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Experimental Production and Modulation of Human Cytotoxic Dermatitis in Human-Murine Chimeras

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Human dermatitis involving cytotoxic interaction between effector lymphocytes and epithelial target cells has thus far been documented* in vivo *only as naturally occurring disease or as an iatrogenic complication of organ engraftment. In this report, we reproduce human cytotoxic dermatitis via local microinjection of heterologous human lymphocytes into human skin xenografted to mice with severe combined immune deficiency syndrome. Injection sites develop progressive T cell epidermotropism culminating in cytotoxic dermatitis resembling human lichen planus within 4 weeks. Effector T cells express a $CD8^+$, $TIA\text{-}1^+$ phenotype, proliferate locally, express interleukin-2 surface receptors, and demonstrate interferon-γ mRNA induction after microinjection. Migration of these T cells into the epidermis is closely linked to experimental induction and coincident expression of intercellular adhesion molecule by keratinocytes. T cell apposition to keratinocytes is associated with endonuclease-mediated DNA fragmentation (apoptosis) in the latter cell type. Intraepidermal T cell migration and related lesion formation is partially abrogated by systemic administration of antisense oligonucleotide to ICAM-1 mRNA. These findings demonstrate that human cytotoxic tissue injury directed against epithelial targets can be produced and modulated in chimeric mice. (Am J Pathol 1997, 150:631–639)

Cytotoxic tissue injury in which alloreactive lymphocytes destroy target epithelial cells is typical of potentially life-threatening disorders such as primary biliary cirrhosis and certain autoimmune diseases and as a complication of organ transplantation.[1] In skin, such lesions often result from interaction between cytotoxic T cells and epidermal target cells and take the form of naturally occurring disorders that include erythema multiforme and lichen planus as well as iatrogenic complications such as graft-*versus*-host disease (GVHD) and certain drug eruptions.[2] Experimental production of cytotoxic tissue injury against epithelial cells *in vivo* using animal chimeras has thus far not been possible, although several informative models of human allograft rejection initiated by microvascular injury and necrosis have recently been established.[3,4] We and others recently have developed human skin-murine chimeras by engrafting neonatal foreskin to immunocompromised mice inbred for severe combined immune deficiency syndrome (SCID mice). Skin xenografts retain human phenotype for at least 4 months and exhibit physiological expression of adhesive glycoproteins for leukocytes upon exposure to recombinant cytokines.[5,6] Because systemic administration of human peripheral blood mononuclear cells (PBMCs) in this system may result in vasculitis and potential secondary ischemic epidermal injury within xenografts,[4] we explored the ability of direct dermal microinjection of heterologous human PBMCs to induce epidermal injury.

We report here that a dermatitis remarkably similar to human lichen planus develops within the human xenografts as a result of cytotoxic interaction between microinjected human effector lymphocytes

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and target epidermal cells. This interaction involves cell-cell contact between intact lymphocytes clustered around degenerating keratinocytes (satellitosis). The lymphocytes involved demonstrate a $CD8^+$, $TIA\text{-}1^+$, interleukin (IL)-2-receptor-positive phenotype, and evidence of local proliferation and active interferon (IFN)-γ synthesis. Epidermal cells apposed by lymphocytes undergo apoptotic cell death, as evidenced by chromosomal fragmentation and ultrastructure. Lesion formation is accelerated by dermal priming with IFN-γ, resulting in induction of intercellular adhesion molecule (ICAM)-1 on epidermal cells. Moreover, inhibition of ICAM-1 expression by antisense oligonucleotide partially abrogates effector cell entry into the epidermis and associated cytotoxic injury. These findings demonstrate that dermatitis closely resembling naturally occurring human disease is inducible *in vivo* in murine chimeras and establish a novel model for exploration of pathogenesis and therapeutic modulation of cytotoxic epithelial injury.

Materials and Methods

Human Skin Transplantation onto SCID Mice

The protocols for skin transplantation have been described previously in detail.[5,7,8] Briefly, neonatal human foreskins were transplanted into size-matched wound beds prepared on each flank of a 4- to 6-week fully immunodeficient SCID mouse (obtained from a colony maintained at the Wistar Institute Animal Facility, Philadelphia, PA). Grafts were characterized extensively by immunohistochemistry and ultrastructure to demonstrate maintenance of the human phenotype and absence of intragraft chimerization. A total of 87 mice (174 grafts) were evaluated at various time points and experimental conditions in this study, with a minimum of 3 mice (6 grafts) for any single time point.

