity.

The purpose of this study was to develop an alternative, more practical, model that would allow study of in vivo human endothelium, with special focus on endothelial cell adhesion molecules and white blood cell migration. To accomplish this, full thickness human skin grafts were transplanted onto severe combined immunodeficient (SCID)[1] mice. After 4–6 wk, the transplanted skin grafts closely resembled normal skin histologically and maintained their human vasculature, with minimal ingrowth of murine endothelium. To determine if these human vessels could participate in an inflammatory reaction, intradermal injections of recombinant human TNF-α were made into the transplanted skin. We found that TNF-α induced an active

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1. *Abbreviations used in this paper:* AEC, 3-amino-9 ethylcarbazole; ICAM-1, intercellular adhesion molecule-1; LFA, lymphocyte function-associated antigen; PECAM, platelet-endothelial cell adhesion molecule-1; SCID, severe combined immunodeficient; VCAM-1, vascular cell adhesion molecule-1.

inflammatory response with adhesion of murine leukocytes to the human endothelium and subsequent migration of these leukocytes into the dermal interstitium. This efflux of leukocytes was accompanied by upregulation of the endothelial cell adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) similar to that which has been reported for normal human skin. These studies demonstrate that the SCID mouse transplanted with full thickness human skin is a valuable model with which to study the in vivo behavior of human microvascular endothelium and endothelial cell–leukocyte interactions.

Methods

Skin transplantation

SCID mice (21), originally obtained from Dr. Melvin Bosma (Fox Chase Cancer Center, Philadelphia, PA) were purchased from a colony maintained at the Wistar Institute Animal Facility. The mice were housed at this facility in a pathogen-free environment without the use of prophylactic antibiotics. At 4–6 wk of age, the plasma of each mouse was tested for IgM production and only fully immunodeficient mice were used.

After anesthesia, 6–8-wk-old mice were prepared for transplantation by shaving the hair from a 5-cm^2 area on each side of the lateral abdominal region. Two circular graft beds, ~ 1.5 cm in diameter were created on the shaved areas by removing full thickness skin down to the fascia. Full thickness human skin grafts of the same size were placed onto the wound beds. The transplants were held in place with 6-0 nonadsorbable monofilament suture material and covered with a Band-Aid clipped to the dorsal and ventral skin of the animal with a surgical staple. An additional layer of micropore cloth tape was applied.

Human skin consisted of neonatal foreskins from elective circumcisions or normal adult skin removed during plastic surgery. Use of such tissue was approved by the Human Subjects Institutional Review Boards of both the Wistar Institute and the University of Pennsylvania.

Experimental protocols

Mice were used for experiments 4–6 wk after human skin transplantation. Only those mice whose grafts grossly showed no signs of inflammation or rejection were used.

To examine the response of the grafts to intradermal injections of cytokines, a skin graft on one side of the mouse was injected intradermally with 50 μl of endotoxin-free saline containing 2 mg/ml of BSA (Sigma Chemical Co., St. Louis, MO) as a protein carrier plus 5% Evans blue dye (Sigma Chemical Co.) to mark the site of injection (control side). The skin graft on the contralateral side was injected with 2,000 U of recombinant human TNF-α (kindly provided by Genentech, Inc., So. San Francisco, CA) diluted in 50 μl of endotoxin-free saline containing 2 mg/ml BSA and Evans blue dye (experimental side).

Mice were killed 2, 8, or 24 h after saline or TNF-α injection, and the skin grafts carefully dissected from the animals. The grafts were cut into two halves through the center of the injection site marked by the Evans blue dye. One-half of each graft was placed in formalin for routine histochemistry. The remaining half of the graft was oriented, placed in OCT compound, and snap frozen for immunohistochemical analysis as described below. All protocols were approved by both the Wistar Institute and University of Pennsylvania Institutional Review Boards.

Immunostaining

5-μm serial sections were cut from the frozen specimens, fixed in cold acetone, and stored at −70°C until use. At the time of staining, the sections were blocked with 5% serum appropriate for the secondary antibodies used, washed in PBS, and treated with primary antibody for 1 h. Each antibody was titrated for optimal reactivity.

Immunofluoresence. After washing in PBS, appropriate secondary (mouse, rat, or rabbit) antibodies labeled with FITC or rhodamine (Cappel Laboratories, West Chester, PA) at a dilution of 1:200 were added for 1 h. The sections were then washed, coverslipped using an antiquench mountant, and viewed with an epifluorescent microscope. Two types of double-labeling experiments were performed including: (*a*) rabbit polyclonal anti-human Factor VIII-related antigen (1:50) plus murine anti-human PECAM-1 monoclonal antibody 1.3 (20 μg/ml) and (*b*) purified rabbit polyclonal anti-human PECAM-1 antibody (2 μg/ml) plus hamster anti-mouse PECAM-1 mAb 2H8 (supernatant). In these studies, both primary antibodies were added simultaneously. After washing, mixtures of rhodamine-labeled anti-rabbit plus FITC-labeled anti-mouse antisera or rhodamine-labeled anti-rabbit and FITC anti-hamster antisera were added. In each experiment, controls performed with preimmune rabbit serum and irrelevant monoclonal antibodies showed minimal staining.

Immunoperoxidase. After washing in PBS, the bound primary antibody was detected with the Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) with 3-amino-9 ethylcarbazole (AEC) as chromagen. No counterstaining was used in order to increase sensitivity.

Antibodies

The following polyclonal antibodies were used in these studies: (*a*) anti-human PECAM-1 raised against human platelet PECAM-1 purified by monoclonal affinity chromatography (22); (*b*) anti-Factor VIII-related antigen (Dako Corp., Carpinteria, CA).

The following monoclonal antibodies were used in these studies: (*a*) murine anti-human PECAM-1 mAb 1.3 (22, 23); (*b*) anti-murine PECAM mAb 2H8, a hamster antibody made against the murine homologue of PECAM-1 (CD31) (24); (*c*) murine anti-human E-selectin mAb 8E4 provided by Dr. Barry Wolitzky (Hoffmann-LaRoche, Inc., Nutley, NJ); (*d*) rat anti-mouse Mac-1 M1/70.15, purchased from American Type Culture Collection, Rockville, MD (25); (*e*) rat anti-mouse CD18 M18/2, purchased from American Type Culture Collection (26); (*f*) murine anti-human VCAM-1 (INCAM110) mAb E16 provided by Dr. M. Bevilacqua (University of California, San Diego) (27), (*g*) murine anti-human ICAM-1 antibody RR1 provided by Dr. Robert Rothlein (Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT) (28). Each antibody was titrated to obtain optimal expression with minimal background staining.

