Epidermal Keratinocytes Express the Adhesion Molecule Intercellular Adhesion Molecule-1 in Inflammatory Dermatoses

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Using indirect immunofluorescence assays on frozen tissue sections of skin from healthy subjects and subjects with inflammatory skin diseases, we found that intercellular adhesion molecule-1 (ICAM-1) was expressed in a cell surface pattern on epidermal keratinocytes at the site of lymphoid infiltration in cutaneous dermatoses. ICAM-1 was not expressed on epidermal keratinocytes in noninflamed skin. Its expression was not related solely to epidermal hyperproliferation, as hyperproliferative, tape-stripped epidermis did not express ICAM-1. We have reported previously that ICAM-1 expression on epidermal keratinocytes was upregulated by treatment with interferon gamma and that activated T lymphocytes bound to cultured epidermal keratinocytes in vitro by lymphocyte function associated-1 (LFA-1) molecules on T cells and ICAM-1 on epidermal keratinocytes. Taken together, these data suggest that upregulation of expression of ICAM-1 is an important feature of cutaneous inflammation. *J Invest Dermatol 92:746–750, 1989*

Infiltration of the skin by activated T lymphocytes is characteristic of many inflammatory dermatoses [1]. In some of these cutaneous dermatoses (i.e., graft versus host disease, allergic contact dermatitis, lichen planus), the infiltration of activated T lymphocytes into the skin is accompanied by expression on epidermal keratinocytes (EK) of major histocompatibility complex (MHC) class II molecules [2–11], which in normal epidermis is limited to Langerhans cells [12–14] and acrosyringial epithelial cells [15]. Major histocompatibility complex class II molecules can be induced in vitro on EK by interferon gamma (IFN-γ), a cytokine produced by activated T lymphocytes [16]. These data have led to the suggestion that activated T lymphocytes migrating to the epidermis may induce expression of MHC class II molecules on the surface of EK by produceing IFN-γ. The functional consequences in vivo of MHC class II expression by EK are not known. Gaspari and Katz [17] reported that MHC class II-positive murine EK can present antigenic peptides to T cell hybridomas and serve as targets for MHC class II restricted cytolytic T cell clones, suggesting that MHC class II-positive keratinocytes can participate in some immune reactions. In addition, Gaspari and colleagues [18] recently reported that in some instances MHC class II-positive murine EK may play a role in suppressing immune responses.

Another molecule whose expression can be regulated by IFN-γ is intercellular adhesion molecule-1 (ICAM-1) [19,20]. ICAM-1 is a cell surface glycoprotein of relative molecular mass 90,000–100,000 that can serve as one ligand for the lymphocyte adhesion molecule LFA-1 in lymphocyte interactions with a variety of different cell types [21–26]. We have previously reported that activated T lymphocytes bound to EK that had been treated with IFN-γ [22], but bound only minimally to EK that were not treated with IFN-γ. Lymphocyte binding to IFN-γ-treated EK was completely abrogated by antibodies to LFA-1 and to ICAM-1, suggesting that ICAM-1/LFA-1 interactions were critical for interactions between activated human T lymphocytes and IFN-γ-treated EK.

If LFA-1/ICAM-1 interactions are important in vivo in inflammatory dermatoses, then ICAM-1 should be expressed on the surface of EK during cutaneous inflammation. In this study, we report that although ICAM-1 was not expressed on normal epidermis, ICAM-1 was focally expressed on EK in a cell surface pattern in a variety of inflammatory dermatoses. The upregulation of ICAM-1 expression was not the result of hyperproliferation of the epidermis, as mechanical induction of hyperproliferation by tape stripping [27] did not induce epidermal expression of ICAM-1. Thus, modulation of the expression of ICAM-1 by EK may be important in the pathogenesis of cutaneous inflammation.