Preparation and Characterization of Mononuclear Cell Fractions

Human PBMCs were separated by Ficoll-Hypaque density gradient centrifugation. For flow cytometric analysis, the isolated PBMCs were labeled by indirect immunofluorescence with a panel of primary antibodies followed by a fluorescein-isothiocyanate-labeled goat anti-mouse secondary antibody. Analysis was done using an Ortho Cytofluorograph 50H cell sorter equipped with a 2150 data handling system (Ortho Instruments, Westwood, MA). A typical profile of the PBMCs utilized was $CD3^+ = 68 \pm 1\%$, $CD4^+ = 50 \pm 4\%$, $CD8^+ = 36 \pm 4\%$, $CD45\text{-}RO^+ = 45 \pm 4\%$, $CD45\text{-}RA^+ = 65 \pm 4\%$. Only a small fraction of the cells were B cells ($CD15^+ = 4\%$) or macrophages ($CD19^+ = 7\%$).

Human Mononuclear Cell Microinjection

A skin graft on one side of each mouse was injected intradermally with 50 μl of endotoxin-free saline containing 2 mg/ml bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) as a protein carrier plus 2 μl of colloidal carbon (Sigma) to mark the site of injection (control side). The skin graft on the contralateral side was injected with the isolated PBMCs at a dose of 10×10^6 cells in 100 μl of saline/BSA with 2 μl of colloidal carbon added to mark the injection site. To determine the effect of ICAM-1 induction on the kinetics of epidermotropism and lesion formation, some grafts were primed with 50 μl of human recombinant IFN-γ (1000 U; Biosource International, Camarillo, CA) or control saline 24 hours before PBMC microinjection.

Syngeneic control experiments were also designed in which discarded adult truncal skin from an elective procedure was engrafted onto SCID mice as described above for neonatal foreskin. PBMCs were isolated from this same patient four weeks later and injected as described above. The response was evaluated histologically and immunohistochemically after 4 weeks. As a control for immunological activity of these PBMCs, we also injected these cells into mismatched donor neonatal foreskin xenografts as well as donor adult facial skin xenografts and evaluated the cytotoxic response after 4 weeks.

Antisense Oligonucleotide Evaluation and Administration

Phosphorothioate oligodeoxyribonucleotides (ODNs; ISIS Pharmaceuticals, Carlsbad, CA) were synthesized as previously described.[9] The following ODNs were used: ISIS 2302, an ICAM-1 antisense 20-mer (AS-ODN) that hybridizes to specific sequences in the 3′-untranslated region of ICAM-1 mRNA and a sense control (SE-ODN) ISIS 8433. Rhodamine-labeled ODN was prepared as previously described.[10] To determine the ability of the ODN to inhibit ICAM-1 expression on the surface of cells, an *in vitro* assay was used as previously described.[11] Human umbilical vein endothelial cells (Clonetics Corp., San Diego, CA) were incubated with 100 nmol/L ODN ISIS 2302 (AS-ODN)

and ISIS 8433 (SE-ODN) in the presence of 10 μg/ml lipofectin (Sigma) for 4 hours at 37°C. Recombinant IFN-γ was added at 1000 U/ml and the cells were incubated for an additional 24 hours at 37°C and analyzed by FACS using 20 μg/ml anti-ICAM-1 monoclonal antibody.

To determine the ability of ODN to function in the xenografts, they were tested for their ability to block up-regulation of keratinocyte ICAM-1 expression induced by intra-graft injection of recombinant IFN-γ. ODNs were injected subcutaneously at a dose of 10 mg/kg/day on the flanks of transplanted SCID mice for 5 days, and 2 days after the start of ODN injections, 100 U of IFN-γ was injected into each graft in 50 μl of saline. Grafts were harvested and stained for ICAM-1 and HLA-DR expression 48 hours later. In experiments in which ODNs were tested for their ability to block epidermotropism after PBMC injection, ODNs were loaded into mini-osmotic pumps (Alza Corp., Palo Alto, CA) at a dose of 250 μg per mouse per day for 14 days. Pumps were surgically implanted subcutaneously beneath the murine neck skin on the same day as PBMC injection. To confirm localization of ODNs in target epidermal cells of the human xenografts, mice were injected subcutaneously on the flank with 250 μg/mouse/day of the rhodamine-isothiocyanate-labeled ISIS 2302 AS-ODN and the skins were explanted after 1, 2, 4, and 24 hours.

Harvesting of Xenografts

After injection of PBMCs, mice were sacrificed after 1 and 4 days and weekly for 4 weeks. After careful macroscopic examination, the skin grafts were removed and bisected through the center of the injection sites, as marked by the colloidal carbon. Each half was oriented appropriately, placed in OCT compound, and snap frozen for immunohistochemistry. Some samples were collected in Karnovsky's fixative to be processed for electron microscopy or in formalin to be embedded in paraffin and processed for histological evaluation, for *in situ* hybridization, or for evaluation by the TUNEL technique.