Quantification of leukocyte infiltration

To identify murine leukocytes, frozen sections of skin grafts were stained as described above with anti-mouse CD11b (Mac-1) or CD18 mAbs raised in rats. Although Mac-1 is preferentially expressed in neutrophils and monocytes, there was little difference observed in the staining of these antibodies since the SCID mice are severely lymphopenic. Routine histology (see Fig. 1 *C* for example) confirmed that most of the leukocytes observed in the early stages of inflammation were, in fact, neutrophils. Multiple sections (four to six) were cut from the center of each skin biopsy to obtain representative samples of the injection site. The number of leukocytes per three to six randomly chosen ×100 microscope fields in each section were directly counted to give an estimate of the number of leukocytes. Because some grafts demonstrated a cuff of leukocytes at the murine–human skin junctional regions, the areas counted were in the immediate subepidermal regions not adjacent to murine skin.

To validate direct counting methods, we also used a computer-enhanced video imaging system. This system is a true color analysis system consisting of an Olympus BH2 microscope, a CCD Color Camera, a Southern Microsystems Image Analysis System, and a Sony Video Printer. Images from slides stained with the anti-Mac-1 mAb using the immunoperoxidase technique with AEC substrate were analyzed using this software. The intensity and color of settings of the system were adjusted so that only the red colored leukocytes were identified by the computer. Subepidermal regions in the section were then outlined and

the computer was programmed to calculate the percentage of the total area occupied by the leukocytes. Using this software, we quantified the degree of leukocyte infiltration by calculation of the percentage of "area" within a given field that showed positive staining.

Quantification of the expression of endothelial cell adhesion molecules

To identify the proportion of vessels staining positively, the total number of vessels present was first determined by staining sections with the endothelial cell specific anti-human PECAM-1 mAb. Since the expression of PECAM-1 appeared unchanged by cytokine injection (see below), the anti-PECAM-1 antibody served as a marker that consistently identified all of the vessels. We therefore defined the total number of vessels by counting the number of PECAM-1-positive vessels per field. A minimum of four fields were counted per section. The number of vessels staining positively for E-selectin, VCAM-1, and ICAM-1 per field on the adjacent serial sections were then counted. The "percentage of positive vessels" staining for each adhesion molecule was subsequently calculated using the following equation:

$$\% \text{ positive vessels} = \frac{\#\text{ of vessels staining positively with adhesion molecule antibody}}{\#\text{ of vessels staining positively for PECAM-1}} \times 100$$

To determine the intensity of staining for each antibody, sections were graded on a semi-quantitative scale (12, 29) using the following scoring system: 0, no staining; +1, weak staining; +2, moderate staining; +3, strong staining. Each section was scored blindly by two observers.

Statistics

Differences among groups were analyzed using two-way analysis of variance. When statistically significant differences were found ($P < 0.05$) individual comparisons were made using the extended Tukey test (30).

Results

Full thickness transplanted human skin grafts are accepted by the mouse and retain a normal histologic appearance. When examined 4 wk after skin grafting, xenographic transplantation of human skin was successful in > 90% of SCID mice as defined by the absence of visible or histologic evidence of inflammation, skin breakdown, skin contraction, or inflammation. The appearance of the transplanted skin remained normal for > 8 wk after transplant. Both neonatal skin (from foreskins) and adult skin (residual tissue from plastic surgery) were used, however, the thicker skin from adults was less vascular and had a somewhat lower success rate.

Fig. 1 *A* shows a hematoxylin and eosin micrograph of the mouse–human skin junctional region of one of our grafts 4 wk after transplantation. The skin on the left hand side of the figure (*m*) is from the mouse and can be distinguished by the very thin epidermis (*arrows*) and hair follicles (*arrowheads*) from the grafted human skin (*h*) on the right. The histological appearance of the human skin was normal, including preservation of the epidermal (*e*) structures (stratum corneum, stratum granulosum, stratum spinosum, stratum basalis, and rete ridge pattern). The dermis showed intact superficial and deep microvascular plexuses. The papillary and reticular dermal collagen appeared normal with normal spindle cell (fibroblast) activity (not hyper- or hypocellular). We also noted that mast cells (identified by anti-chymase antibodies), Langerhans cells (identified by anti-CD1a antibodies), and melanocytes persisted (data not shown).

The vessels in the grafts are of human origin. The endothelial cells within the graft were initially identified using a polyclonal antibody against Factor VIII–related antigen (data not shown). However, this antibody was not species specific and also stained mouse endothelium. To determine specifically the origin of the graft vasculature, we used species-specific anti-PECAM-1 antibodies. PECAM-1 is a member of the immunoglobulin superfamily that is expressed strongly by endothelial cells in culture and tissues (31–33). Fig. 1 *B* is an immunofluorescence micrograph from a section of the mouse–human skin junction of one of the grafts (similar to that shown in Fig. 1 *A*) that has been stained with the human specific anti-PECAM-1 monoclonal antibody. The skin had been transplanted 4 wk previously. The skin on the left hand side of the figure is from the mouse (*M*) and the grafted human skin (*H*) is on the right. Strong expression of human PECAM-1 can be seen in the vessels (*arrowheads*) of the dermis of the human portion of the graft, while no staining of the murine vessels is present.

To determine the amount of infiltration by murine vessels, we stained a graft transplanted 4 wk earlier (Fig. 2, *A* and *B*) and adjacent murine skin (Fig. 2, *C* and *D*) with the anti-human PECAM polyclonal antibody (Fig. 2, *A* and *C*) and a recently developed anti-murine PECAM-1 monoclonal antibody raised in hamsters that reacts exclusively with murine vasculature (24) (Fig. 2, *B* and *D*). Virtually no ingrowth of murine vessels was seen. The anti-murine PECAM-1 antibody strongly labeled the endothelium within the murine skin (Fig. 2 *D*) but did not react with the vessels in the human graft (Fig. 2 *B*). Conversely, the anti-human PECAM-1 antibody reacted with the vessels in the graft (Fig. 2 *A*) but not in the murine skin (Fig. 2 *C*).

The human vessels within the graft constitutively express relatively high levels of ICAM-1, but low levels of E-Selectin and VCAM-1. To assess the baseline expression of endothelial cell adhesion molecules, frozen sections from 15 human skin grafts transplanted in the mice for 4 wk were stained with anti-human-specific monoclonal antibodies directed against the endothelial cell adhesion molecules PECAM-1, E-selectin, VCAM-1, and ICAM-1 (Fig. 3, *A, C, E,* and *G*). The percentage of positive vessels and the level of expression of each cell adhesion molecule are tabulated in Table I (*Baseline*). PECAM-1 was expressed strongly (+3) by all endothelium. E-selectin was identified on only 18% of the vessels with weak to moderate staining (+1.4 [SEM = 0.02]). VCAM-1 staining was limited to < 10% of vessels and showed only weak expression (+0.9 [SEM = 0.16]). In contrast, ICAM-1 was expressed at moderate to strong levels (average staining intensity of +2.6 [SEM = 0.24]) in ~ 50% of vessels.