MATERIALS AND METHODS

Tissue Specimens Three-millimeter punch biopsies were obtained after informed consent from patients or from healthy volunteers. The patients' diagnoses were established by clinical criteria and routine histopathology of the biopsies. We examined biopsies from 5 patients with atopic dermatitis, 2 patients with graft-versus-host disease, 2 patients with psoriasis, 2 patients with lichen simplex chronicus, and 5 patients with spongiotic dermatitis including 2 with contact dermatitis and 3 with spongiotic dermatitis of unknown etiology. We also examined biopsies of 6 patients with

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Abbreviations:
EK: epidermal keratinocytes
ICAM-1: intercellular adhesion molecule-1
IFN-γ: interferon gamma
LFA-1: lymphocyte function associated molecule-1
MHC: major histocompatibility complex

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cutaneous T cell lymphoma. Each biopsy was snap frozen, mounted in O.C.T. 4583 embedding compound (Miles Laboratories, Elkhart, IN), cut into 4-μm sections using an AO Histostat Microtome (Reichert-Jung, Buffalo, NY), and processed for immunofluorescence. In each case, sample cryostat sections were stained with hematoxylin and eosin. To induce epidermal hyperproliferation for control experiments, skin of the inner, upper arm of two volunteers was stripped using Scotch brand tape, type 810 (3M, St. Paul, MN), until the skin glistened (approximately 50 times). Biopsies were taken 48 h later [28].

Indirect Immunofluorescence Indirect immunofluorescence was performed on cryostat-sectioned tissues as previously described [29]. In some cases, immunofluorescence was performed on consecutive sections with specific monoclonal reagents. The following dilutions of monoclonal antibodies were used: 1:1000 AE1 (antikeratin) [30], 1:1000 RR1/1 (anti-ICAM-1) [20], 1:500 L243, which reacts with a nonpolymorphic epitope of MHC class II (HLA-DR) molecules [31], 1:500 TS1/22 (anti-LFA-1) [32], and 1:500 Leu4, which reacts with the epsilon chain of the CD3 molecule associated with the T cell antigen receptor [33].

RESULTS

Expression of Intercellular Adhesion Molecule-1 on Tissue Sections of Inflamed Skin Normal human skin from several body sites including upper arm, foreskin, and breast was examined for expression of ICAM-1. As shown in Fig 1*A*, ICAM-1 expression was not detected within the epidermis. Although it is not readily seen in Fig 1*A*, vascular endothelium did express ICAM-1. In contrast, in a variety of cutaneous dermatoses, EK expressed ICAM-1 in a cell surface pattern (Fig 1 *B–D*). Intercellular adhesion molecule-1 expression was detected on EK in lesional skin of atopic dermatitis (3 of 5 patients), allergic contact dermatitis (2 of 2 patients), spongiotic dermatitis of unknown etiology (2 of 3 patients), lichen simplex chronicus (2 of 2 patients), psoriasis (2 of 2 patients), graft-versus-host disease (2 of 2 patients), and cutaneous T cell lymphoma (5 of 6 patients). In addition, vascular endothelium was strongly reactive with antibody to ICAM-1, as was the dermal mononuclear cell infiltrate (Fig 1*B,D*).

Expression of ICAM-1 by EK was frequently focal in nature and correlated well with the extent of inflammation (Fig 2*A*). When sequential sections were evaluated for expression of ICAM-1, CD3, and LFA-1, the expression of ICAM-1 on EK correlated well with the presence of a CD3-positive infiltrate. CD3-positive cells were seen in the perivascular, superficial dermis and frequently within the epidermis as well as (Fig 2*B*). The T cell infiltrate was also reactive with antibody TS1/22 (anti-LFA-1) (Fig 2*C*). Cells reactive with anti-LFA-1 but not anti-CD3 were also detected. The sections shown in Fig 2*A–C* were from a patient with atopic dermatitis. In agreement with previous reports [34,35], we found that lesions of acute atopic dermatitis were characterized by a mononuclear infiltrate of primarily lymphocytes with CD4+ cells predominating. As reported by Zachary et al [35], infiltrating T cells expressed HLA-DR antigens as detected by L243 (not shown), and thus are activated. Expression of both ICAM-1 and HLA-DR was seen in focal areas of inflammation. Although typically EK that expressed ICAM-1 also expressed HLA-DR, one exception was a case of spongiotic dermatitis in which ICAM-1 expression was detected in a cell surface pattern on EK located near a focal infiltration of CD3-positive cells, however, no HLA-DR expression by epidermal keratinocytes was observed.