Immunohistochemistry

Immunoperoxidase staining was performed as previously described.[6] The following monoclonal antibodies were used for immunostaining: anti-CD3, anti-CD4, anti-CD8, anti-CD15, anti-CD19 and anti-HLA-DR (Becton Dickinson, Mountain View, CA), anti-ICAM-1 (AMAC, Westbrook, ME), anti-TIA-1 (Coulter Immunology, Hialeah, FL), and anti-Ki-67 and anti-IL-2 receptor (DAKO, Carpinteria, CA).

In Situ *Hybridization*

Expression of IFN-γ was detected by *in situ* hybridization using standard techniques[12] and with the generous assistance of Dr. Benjamin Vowels. The IFN-γ probe (DNA synthesis service, University of Pennsylvania) was labeled using the digoxigenin-11dUTP Boeringer Mannheim Biochemicals DNA tailing kit.

TUNEL Analysis

Determination of apoptotic target cell injury was performed as previously described for the terminal uridine deoxynucleotidyl transferase end ligation technique.[13]

Ultrastructural Analyses

Conventional transmission electron microscopy was performed as previously described.[14]

Quantitation and Statistics

Human lymphocytes were identified immunohistochemically using the anti-CD3 monoclonal antibody. Multiple 5-μm sections were cut from the center of each skin biopsy as marked by colloidal carbon to obtain representative samples of the injection site. In experiments correlating ICAM-1 expression with epidermotropism, sections were obtained along planes tangential to the dermal-epidermal junction to maximize epidermal cross-sectional area represented. The number of leukocytes that infiltrated the entire epidermis spanning each section were counted using an ocular grid micrometer at ×40 and expressed as cells/per square millimeter. In these and subsequent analyses (below), a minimum of 0.5 mm^2 of epidermis was evaluated per specimen. In experiments assessing effects of ODNs on epidermotropism, CD3-positive intraepidermal cells were evaluated only in epidermal regions showing responsiveness to IFN-γ stimulation, as determined by HLA-DR induction in adjacent sections. Differences among groups were analyzed using one-way analysis of variance. When statistically significant differences were found ($P < 0.05$), individual comparisons were made using the Bonferroni/Dunn test.

Computer-assisted image analysis using planar morphometry in conjunction with microdensitometry software was also employed for analysis of correla-

tive $CD3^+$ T cell epidermotropism and ICAM-1 expression by epidermal cells (Southern Micro Instruments (Atlanta, GA) system using Microcomp software). This enabled the automated counting of CD3-positive cells in the epidermal compartment of tangentially sectioned skin and correlation with density of ICAM-1 epidermal intensity in these regions (pixels/per square micron). Multiple measurements (six to eight) were obtained for each time point (1 and 4 days and 1, 2, and 4 weeks).

In experiments involving assessment of large epidermal regions for induction or suppression of HLA-DR or ICAM-1 reactivity, a semiquantitative method for measuring relative staining intensity was employed. Levels of expression were assigned as follows: 0, no expression; 1, faint diffuse expression; 2, strong basal layer expression; 3, strong diffuse expression. The average expression (expression index) was calculated for the entire epidermal area when more than one pattern co-existed.

Results

Production of Human Lichen Planus-Like Cytotoxic Dermatitis in Xenografts

Human neonatal foreskin transplanted to the backs of SCID mice retained a normal gross and histological phenotype up to 3 months after transplantation, as previously described.[5,6,8] However, 4 weeks after intradermal injection of heterologous human PBMCs into the grafts, the grafts developed progressive scaling (Figure 1B) that was not seen in the control xenografts injected with saline (Figure 1A). Conventional histology at 4 weeks after heterologous intradermal PBMC injection revealed a band-like lymphocytic infiltrate selectively beneath a hyperplastic human epidermis but not affecting a normal murine epidermis at the graft anastomotic junction (Figure 1C). This infiltrate diffusely involved the human graft and focally obscured the dermal-epidermal junction where degenerating basal keratinocytes (Figure 1D) and clefts due to basal layer cytolysis were observed (Figure 1E). At higher magnification, many injured basal cells were surrounded by lymphocytes that had infiltrated into the epidermal layer (Figure 1F). There was no evidence of diffuse necrotizing vascular injury, as may occur after systemic PBMC administration.[4] Evaluation by electron microscopy (not shown) confirmed injury consistent with apoptosis[15] of basal keratinocytes surrounded by viable lymphocytes, and TUNEL technique revealed DNA fragmentation consistent with apoptotic death of some basal cells (Figure 1F, inset). When compared with naturally occurring human dermatitis, the xenografts most resembled changes of epidermal-type chronic GVHD and lichen planus, namely, epidermal hyperplasia associated with an underlying band-like lymphocytic infiltrate, degeneration and lytic change in the basal cell layer (Figure 1G), and epidermotropism of lymphocytes associated with basal keratinocyte cell death (Figure 1H).