Intradermal injection of recombinant human TNFα upregulates human endothelial cell adhesion molecules. The cytokine TNFα has been shown to upregulate the expression of cell adhesion molecules on endothelium in vitro (34), in organ culture (17), and in vivo (18). To determine the effects of this cytokine in our model, skin grafts were injected intradermally with recombinant human TNFα or saline and the endothelium examined for the expression of the inducible endothelial cell adhesion molecules.

PECAM-1. PECAM-1 was strongly expressed on all endothelial cells in a diffuse pattern. Intradermal injection of 2,000 U of human TNFα did not affect the expression or distribution of PECAM-1 at 2, 8 (Fig. 3, *A* and *B*), or 24 h after injection.

E-selectin. In contrast to the stability of PECAM-1 expres-

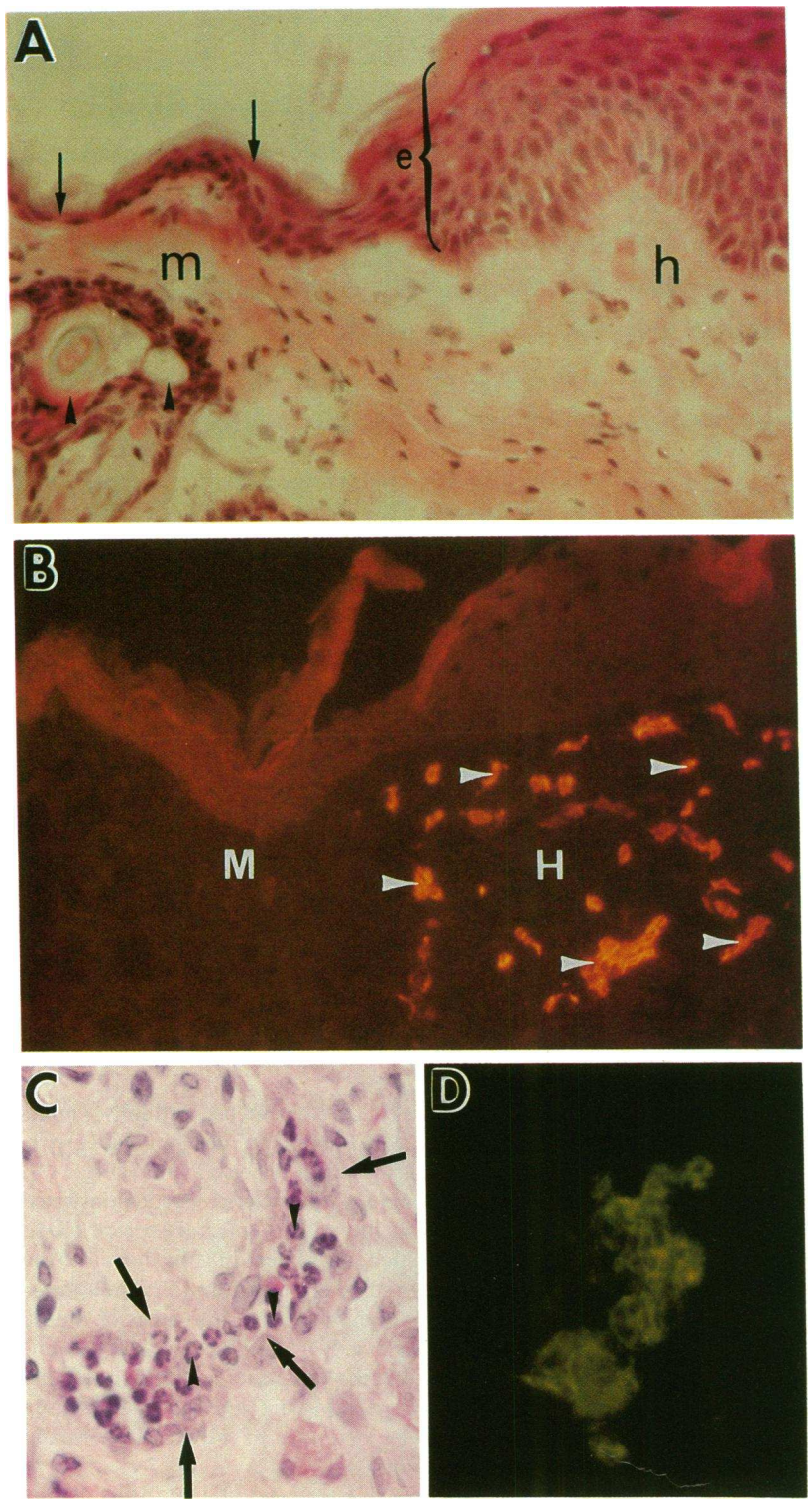

Figure 1. Histology of the human skin transplants. (*A*) A hematoxylin and eosin micrograph of the junction between the mouse skin (*m*) and the human skin graft (*h*). The mouse skin can be identified by the thin epidermis (*arrows*) and the hair follicles (*arrowheads*). The human skin maintains its normal stratified squamous epithelium (*e*) and a relatively thick dermal region. Magnification, 400. (*B*) Immunofluorescence micrograph of mouse/human junction reacted with anti-human PECAM-1 antibody and stained with a rhodamine-labeled secondary antibody. The vasculature of the human skin (*H*) stains strongly with the anti-human PECAM antibody (*arrows*) while the mouse skin (*M*) shows no reactivity. Magnification, 400. (*C*) A hematoxylin and eosin micrograph of a vessel (*arrows*) within a portion of the human skin transplant that had been injected with recombinant human TNF-α 2 h previously shows that the lumen of the vessel is filled with neutrophils (*arrowheads*). Magnification, 1,000. (*D*) Immunofluorescence micrograph of a similar vessel stained with a mouse-specific anti-CD18 monoclonal antibody reveals that the leukocytes within the vessel are of murine, rather than human, origin. Magnification, 1,000.

sion, alterations in E-selectin expression were noted (Table I, Fig. 3, *C* and *D*, and Fig. 4 *A*). 2 h after intradermal TNF-α injection, the percentage of vessels expressing E-selectin was 47% compared with 19% in the control injected sites. This difference was statistically different ($P < 0.05$) from both baseline (time 0) and from the saline injected control grafts at 2 h. The intensity of expression also increased in the TNF-α injected sites compared with control sites (+3 vs +1.4). As shown in Figs. 3 *D* and 4 *A*, this increased expression was maintained at 8 h, where 41% of vessels stained positively with an average intensity of +2.5. After 24 h, E-selectin expression had declined to baseline, both in number of vessels positive and in intensity of expression (Table I, Fig. 4 *A*).