Intercellular Adhesion Molecule-1 Expression by Epidermal Keratinocytes Not a Result of Hyperproliferation Because most of the diseases in which we detected EK ICAM-1 expression also involved hyperproliferation of the epidermis, we considered the possibility that the expression of ICAM-1 was a result of epidermal hyperproliferation. Epidermal hyperproliferation is accompanied by an alteration in expression of keratin polypeptides that can be monitored using the antikeratin antibody AE1. In healthy skin, AE1 binds primarily to the basal layer of epidermis [31], whereas in pathologic skin conditions involving epidermal hyperproliferation and in mechanically tape-stripped skin, the pattern of reactivity of AE1 changes within 24 h to a suprabasal pattern [29]. In most of the biopsies we studied, AE1 stained in a pattern consistent with hyperproliferation of the epidermis (Fig 2*D*). To examine ICAM-1 expression on hyperproliferative epidermis in the absence of a lymphoid infiltrate, we examined biopsies of skin that had been mechanically tape stripped and shown to express a pattern of reactivity with AE1 consistent with that seen in hyperproliferative epidermis. Frozen sections were evaluated for expression of ICAM-1 on EK. Tissue sections were also evaluated with antibodies to CD3 and LFA-1 to confirm that tape stripping did not induce lymphoid infiltration of the skin. Figure 2*D* shows the pattern of reactivity expected for AE1 on hyperproliferative epidermis from a patient with spongiotic dermatitis. Tape-stripped epidermis showed a similar pattern of reactivity with AE1 (Fig 2*E*) but was not reactive with antibody to ICAM-1 (Fig 2*F*). Thus, tape stripping clearly induced changes in keratin expression associated with hyperproliferation of epidermis but did not induce ICAM-1 expression on EK. No HLA-DR expression was detected on EK In tape-stripped epidermis (not shown). Companion sections reacted with antibodies to lymphoid cells confirmed that tape stripping did not induce lymphoid infiltration (not shown).

DISCUSSION

In this study, we report that ICAM-1 was expressed in a cell-surface pattern on EK at the site of lymphoid infiltration in cutaneous dermatoses. Furthermore, ICAM-1 expression was not associated with epidermal hyperproliferation in the absence of lymphoid infiltration. It is likely that ICAM-1 expression is related to infiltration of activated lymphocytes into the skin.

Adhesion of T lymphocytes to hematopoietic and nonhematopoietic cells is required for induction and maintenance of immune responses. In recent years, attention has been focused on the role of molecules, other than the B and T cell receptor for antigen, in mediating adhesion of lymphoid cells [reviewed in Ref 36]. The CD4 and CD8 molecules have been implicated in direct interaction with MHC class II and class I products, respectively [37]. In addition, two pairs of molecules have been implicated as serving important roles in lymphoid adhesion. The CD2 interaction with its ligand LFA-3 is involved in cytotoxic T lymphocyte binding to target cells [38] and in thymocyte binding to thymic epithelial cells [39]. The importance of these two molecules goes beyond their role in binding, as recent reports have demonstrated that, in appropriate combination with other signals, purified LFA-3 molecules can deliver activation signals to thymocytes [40] and to T cells [41,42] by CD2 molecules. The adhesion molecule LFA-1 is expressed on hematopoietic cells and has been shown to participate in a variety of T and B cell functions as well as myeloid functions [25,42–45]. A clinical syndrome has been described in which a heritable deficiency of LFA-1 and two other molecules, which share a common beta chain, Mac-1 and p150,95, result in a deficiency of cellular inflammatory responses [46].

Although other LFA-1 ligands probably exist, ICAM-1 has been shown to function as a ligand for LFA-1 in binding of T lymphocytes to endothelial cells [24], target cells for cytotoxic T lymphocytes [26], and in binding of activated T lymphocytes to IFN-γ-treated EK [22] and to IFN-γ-treated thymic epithelial cells [Singer KH, Tuck DT, Smith J, Haynes BF, unpublished results). The gene for ICAM-1 has recently been sequenced [47,48] and demonstrated to share homology with members of the immunoglobulin gene superfamily, whereas LFA-1 has been shown to have structural homology with the integrin family of adhesion molecules [49]. Thus, the interaction of LFA-1 and ICAM-1 is an interaction between a member of the integrin family of molecules, LFA-1, and a member of the immunoglobulin family of molecules, ICAM-1.