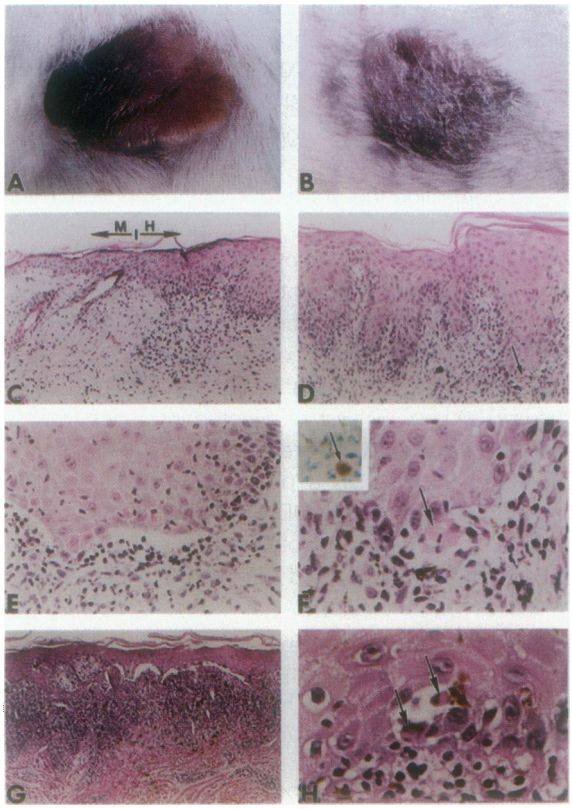

Figure 1. *Development of lichen planus-like cytotoxic dermatitis in human skin xenografted to SCID mice.* **A** *and* **B**: *As compared with PBS-injected controls* (**A**), *grafts show clinical hyperkatosis 4 weeks after intradermal injection of allogeneic PBMCs* (**B**). **C** *to* **F**: *H&E staining shows cytotoxic inflammation that involves only human skin to the right of the murine* (*M*)*/human* (*H*) *anastomosis site* (**C**, ← | →); *lesions show hyperkatosis, epidermal thickening* (**D**), *destruction of the basal cell layer* (**E** *and* **F**) *with clefts due to basal layer cytolysis* (**E**), *and necrotic basal keratinocytes* (**F**, **arrow**) *that also are TUNEL positive* (**F**, **inset** *with* **arrow**). **G** *and* **H**: *Lichen planus lesions from patients show similar to identical findings* (**arrow**, *necrotic basal keratinocytes*). *Magnification,* ×*100* (**C**, **D**, *and* **G**) *and* ×*400* (**E**, **F**, *and* **H**).

To confirm that these changes represented an allogeneic response rather than a nonspecific inflammatory reaction, PBMCs from a single donor were injected into both allogeneic and syngeneic skin grafts. PBMC injection into syngeneic xenografts failed to induce clinical or histological evidence of epidermal lymphocyte infiltration or related cytotoxic injury, whereas injection in both neonatal

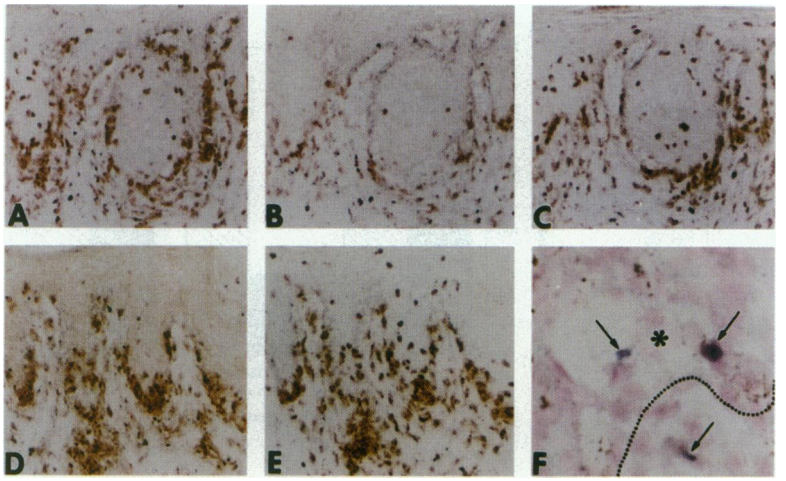

Figure 2. *Phenotype of epidermotropic CD3⁺ PBMCs in xenografts 4 weeks after injection determined by immunohistochemistry and* in situ *hybridization.* **A:** *CD3.* **B:** *CD4.* **C:** *CD8 (adjacent sections).* **D:** *CD8.* **E:** *TIA-1 (adjacent sections).* **F:** In situ *hybridization for IFN-γ mRNA* (**arrows,** *positive cells; *necrotic keratinocyte; · · · ·, basement membrane zone). Magnification, ×100* (**A** *to* **E**) *and ×400* (**F**).

and adult allogeneic skins resulted in changes similar to those seen in Figure 1.