VCAM-1. VCAM-1 was also induced after intradermal TNF-α injection (Table I, Fig. 4 *B*), although with a slightly different time course than E-selectin. Whereas VCAM-1 stain-

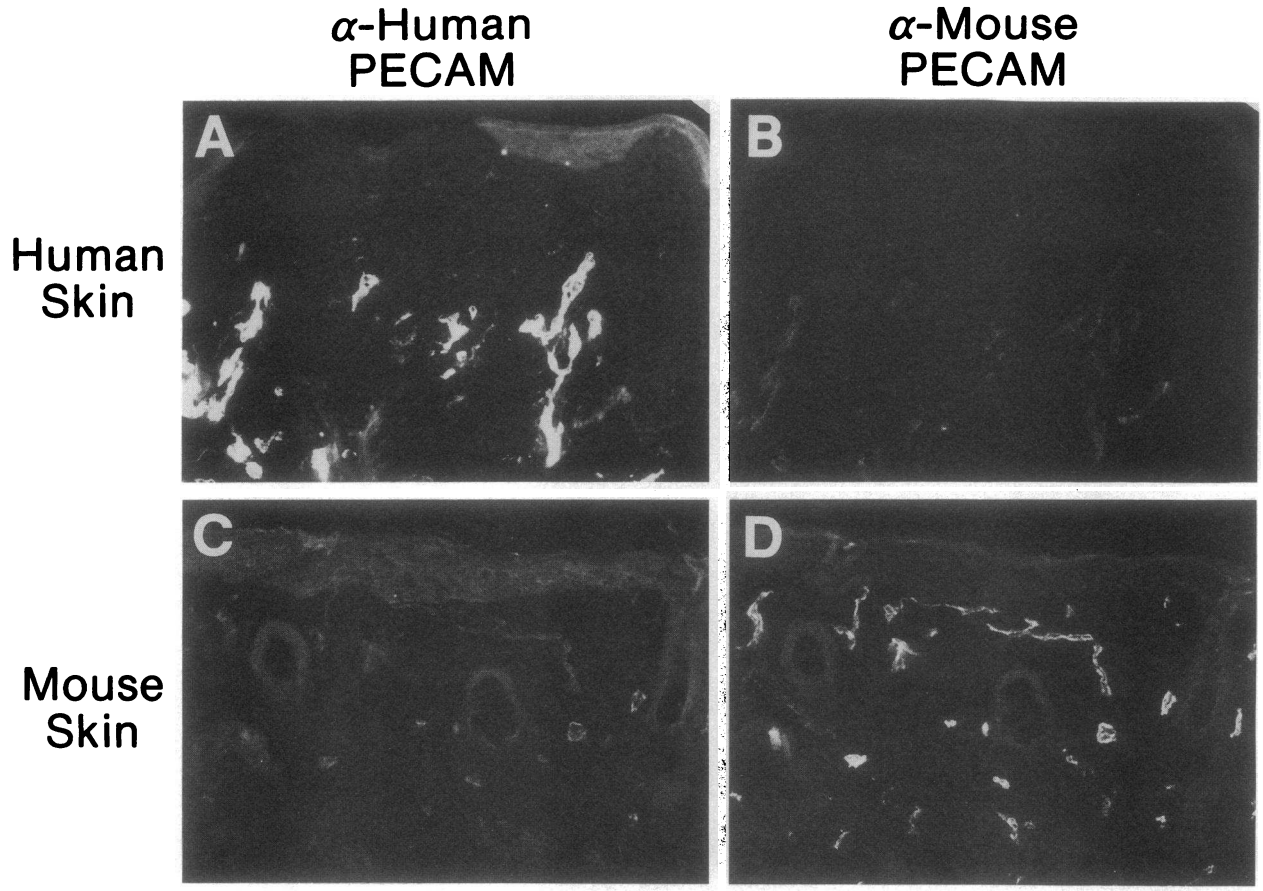

Figure 2. Identification of skin vessels using species-specific anti-PECAM-1 antibodies. Frozen tissue sections from transplanted human skin (*A* and *B*) and adjacent murine skin (*C* and *D*) were reacted with polyclonal rabbit anti-human PECAM-1 antiserum and a mouse-specific anti-PECAM-1 hamster monoclonal antibody. Sections were then stained with either a rhodamine-labeled anti-rabbit antiserum (*A* and *C*) or an FITC-labeled anti-hamster antiserum (*B* and *D*) and photographed using epifluorescence. Magnification, 400. (*A*) Transplanted human skin stained with anti-human PECAM-1 polyclonal antibody. (*B*) Transplanted human skin stained with anti-murine PECAM-1 monoclonal antibody. (*C*) Mouse skin stained with anti-human PECAM-1 antibody. (*D*) Mouse skin stained with anti-murine PECAM-1 antibody.

ing was minimal under baseline conditions (Fig. 3 *E*), 2 h after injection, VCAM-1 staining was seen on 24% of vessels. After 8 h, 60% of the vessels were expressing VCAM-1 at moderate intensity (+2.5) (Fig. 3 *F*). This difference was statistically different ($P < 0.05$) from baseline (time 0), from the saline injected control graft at 8 h, and from the TNF-α-injected skin at 2 h and 24 h. By 24 h after injection, only 23% of vessels were expressing VCAM-1 and at weak intensity levels (+1.5).

ICAM-1. Under baseline conditions, expression of ICAM-1 was relatively high (48% of vessels). Exposure to TNF-α appeared to increase the proportion of vessels positive, with 87% of the vessels staining for ICAM-1 after 8 h (Table I, Fig. 3, *G* and *H*, and Fig. 4 *C*). This difference was statistically different from baseline. Expression declined toward baseline levels after 24 h. In addition to increased expression of ICAM-1 on endothelial cells after 8 h, there was staining of the basal keratinocytes in the epidermis and of the interstitial cells, possibly dendritic cells or fibroblasts, in the dermis (see Fig. 3 *H*).

Intradermal injection of recombinant human TNF-α induces leukocyte migration. Under baseline conditions, some murine white blood cells were present in the dermal regions (Fig. 6). However, the injection of TNF-α, but not saline control solution, into the skin grafts elicited a brisk inflammatory reaction with intravascular and interstitial accumulation of leukocytes (Table I, Fig. 1 *C*, Figs. 5 and 6). Within two hours after injection, leukocytes, primarily neutrophils (*arrowheads*), were seen filling the dermal vessels (*arrows*) (Fig. 1 *C*). These white blood cells were of murine origin, as demonstrated by positive staining with the rat anti-murine-specific CD18 mAb (Fig. 1 *D*). In addition, movement of leukocytes into the dermal interstitium was also noted (Figs. 5 and 6). The appearance of murine leukocytes extravasating into areas of mouse skin injected with TNF-α (Fig. 5, *A* and *B*) was virtually identical to the appearance of murine leukocytes migrating out of the human vessels in the skin graft (Fig. 5, *C* and *D*) demonstrating that the mouse leukocytes were able to extravasate normally into the human skin graft.

