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Immunology

Epidermal expression of intercellular adhesion molecule 1 is not a primary inducer of cutaneous inflammation in transgenic mice

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ABSTRACT **Keratinocytes at sites of cutaneous inflammation have increased expression of intercellular adhesion molecule 1 (ICAM-1), a cytokine-inducible adhesion molecule which binds the leukocyte integrins LFA-1 and Mac-1. Transgenic mice were prepared in which the expression of mouse ICAM-1 was targeted to basal keratinocytes by using the human K14 keratin promoter. The level of constitutive expression attained in the transgenic mice exceeded the peak level of ICAM-1 expression induced on nontransgenic mouse keratinocytes *in vitro* by optimal combinations of interferon γ and tumor necrosis factor α or *in vivo* by proinflammatory stimuli such as phorbol 12-myristate 13-acetate. *In vitro* adhesion assays demonstrated that cultured transgenic keratinocytes were superior to normal keratinocytes as a substrate for the LFA-1-dependent binding of mouse T cells, confirming that the transgene-encoded ICAM-1 was expressed in a functional form. However, the high level of constitutive ICAM-1 expression achieved on keratinocytes *in vivo* in these transgenic mice did not result in additional recruitment of CD45$^+$ leukocytes into transgenic epidermis, nor did it elicit dermal inflammation. Keratinocyte ICAM-1 expression also did not potentiate contact-hypersensitivity reactions to epicutaneous application of haptens. The absence of a spontaneous phenotype in these transgenic mice was not the result of increased levels of soluble ICAM-1, since serum levels of soluble ICAM-1 were equal in transgenic mice and controls. We conclude that elevated ICAM-1 expression on keratinocytes cannot act independently to influence leukocyte trafficking and elicit cutaneous inflammation.**

Cell surface adhesion molecules facilitate the migration of leukocytes to the site of a localized inflammatory stimulus (1). Adhesion molecules relevant to the trafficking of leukocytes include those expressed by leukocytes and those found on cells that leukocytes encounter during the process of extravasation, including endothelial cells and epithelial cells. The best characterized inducible adhesion molecule on epithelial cells is intercellular adhesion molecule-1 (ICAM-1), a counterligand for the leukocyte integrins LFA-1 and Mac-1 (2, 3). Binding of ICAM-1 to its β_2-integrin counterligands plays a pivotal role in facilitating leukocyte function in multiple immune and inflammatory responses, including antigen presentation to T lymphocytes, leukocyte attachment to endothelium, and cell-mediated cytotoxicity (1).

The pattern of expression of ICAM-1 differs from that of the homologous immunoglobulin superfamily molecules ICAM-2 and ICAM-3, two additional LFA-1 counterligands (4, 5). ICAM-1 is the only one of these molecules known to be expressed on cells of epithelial origin (4, 5). On epithelial cells, including epidermal keratinocytes, basal ICAM-1 expression is low or absent (6–8), but proinflammatory stimuli can dramatically augment its expression (6, 7). A close spatial and temporal association between elevated ICAM-1 expression and the appearance of leukocytes in cutaneous inflammatory lesions has been described (7, 9, 10). This suggests that ICAM-1 expression may be an important signal required for the initiation and/or propagation of localized skin inflammation (9, 10), but this hypothesis has not been formally tested *in vivo*. The use of epidermal keratin gene promoters such as the human K14 promoter in transgenic mice (11) is an effective means of achieving overexpression of gene products in defined subsets of keratinocytes. In this report, we describe the effects of targeting overexpression of ICAM-1 to basal keratinocytes in transgenic mice. Our results show that elevated expression of ICAM-1 on keratinocytes, as an isolated event, is not sufficient to provoke cutaneous inflammation.

METHODS

DNA Constructs and Transgenic Mice. A 1.9-kb cDNA for mouse ICAM-1 (12) was converted into a *Bam*HI fragment with linkers and cloned into the single *Bam*HI site of the K14/human growth hormone (hGH) expression vector (13) to generate the K14/ICAM-1 transgenic construct (Fig. 1). Transgenic mice were prepared in the inbred FVB/N strain (Taconic Farms) by pronuclear injection with a 6.15-kb *Eco*RI fragment (14). Five transgenic founder mice were identified by PCR amplification of ear skin DNA (15), using hGH exon 1 sense primer (5′-CTCACCTAGCTGCAATGG-3′) and exon 2 antisense primer (5′-AAGGCACTGCCCTCT-TGAAGC-3′). Four of these founder mice were successfully bred to establish transgenic lines (I12, I13, I21, I31). Transgene copy number from PhosphorImager (Molecular Dynamics) analysis ranged from 20 for the I31 line to 75 for the I12 line.