PBMC Infiltrating Xenografts Are of a Cytotoxic Phenotype

Immunophenotypic characterization of PBMC infiltrating xenografts at 4 weeks revealed the majority (>95%) to be $CD3^+$ T cells (Figure 2A). In serial adjacent sections, a minority (<25%) expressed CD4 (Figure 2B), whereas a majority (>75%) expressed CD8 (Figure 2C). There was a close association between $CD8^+$ epidermal T cells with cells expressing the TIA-1 marker for cytotoxic granules (Figure 2, D and E). Approximately 25% of the T cells expressed IL-2 membrane receptors, and in addition to basal keratinocytes, some (10 to 20%) of these T cells showed proliferative capacity, as judged by Ki-67 nuclear immunoreactivity (data not shown). By 2 weeks, occasional infiltrating T cells (<10%) demonstrated positive mRNA hybridization with a probe for IFN-γ (Figure 2F).

Epidermotropism of PBMCs in Xenografts Involves Association of $CD3^+$ T Cells with $ICAM\text{-}1^+$ Keratinocytes

Sequential analysis of the number of epidermotropic $CD3^+$ cells disclosed intraepidermal immigration to occur primarily 2 to 4 weeks after PBMC injection (Figure 3A). Graft priming with IFN-γ at the time of PBMC injection altered the kinetics of epidermotropism, enhancing intraepidermal immigration during the first 2 weeks (Figure 3A) but not thereafter. By 4 weeks, the extent of epidermotropism of $CD3^+$ cells did not differ significantly between IFN-γ-primed and nonprimed grafts.

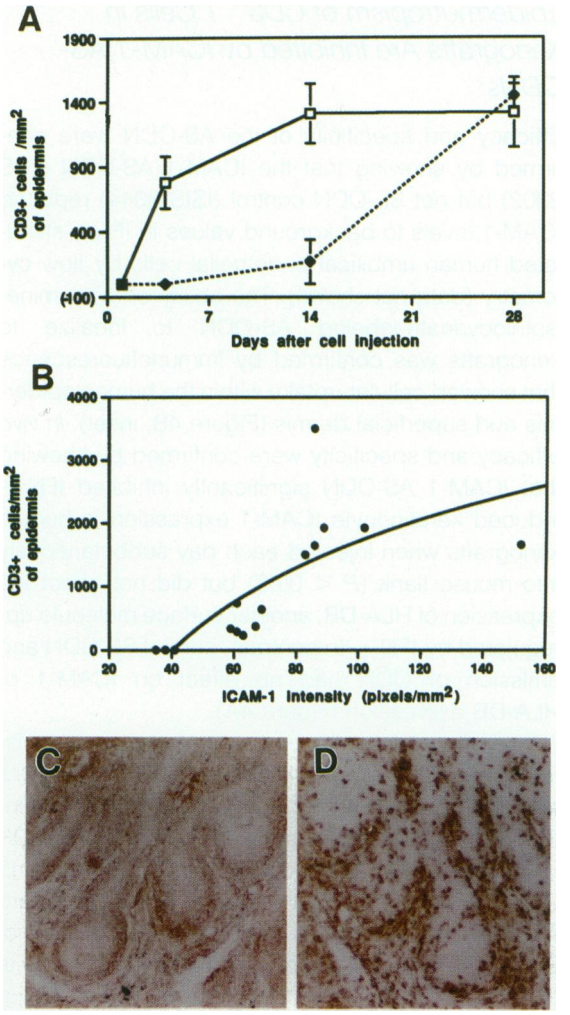

Figure 3. A: *Sequential analysis of epidermotropic $CD3^+$ PBMCs after graft priming with IFN-γ (□) or PBS (▲).* **B:** *Correlation of degree of epidermotropism with ICAM-1 intensity expressed by surrounding keratinocytes at 28 days after PBMC injection into IFN-γ-primed grafts.* **C** *and* **D:** *Qualitative relationship of ICAM-1 expression* (**C**) *with sites of $CD3^+$ T cell epidermotropism* (**D**) *in adjacent sections represented here as mirror images of apposing tissue planes. Magnification, ×100.*

Because IFN-γ is a potent inducer of epidermal ICAM-1 in xenograft epidermis (unpublished), we next sought to determine whether sites of $CD3^+$ T cell epidermotropism correlated spatially with zones of ICAM-1 expression by graft keratinocytes. The number of intraepidermal T cells was closely associated with ICAM-1 intensity at 2 and 4 weeks as measured microdensitometrically in adjacent keratinocytes (Figure 3B). Moreover, epidermal regions selectively infiltrated by $CD3^+$ T cells (Figure 3D) corresponded quantitatively to zones of ICAM-1 keratinocyte expression in adjacent sections (Figure 3C).