The presence of lymphocytes within the epidermis is a frequent feature of many inflammatory dermatoses; however, the mechanism of exocytosis is not known. The expression of adhesion molecules on the surface of EK provides a mechanism for binding of

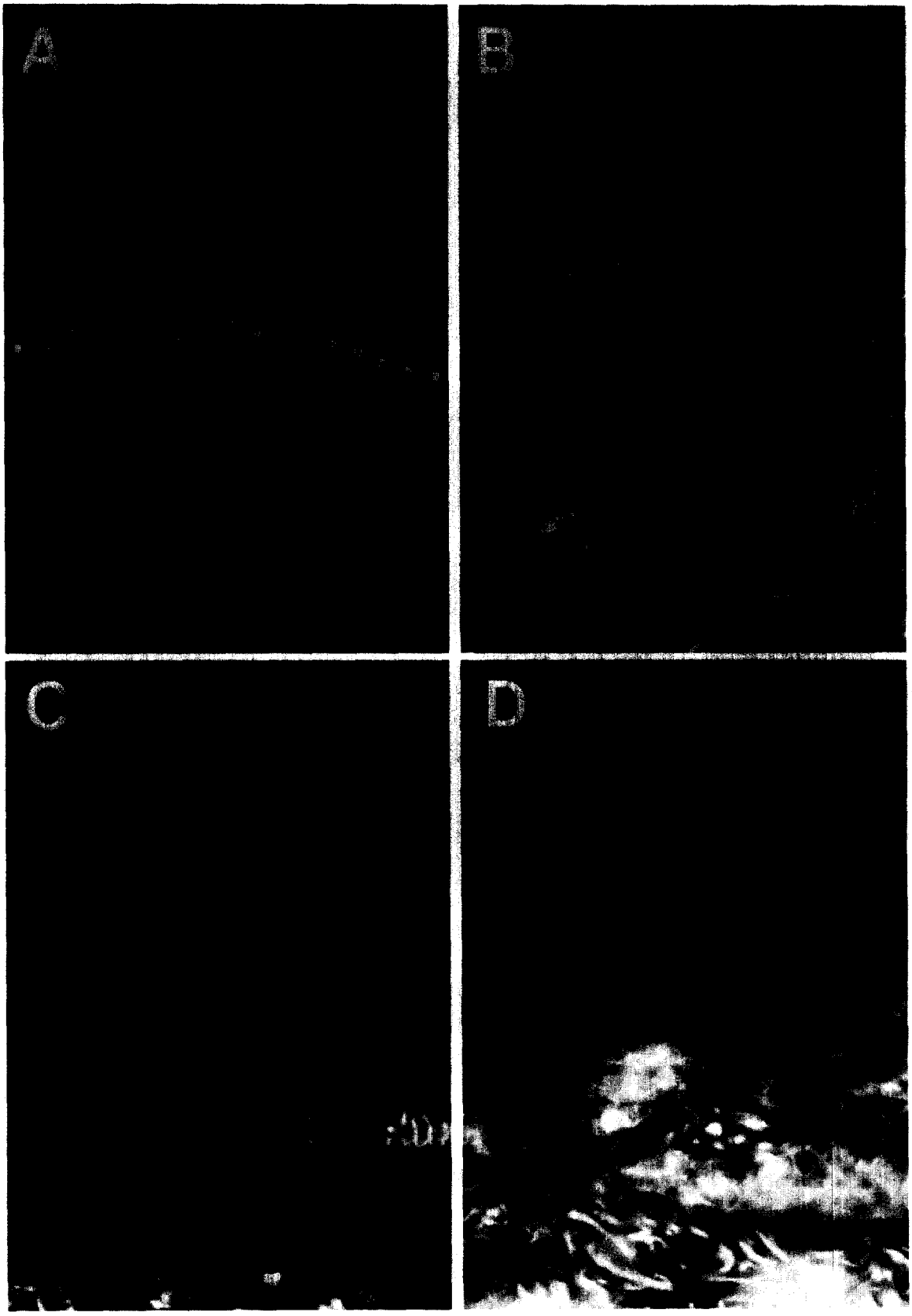

Figure 1. Reactivity of antibody to ICAM-1 on cryostat sections of noninflamed and inflamed skin. Skin biopsies were taken from *(A)* normal skin, *(B)* atopic dermatitis, *(C)* psoriasis, and *(D)* spongiotic dermatitis. The interrupted line represents the basement membrane zone observed by light microscopy. (×400)