After 2 h, the number of leukocytes present in the dermis increased by approximately four- to fivefold ($P < 0.05$). The number of leukocytes in the tissues declined by 8 and 24 h but remained significantly elevated when compared to baseline (Fig. 6).

To validate our method of manually counting leukocytes, the degree of leukocyte infiltration was also calculated in one experiment using computerized image analysis. The intensity and color settings of digitized images were adjusted so that only the red colored leukocytes were identified by the computer. Subepidermal regions were outlined and the computer pro-

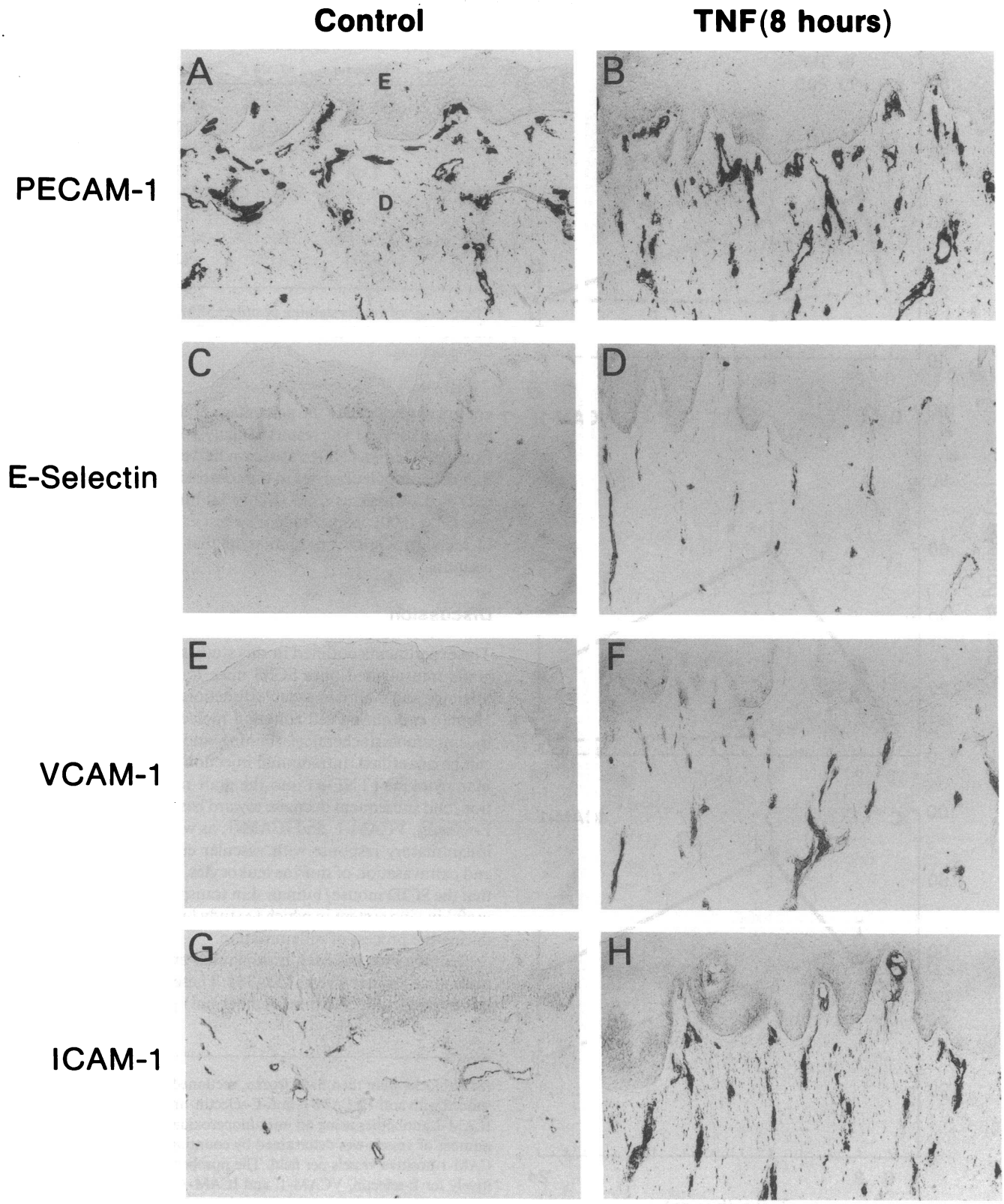

Figure 3. Expression of endothelial cell adhesion molecules in control and cytokine-injected human skin transplants. Transplanted human skin grafts (*E*, epidermis, *D*, dermis) were injected intradermally with either PBS with 2 mg/ml BSA (*Control*) or PBS with 2 mg/ml BSA containing 2,000 U of recombinant human TNF-α (*TNF*). After 8 h, the grafts were harvested, flash frozen, sectioned, fixed in acetone, and stained using an immunoperoxidase technique with the following specific antiendothelial cell adhesion molecule antibodies: *A* and *B*, polyclonal anti-human PECAM-1; *C* and *D*, monoclonal anti-E-selectin; *E* and *F*, monoclonal anti-VCAM-1; *G* and *H*, monoclonal anti-ICAM-1. Magnification, 400.