Immunoperoxidase Staining. Ear skin punch biopsies were embedded in optimal-cutting-temperature (OCT) compound (Miles) and frozen in cold isopentane. Cryostat sections (5 μm) were stained with 3E2 anti-ICAM-1 monoclonal antibody (mAb) (16) as the primary reagent and the Vectastain Elite ABC kit (Vector Laboratories).

Flow Cytometric Analysis of Epidermal Cells. Epidermal cell suspensions were prepared by sequential dispase and trypsin treatments of split adult mouse ear skin (17, 18). The mAbs used for staining were YN1 and 3E2 (anti-ICAM-1), 30F11.1 (anti-CD45), and KH116 (anti-I-A^q). The YN1 hybridoma (19) was obtained from the American Type Culture Collection. The remaining primary mAbs were purchased in unconjugated, biotinylated, fluorescein isothiocyanate (FITC)-conjugated, or phycoerythrin-conjugated forms from PharMingen. Secondary reagents used were FITC-labeled goat anti-rat immunoglobulin and goat anti-hamster immunoglobulin (Southern Biotechnology Associates) and streptavidin conjugated to FITC or phycoerythrin (AMAC, Westbrook, ME). Propidium iodide was added to all samples to

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Abbreviations: CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene; FITC, fluorescein isothiocyanate; hGH, human growth hormone; ICAM, intercellular adhesion molecule; IFN, interferon; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; TNCB, 2,4,6-trinitrochlorobenzene; TNF, tumor necrosis factor.

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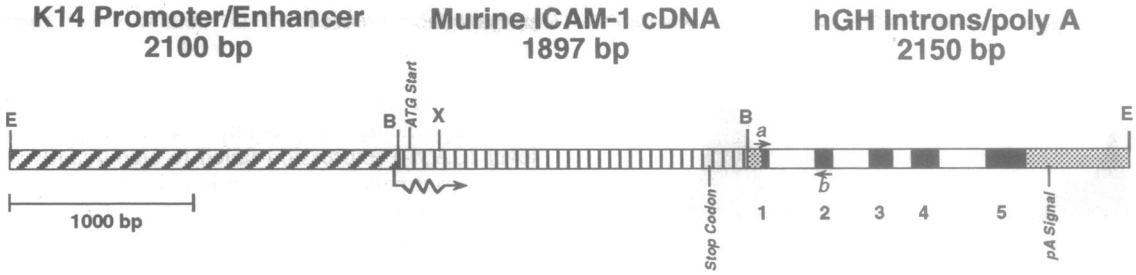

FIG. 1. K14/ICAM-1 transgenic construct with the human K14 promoter/enhancer (diagonal lines), a murine ICAM-1 cDNA containing all coding sequences and a stop codon (vertical lines), and a portion of the hGH gene including four introns and a polyadenylylation (pA) site. Lowercase letters next to small arrows indicate sites of annealing of sense (*a*) and antisense (*b*) PCR primers. E, *Eco*RI; B, *Bam*HI; X, *Xho* I.

allow electronic exclusion of dead cells. For each sample, 10,000 gated events were analyzed on a FACScan (Becton Dickinson).

Keratinocyte Cultures. Cultures of neonatal keratinocytes were prepared by using medium with 0.05 mM Ca^{2+} (20), to allow the keratinocytes to maintain expression of basal keratins (21). After 4 days of culture, some keratinocyte cultures were stimulated with recombinant interferon γ (IFN-γ; provided by Genentech) and/or tumor necrosis factor α (TNF-α; gift of Robert Schreiber, Washington University, St. Louis) at 1000 units/ml. Two days after cytokine stimulation, the keratinocytes were detached with trypsin and stained for flow cytometry.