Epidermal ICAM-1 Expression and Related Epidermotropism of $CD3^+$ T Cells in Xenografts Are Inhibited by ICAM-1 AS-ODNs

Efficacy and specificity of the AS-ODN were confirmed by showing that the ICAM-1 AS-ODN (ISIS 2302) but not SE-ODN control (ISIS 8344) reduced ICAM-1 levels to background values in IFN-γ-stimulated human umbilical endothelial cells by flow cytometry (data not shown). The ability of rhodamine-isothiocyanate-labeled AS-ODN to localize to xenografts was confirmed by immunofluorescence that showed cellular uptake within the human epidermis and superficial dermis (Figure 4B, inset). *In vivo* efficacy and specificity were confirmed by showing that ICAM-1 AS-ODN significantly inhibited IFN-γ-induced keratinocyte ICAM-1 expression in human xenografts when injected each day subcutaneously into mouse flank ($P < 0.05$) but did not affect the expression of HLA-DR, another surface molecule up-regulated by IFN-γ. In contrast, control SE-ODN and omission of ODN had no effect on ICAM-1 or HLA-DR expression (Figure 4A).

The effect of continuous subcutaneous administration by osmotic pump of saline, ICAM-1 SE-ODN, and ICAM-1 AS-ODN for 2 weeks after PBMC injection into xenografts was next evaluated. HLA-DR remained high in all conditions (Figure 5, A, D, and G). Qualitatively, ICAM-1 in the epidermal compartment was up-regulated after saline (Figure 5B) or SE-ODN (Figure 5E) but reduced after treatment with ICAM-1 AS-ODN (Figure 5H). As summarized in Figure 4B, epidermotropism of $CD3^+$ T cells within human xenografts was significantly inhibited by AS-ODN (Figure 5I; $P < 0.05$) but not by SE-ODN administration (Figure 5F) or by omission of ODN, (Figures 4B and 5C). Although difficult to quantitate,

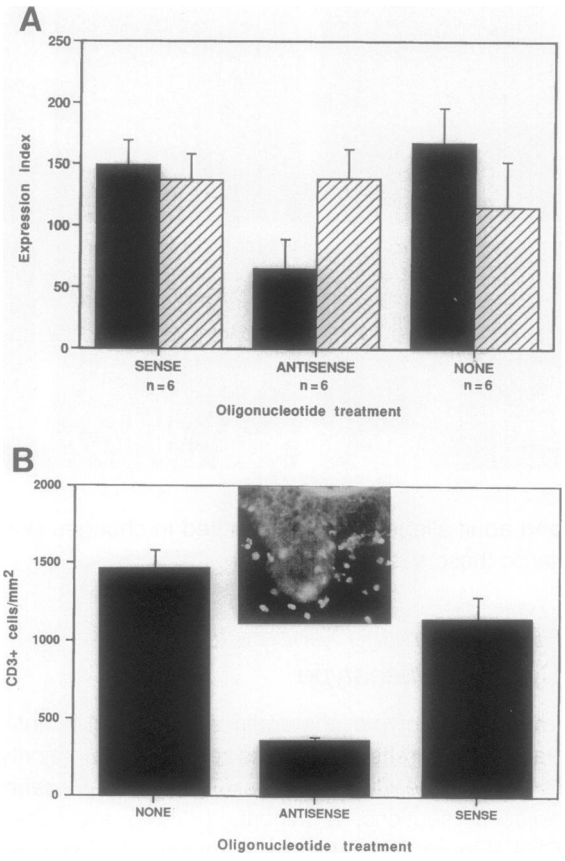

Figure 4. A: *Inhibition of epidermal ICAM-1 expression* **(solid bars)** *in xenografts by daily subcutaneous AS-ODN treatment for 5 days beginning 3 days before intradermal IFN-γ injection. No effect was seen with SE-ODN or with saline control. Expression index (see Materials and Methods) was calculated 48 hours after IFN-γ administration.* **Hatched bars,** *epidermal HLA-DR expression in adjacent sections.* **B:** *Inhibition of epidermotropism of $CD3^+$ PBMCs by AS-ODN administered continuously s.c. for 2 weeks, 2 weeks after PBMC injection i.d. into xenografts (time of earliest epidermotropic response when PBMCs are administered alone; see Figure 3A).* **Inset** *represents rhodamine isothiocyanate AS-ODN staining 24 hours after subcutaneous administration; bright zones indicate cellular uptake within the lower epidermis and superficial dermis.*

there appeared to be less cytotoxic injury in the AS-ODN treated animals (Figure 5F *versus* 5I).