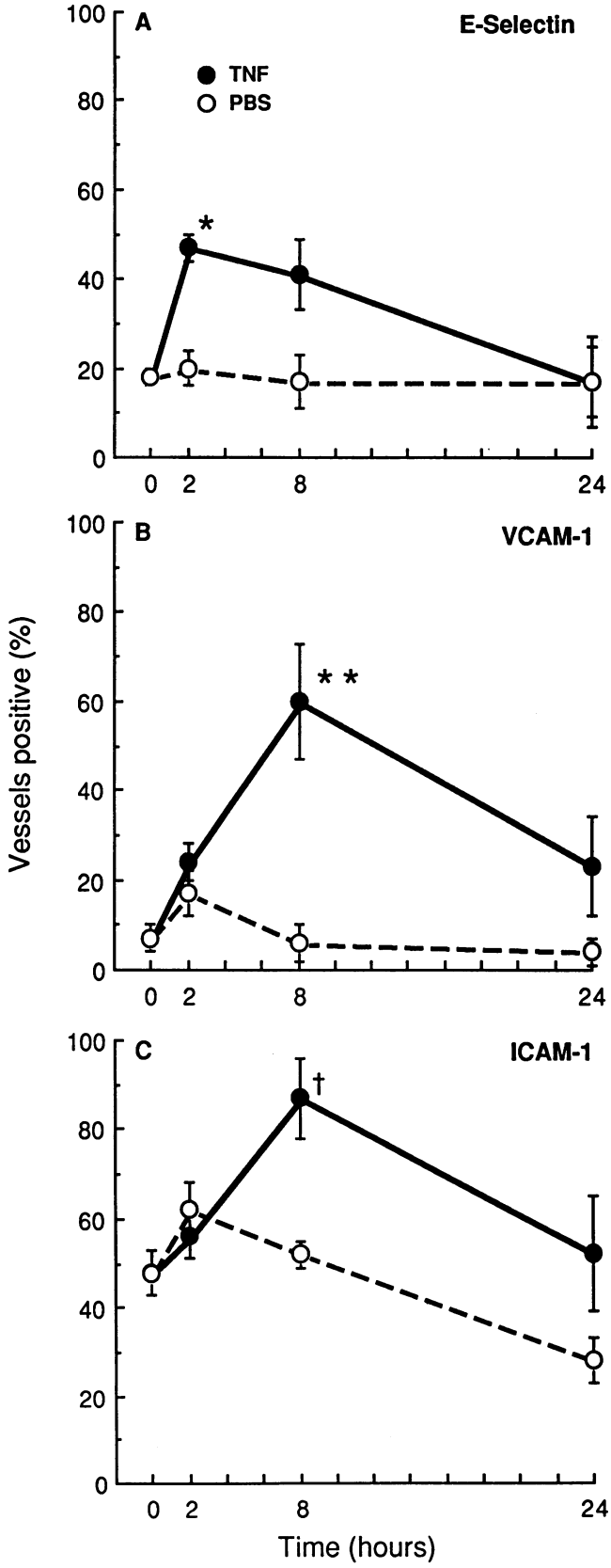

Figure 4. Kinetics of expression of inducible endothelial cell adhesion molecules after intradermal injection of TNF-α. Transplanted human skin grafts were injected intradermally with either PBS with 2 mg/ml BSA (*PBS*) or PBS with 2 mg/ml BSA containing 2,000 U of recombinant human TNF-α (*TNF*). The grafts were harvested immediately before injection (time 0) or 2, 8, or 24 h after injection. The tissue blocks were then flash frozen, sectioned, fixed in acetone, and stained with anti-PECAM-1, anti-E-selectin, anti-VCAM-1, and anti-ICAM-1 antibodies using an immunoperoxidase technique. The total number of vessels was determined by counting the number of PECAM-1-positive vessels per field. The number of vessels staining positively for E-selectin, VCAM-1, and ICAM-1 per field was then counted. The percentage of vessels staining positively (*Vessels positive %*) was determined by dividing the number of vessels staining with the specific antiendothelial cell antibody by the number of vessels staining positively for PECAM-1 and multiplying by 100. (*A*) E-selectin; (*B*) VCAM-1; (*C*) ICAM-1. *Significantly different ($P < 0.05$) from time 0 and PBS control value. **Significantly different ($P < 0.05$) from time 0, 2 h, 24 h, and PBS control value. †Significantly different ($P < 0.05$) from time 0.

Table I. Expression of Endothelial Cell Adhesion Molecules and Leukocyte Accumulation

	E-Selectin	VCAM-1	ICAM-1	Leukocytes/mm²
Baseline	18%±2%* (1.4)‡	7%±3% (0.9)	48%±5% (2.4)	88±8
TNF: 2 h	47%±3% (3.0)	24%±4% (2.1)	56%±5% (3.0)	408±64
TNF: 8 h	41%±8% (2.5)	60%±13% (2.3)	87%±9% (3)	316±44
TNF: 24 h	17%±10% (1.1)	23%±11% (1.5)	52%±13% (2.5)	244±48

* Percentage of vessels staining positive±SEM. ‡ Average intensity of staining: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining.

grammed to calculate the percentage of the total area occupied by the leukocytes. The results of analysis of 14 fields in sections from vehicle and TNF-treated skin harvested after 8 h revealed that the area of leukocytes in the control injected side was 1.7% (SD = 0.2%) versus 6.5% (SD = 1.1) in the TNF-α-injected side ($P < 0.001$ using Student's t test). This 3.8-fold increase in leukocytes was very similar to that obtained by manual counting.

Discussion

The experiments outlined in this study show that human skin grafts transplanted onto SCID mice for up to 8 wk are well tolerated and maintain a viable, functional human vasculature. Human endothelial cell adhesion molecules can be identified by immunohistochemical staining and leukocyte migration can be quantified. Intradermal injection of a recombinant human cytokine (TNF-α) into the graft results in an upregulation, and subsequent decrease toward baseline, in expression of E-selectin, VCAM-1, and ICAM-1, as well as inducing a brisk inflammatory response with vascular endothelial attachment and extravasation of murine leukocytes. These results indicate that the SCID mouse/human skin transplant model provides a useful in vivo system in which to study human endothelial cells during the process of inflammation.

Human skin has been transplanted onto immunodeficient nude mice for many years (35–38). These studies have primarily involved split thickness (epidermal) grafts and have been