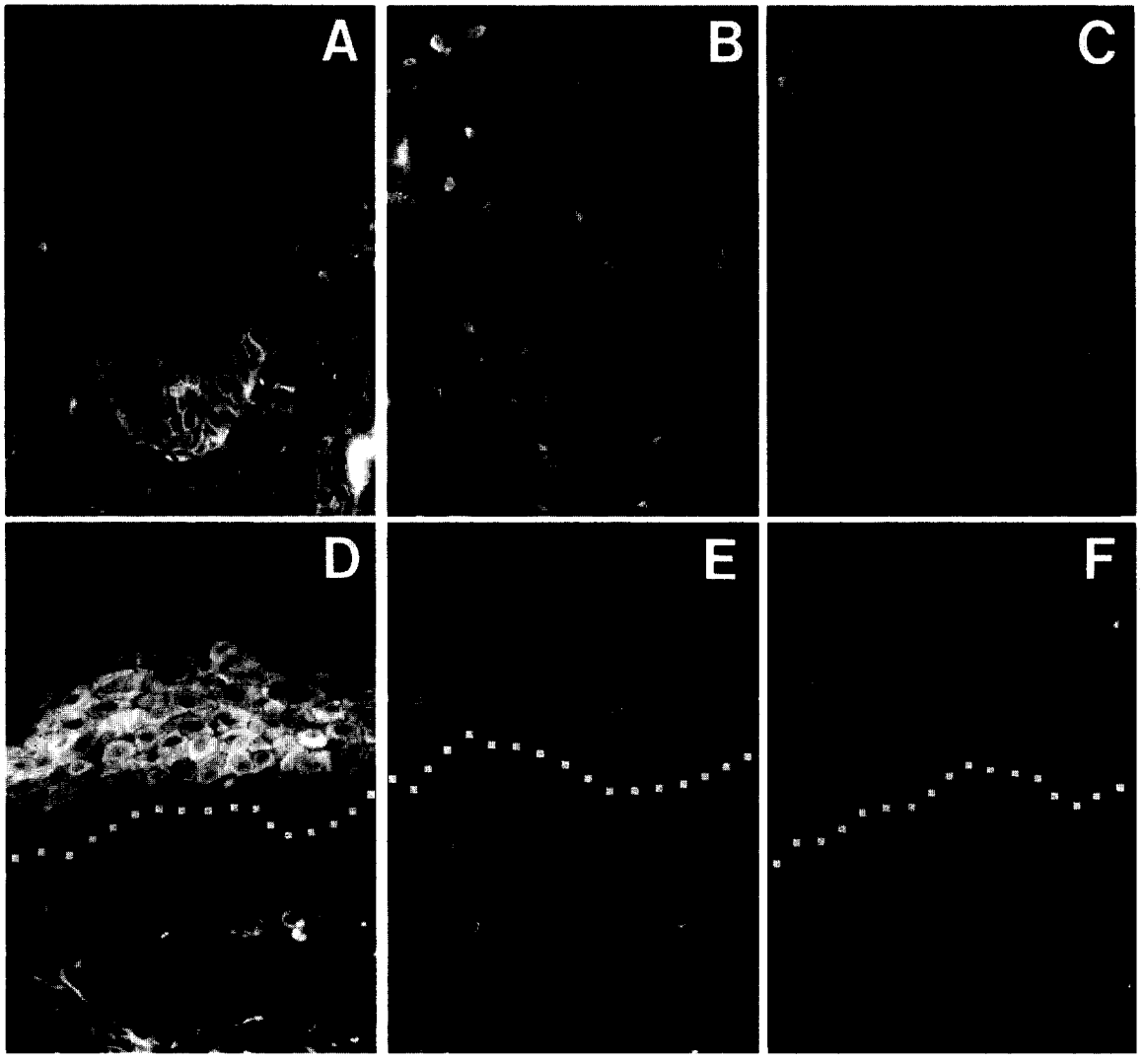

Figure 2. Reactivity of antibodies to *(A)* ICAM-1, *(B)* CD3, and *(C)* LFA-1 with consecutive cryostat sections of skin from a patient with atopic dermatitis. Reactivity of antikeratin antibody AE1 with cryostat sections of *(D)* a biopsy from a patient with spongiotic dermatitis and *(E)* a biopsy of tape-stripped skin. Both show a pattern of reactivity consistent with epidermal hyperproliferation. *F*: Reactivity of anti-ICAM-1 antibody with tape-stripped skin from the same individual shown in *(E)*. The interrupted line represents the basement membrane zone observed by light microscopy. (×400)

activated T lymphocytes to epidermal cells at the site of cutaneous inflammation. The functional consequence of the interaction of activated T lymphocytes with EK is currently under investigation.

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