Discussion

We demonstrate here that human cytotoxic dermatitis with phenotypic similarity to naturally occurring lichen planus is experimentally reproducible in human skin/SCID mouse chimeras. Human PBMCs responsible for this phenomenon express an allostimulated cytotoxic T cell phenotype and migrate into the human epidermis in direct association with ICAM-1 induction. The potential importance of ICAM-1 expression by epidermal cells in promoting cytotoxic interactions with target cells is emphasized by the

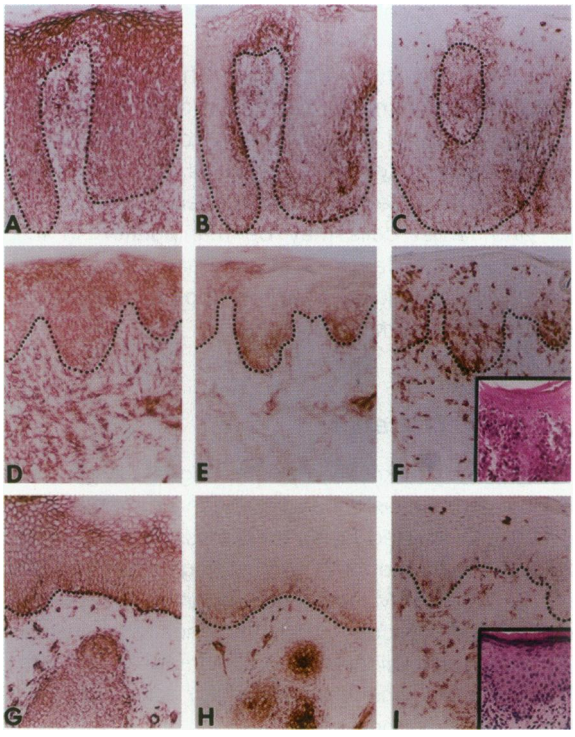

Figure 5. *Immunohistochemical assessment of effects of ICAM-1 AS-ODN administration on epidermal HLA-DR and ICAM-1 expression and CD3$^+$ epidermotropism within xenografts 4 weeks after PBMC injection.* A *to* C: *Control (no ODN) pattern for HLA-DR* (A), *ICAM-1* (B), *and CD3$^+$* (C). D *to* F: *ICAM-1 SE-ODN control for HLA-DR* (D), *ICAM-1* (E), *and CD3$^+$* (F). G *to* I: *ICAM-1 AS-ODN effects on HLA-DR* (G), *ICAM-1* (H), *and CD3$^+$* (I). *Note marked reduction of ICAM-1 and CD3$^+$ immunoreactivity as a consequence of antisense treatment* (H *and* I). Insets *in* F *and* I *show conventional histology indicating cytotoxic injury obscuring the dermal-epidermal junction in SE-ODN control-treated animals* (F) *compared with relatively intact dermal-epidermal interface in AS-ODN-treated animals* (I) (· · · ·, *dermal-epidermal junction indicated for immunohistochemical preparations*). *Magnification,* ×*100.*

ability to lessen intraepidermal T cell migration and lesion severity with AS-ODN to ICAM-1 mRNA.

Lichen planus is a relatively common skin disorder characterized by multiple scaling skin and oral mucosal papules.[16] The provocative cause is unknown, although remarkably similar lesions may occur in association with ingestion of certain drugs[17] and GVHD.[18] The histopathology of lichen planus is characteristic, consisting of a band of lymphocytes within the superficial dermis associated with their migration into the epidermal layer (epidermotropism) and their apposition to degenerating keratinocytes (satellitosis).[2] The epidermal layer responds with hyperplasia and increased scale formation. The immunophenotype of the lymphoid infiltrate in lichen planus indicates T cell lineage,[19] and although CD4$^+$ cells may participate in early antigen presentation, cytotoxic injury is now regarded to reside in a CD8$^+$ T cell subpopulation.[20,21] Heretofore, *in vivo* models for reproduction and study of human lichen planus-like cytotoxic dermatitis have not existed. In the human skin/SCID mouse xenograft model, heterologous human PBMCs were injected directly into graft dermis, bypassing the microvasculature, as previous studies have indicated inefficiency in inducing homing of systemically administered T cells to skin.[3] Moreover, when homing is achieved, vascular injury mimicking allogeneic skin graft rejection has been documented,[3,4] which is a potential source for secondary ischemic epidermal injury and intraepidermal T cell migration.[22,23] Indeed, it has recently been emphasized that endothelial cells are capable of stimulating proliferation of allogeneic T cells,[4] a finding relevant to previous studies of SCID mice in which systemically administered human PBMCs must first interact with dermal microvessels within human xenografts.[3,4,22,23]