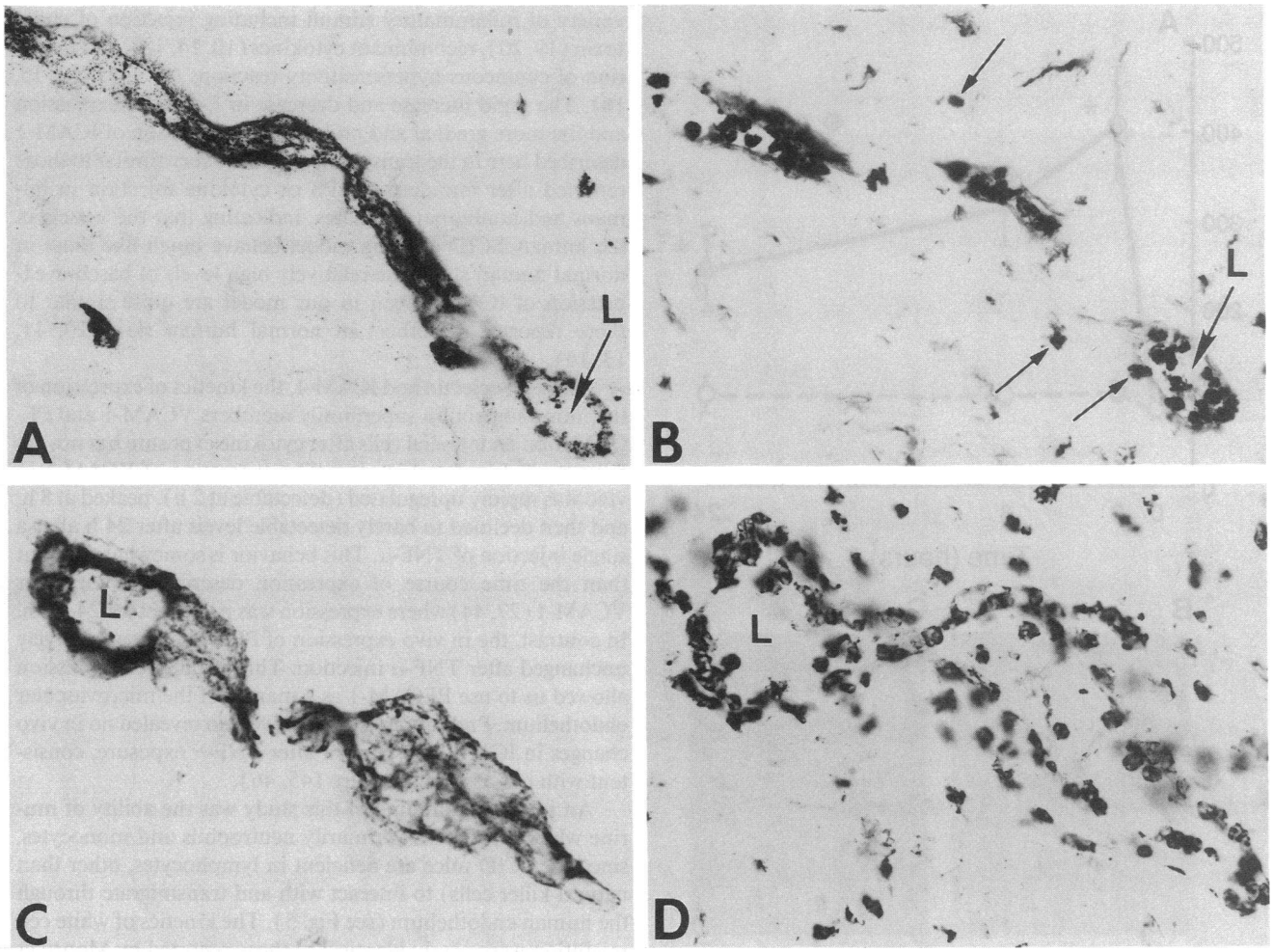

Figure 5. Migration of murine leukocytes in murine and human skin after intradermal injection of TNF-α. Murine skin (*A* and *B*) or transplanted human skin grafts (*C* and *D*) were injected intradermally with PBS with 2 mg/ml BSA containing 2,000 U of recombinant human TNF-α (*TNF*). The mouse skin or graft was harvested, flash frozen, sectioned, fixed in acetone, and stained with antibody against murine PECAM-1 to label murine endothelium (*A*), antibody against human PECAM-1 to label human endothelium (*C*), or an anti-murine Mac-1 antibody to label leukocytes (*B* and *D*) using an immunoperoxidase technique. White blood cells are clearly seen within the lumen (*L*) of murine (*B*) and human (*D*) vessels, as well as extravasating outside of the vessels (*arrows*). Magnification, 800.

useful for studying keratinocyte biology. The use of split thickness grafts, however, which contain minimal amounts of dermal tissue, has limited the utility of this model in studying endothelial cell biology. In addition, at least one group has reported that the human endothelial cells disappeared rapidly from human skin grafts and were replaced by mouse endothelial cells within 2 wk of transplantation (37). The lack of antibodies specific for human or mouse endothelial cells has also hampered careful studies. Another problem with using nude mice in transplantation studies is that immunohistochemical studies using murine monoclonal antibodies, even in the transplanted skin, are difficult because of high background staining caused by the endogenous mouse immunoglobulins present in the tissues.

The SCID mouse is a mutant in which the differentiation of both T and B lymphocytes is severely impaired because of alterations in the DNA recombinase system that is responsible for rearranging the variable diversity joint of immunoglobulins and T cell receptors (21, 39). The SCID mouse has a number of advantages over the nude mouse. Despite the presence of some natural killer cells (40), xenographic tissues such as human skin or human tumors can easily be grafted onto these animals with minimal rejection and without any further type of immunosuppression (41, 42, 43). Our success rate in transplanting full thickness human skin grafts was > 90%. In addition, the extremely low level of endogenous immunoglobulins in the SCID mouse has made it possible to use murine monoclonal antibodies for indirect immunohistochemical studies without the high background staining levels that we observed in nude mice.

In contrast to the lack of persistence of human vasculature in the nude mouse reported by Demarchez et al. (37), we have found that full thickness grafts of human skin transplanted onto SCID mice maintain a viable, fully functional human microvasculature, even up to 8 wk (data not shown) after transplantation. The species origin of the vessels was directly addressed by the use of species-specific anti-PECAM-1 antibodies that reacted exclusively with either mouse or human endothelium. Our studies are in agreement with the recent report of Kim et al. (42), who also found maintenance of the human