Intradermally administered PBMCs presumably undergo allostimulation *in situ* in our model, obviating the need for *in vitro* antigen priming or use of HLA-presensitized donors.[3] Evidence of allogeneic T cell activation includes inability to produce lesions subsequent to identically administered syngeneic PBMC controls, the T cell phenotype associated with epidermal injury (CD3$^+$, CD8$^+$, TIA-1 cytotoxic granule positive, Ki-67$^+$, and IFN-γ mRNA$^+$) and the presence of apoptotic TUNEL$^+$ keratinocytes surrounded by epidermotropic lymphocytes. Although only a minority of T cells were positive for IFN-γ mRNA and IL-2 receptors, *in situ* hybridization of infiltrating cells in acute human renal allograft rejection has revealed similar percentages,[24] suggesting that only a relatively small number of effector cells may express this activated phenotype. Because human lichen planus and lichen planus-like chronic GVHD are considered to be clinical paradigms for cytotoxic injury to skin epithelium, the ability to reproduce similar cellular interactions in this model has implications for understanding basic molecular mechanisms for these disorders.

Cutaneous alloreactivity directed against putative target antigens within the epidermal layer implies a complex and coordinated sequence of adhesive and migratory steps. In lichen planus[25,26] and chronic GVHD,[27] induction of HLA-DR and ICAM-1 on keratinocytes and subsequent lymphocyte binding *via* membrane ligand LFA-1 have been regarded as critical to the epidermotropic response and resultant target cell injury. ICAM-1 expression alone, however, may not be sufficient to mediate such events in view of recent studies failing to observe a relationship between intraepidermal lymphocyte homing and epidermal cell expression of the ICAM-1 transgene *in vivo*.[28] In the present study, association between T

cell epidermotropism and ICAM-1 expression by epidermal keratinocytes consisted of 1) linkage between graft priming with the ICAM-1-inducing cytokine IFN-γ and T cell epidermotropism during the first 2 weeks after PBMC injection (before significant allostimulation is likely to have occurred), 2) temporal association of ICAM-1 expression with degree of T cell epidermotropism, and 3) spatial association between zones of ICAM-1$^+$ epidermal cells and intraepidermal T cells. The existence of an *in vivo* animal model reproducing these molecular and cellular associations now permits experimental manipulation to determine the relative importance of ICAM-1 expression in the epidermotropic response prerequisite for cutaneous cytotoxicity.

AS-ODNs have recently been employed to inhibit transcription of specific adhesive proteins by selective reduction of mRNA levels. Bennet et al[9] have utilized anti-ICAM-1 ODNs to abrogate *in vitro* endothelial and keratinocyte expression of cell adhesion molecules important in inflammation. The relevance of these data to human cells *in vivo* has until now been unclear due to limitations in experimental systems. In this study, the ICAM-1 AS-ODN ISIS 2302 was first evaluated for showing successful inhibition of endothelial ICAM-1 expression *in vitro* by FACS analysis, ability of systemically administered ODN *in vivo* to be taken up by relevant epidermal cells in human xenografts, and efficacy in inhibiting IFN-γ-induced epidermal ICAM-1 expression in human xenografts. Controls consisted of omission of probe as well as SE-ODN, the latter of which may in certain experimental settings demonstrate nonspecific inhibitory effects on ICAM-1 expression.[29] AS-ODNs were administered during the 3rd and 4th weeks after PBMC injection, the time interval of T cell epidermotropism in xenografts not primed with IFN-γ. Our data suggest that AS-ODN, but not SE-ODN, administration significantly inhibits T cell epidermotropism and qualitatively ameliorates cytotoxic lesion formation in this model. It is most likely that this effect is mediated at the level of the anchoring cytotoxic T cells within the epidermal layer subsequent to upward migration due to chemotactic or chemokinetic gradients and/or inhibition of cell-cell contact necessary for T-cell-mediated keratinocyte injury. It is alternatively possible that inhibition involved ICAM-1/LFA-1 interactions implicit to allostimulation, although this is considered less likely as ODNs were administered only during the 3rd and 4th weeks after PBMC injection, a time interval that followed development of the activated cytotoxic phenotype. Moreover, recent evidence implicates molecules other than ICAM-1 in cell-cell binding necessary for alloantigen presentation.[30]

In summary, this study establishes human skin/SCID mouse chimeras as the first model for *in vivo* reproduction and sequential study of the pathogenesis of human cytotoxic dermatitis. Specific molecules that mediate interaction between human effector and target cells may be manipulated in this experimental system in an effort to better understand and more specifically treat this important and clinically relevant form of tissue injury.

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