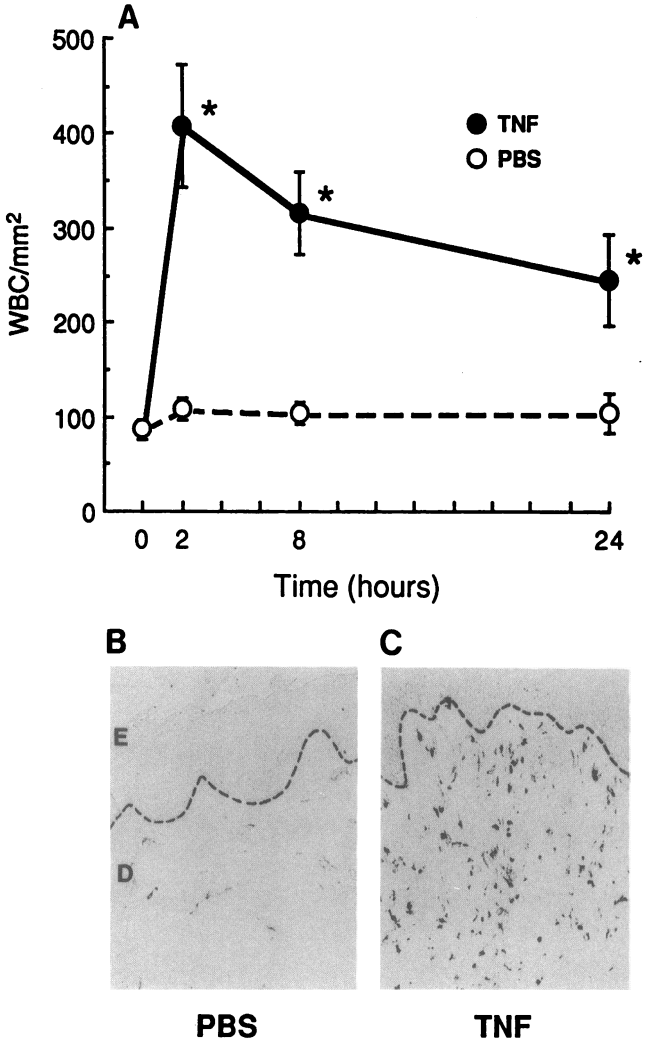

Figure 6. Kinetics of migration of leukocytes after intradermal injection of TNF-α. Transplanted human skin grafts were injected intradermally with either PBS with 2 mg/ml BSA (*PBS*) or PBS with 2 mg/ml BSA containing 2,000 U of recombinant human TNF-α (*TNF*). The grafts were harvested immediately before injection (time 0) or 2 h, 8 h, or 24 h after injection. The tissue blocks were flash frozen, sectioned, fixed in acetone, and stained with a leukocyte-specific anti-murine Mac-1 antibody using an immunoperoxidase technique. Magnification, 400. (*A*) The number of leukocytes per mm^2 was determined by direct counting and plotted. (*B*) Immunoperoxidase stain of skin graft 8 h after injection of PBS with 2 mg/ml BSA. (*C*) Immunoperoxidase stain of skin graft 8 h after injection of PBS with 2 mg/ml BSA containing 2,000 U of TNF-α. *Significantly different ($P < 0.05$) from time 0 and PBS control value.

phenotype in skin transplanted onto SCID mice. The reason for the differences between the report of Demarchez et al. and the experiments reported here and those of Kim et al. is not known for certain, but may be related to our use of full thickness rather than split thickness grafts and to a possible difference between the SCID and nude mouse models.

In addition to maintaining their human phenotype for up to 8 wk, the microvessels within the grafts were able to respond to the injection of recombinant human TNF-α by upregulation of the cell adhesion molecules E-selectin, VCAM-1, and ICAM-1. The in vivo expression of E-selectin and ICAM-1 have been studied in both primate and human models after a variety of inflammatory stimuli including injection of endotoxin (19, 20), recombinant cytokines (10, 14, 18), and induction of cutaneous hypersensitivity reactions (8, 9, 11–13, 15, 16). The rapid increase and decrease in E-selectin expression and the more gradual and prolonged upregulation of ICAM-1 described here in the transplanted skin are very similar to those reported after intradermal LPS or cytokine injection in humans and nonhuman primates, indicating that the vessels in the human/SCID chimera model behave much like those in normal human skin. The relatively high levels of baseline expression of ICAM-1 seen in our model are quite similar to those reported by others in normal human skin (10, 11, 13, 16).

Unlike E-selectin and ICAM-1, the kinetics of expression of the immunoglobulin superfamily members VCAM-1 and PECAM-1 on endothelial cells after cytokine exposure has not yet been studied in detail in vivo. The expression of VCAM-1 in vivo was rapidly upregulated (detectable at 2 h), peaked at 8 h, and then declined to barely detectable levels after 24 h after a single injection of TNF-α. This behavior is somewhat different than the time course of expression described in vitro for VCAM-1 (27, 44) where expression was persistent for 24–48 h. In contrast, the in vivo expression of PECAM-1 was relatively unchanged after TNF-α injection. This stability of expression allowed us to use PECAM-1 as a marker of the microvascular endothelium. Preliminary studies have also revealed no in vivo changes in ICAM-2 expression after TNF-α exposure, consistent with observations in vitro (45, 46).

An interesting finding of this study was the ability of murine white blood cells (primarily neutrophils and monocytes, since the SCID mice are deficient in lymphocytes, other than natural killer cells) to interact with and transmigrate through the human endothelium (see Fig. 5). The kinetics of white cell migration were nearly identical to those reported by Monro et al. (18) after injection of TNF-α into baboon skin. Although the mouse leukocytes were able to effectively adhere and migrate into the human tissue, the detailed mechanisms of mouse leukocyte ligand–human endothelial cell-receptor interactions have not yet been well explored. Heterotypic mouse–human interactions may not be totally analogous to their homotypic mouse–mouse or human–human counterparts. This may be due not only to primary sequence differences in the structures of mouse versus human adhesion molecules, but also to other differences, for example, in the composition of the cell membrane of the respective leukocyte and/or endothelial cells. It is also possible that factors other than those locally generated could be important in leukocyte-endothelial cell interactions in inflammation. These systemic mediators (i.e., contributions from circulating complement components or adhesion co-factors) may be different in mouse and man.

When in vitro binding of heterotypic cells have been studied, Wu et al. (47) found that murine lymphocytes were able to adhere quite well to human endothelium, supporting a conservation of lymphocyte-endothelial recognition mechanisms. On the other hand, Johnston et al. (48) reported that murine cell lines expressing lymphocyte function–associated antigen-1 (LFA-1) did not bind to purified human ICAM-1. The ability of the murine leukocytes to interact with the human endothelium in our in vivo system may be due to (*a*) the fact that neutrophils bind to endothelium primarily using Mac-1 rather than LFA-1 (Mac-1 binds to a different site on the ICAM-1 molecule than does LFA-1 [49]), (*b*) inherent differ-

ences between the in vivo and in vitro models, or (*c*) the possibility that ICAM-1 binding may not be required for neutrophil emigration in this model. Experiments are currently underway to explore which hypothesis is correct. In the future, we also plan to compare our observations of murine leukocyte behavior with that of human leukocytes injected intravenously into the transplanted mice.

In summary, the human skin/SCID mouse chimera described here should provide a useful system to study human vascular biology, with the important caveat that murine white blood cells may not behave identically to human leukocytes under some conditions (i.e., not respond to certain human cytokines). The advantages of this model include: (*a*) it is an in vivo model that will allow study of both the expression of human endothelial cell molecules and quantitative measures of leukocyte migration; (*b*) it will allow study of human microvessels, the most relevant part of the vascular system with regard to inflammation; (*c*) it will allow the use of existing murine anti-human monoclonal antibodies and other human specific reagents in both immunohistochemical studies and blocking studies; and (*d*) the small size of the animals will allow blocking studies to be done with relative small quantities of antibodies, carbohydrates, or peptide blocking reagents. This model will allow the roles of a number of human endothelial cell adhesion molecules (i.e., P-selectin, E-selectin, ICAM-1, PECAM) implicated in the adhesion cascade to be directly determined by systemic infusion of specific blocking monoclonal antibodies simultaneously with the injection of cytokines into the transplanted human skin.

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