Keratinocyte/T-Cell Adhesion Assay. Adult mouse ear keratinocytes were cultured in 24-well plates for 3 days. YAC-1 T-lymphoma cells labeled with 2',7'-bis(2-carboxyethyl)-5 (and 6)-carboxyfluorescein (Molecular Probes) (5) were resuspended in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM $MgCl_2$ and added to wells containing washed keratinocyte monolayers. The plates were incubated at 37°C for 45 min, washed twice by aspiration through a 22-gauge needle, and analyzed by fluorescence microscopy. The blocking anti-LFA-1 mAb FD441.8 (American Type Culture Collection) was added to some wells.

Contact Hypersensitivity (CHS) Responses. ICAM-1 transgenic and control nontransgenic mice were sensitized by epicutaneous application of 2,4,6-trinitrochlorobenzene (TNCB; Eastern Chemical, Smithtown, NY) or 2,4-dinitrofluorobenzene (DNFB; Sigma) to shaved abdominal skin. TNCB sensitization was done with 100 μl of 0.05–1.25% TNCB in 3:1 acetone/olive oil. The sensitizing dose of DNFB was 50 μl of 0.005–0.5% DNFB in the same diluent. Five days after sensitization, the mice were challenged on one ear with 20 μl of 1% TNCB or 0.2% DNFB in 3:1 acetone/olive oil. Ear thickness was measured with engineer's calipers (Dyer, Lancaster, PA) at three locations on each ear 24 hr after challenge.

ELISA for Soluble Mouse ICAM-1. Serum levels of soluble mouse ICAM-1 were determined with a dual monoclonal sandwich ELISA kit (Endogen, Cambridge, MA). Some serum samples tested were obtained from FVB mice pretreated 1 day earlier with an i.p. injection of phosphate-buffered saline containing 25 μg of lipopolysaccharide from *Escherichia coli* O26:B6 (Sigma).

RESULTS

Immunoperoxidase Analysis of Transgene Expression in Skin. Frozen sections of skin from K14/ICAM-1 mice and control littermates were stained with mAb to ICAM-1. In nontransgenic mice, constitutive ICAM-1 expression was detected on microvascular endothelial cells and scattered cells within the dermis, but no staining was detected on follicular or epidermal keratinocytes (Fig. 2). In each of the four K14/ICAM-1 lines, staining for ICAM-1 was present in the basal layer of epidermis and on cells of the outer root sheath of the hair follicle.

Flow Cytometric Analysis of Transgene Expression. Quantitative analysis of cell surface ICAM-1 expression by keratinocytes from the K14/ICAM-1 transgenic lines was done by flow cytometry of freshly isolated epidermal cells stained with the YN1 mAb (19). A low basal level of ICAM-1 expression was detected on a majority of nontransgenic epidermal cells (Fig. 3). In each of the K14/ICAM-1 lines, a marked increase in cell surface ICAM-1 expression was observed. I12 epidermal cells had the highest level of ICAM-1 expression, with a peak channel fluorescence intensity 36-fold higher than FVB epidermal cells.

We next asked how the levels of ICAM-1 expression attained in the transgenic mice compared with the level of ICAM-1 that can be induced on normal keratinocytes by various stimuli. ICAM-1 expression by cultured transgenic keratinocytes was compared with ICAM-1 expression by normal keratinocytes treated with a combination of proinflammatory cytokines (IFN-γ and TNF-α) known to maximally induce ICAM-1 expression on cultured keratinocytes (6). Unstimulated cultured FVB keratinocytes expressed a basal level of ICAM-1 which was increased by the synergistic action of IFN-γ and TNF-α (Table 1). Keratinocytes from the I12 transgenic line cultured without cytokines expressed higher levels of ICAM-1 than nontransgenic keratinocytes optimally stimulated with IFN-γ and TNF-α. A modest further increase in the expression of ICAM-1 by cultured I12 keratinocytes was obtained by IFN-γ and TNF-α treatment as a result of cytokine-induced stimulation of the endogenous ICAM-1 gene.

Effect of K14/ICAM-1 Transgene on Leukocyte Populations in Epidermis. As an initial screening technique to detect inflammatory changes in the skin of the K14/ICAM-1 mice, we compared hematoxylin/eosin-stained sections of skin biopsies from several anatomic sites of I12 and nontransgenic mice. These biopsies showed no discernible evidence of inflammatory changes in the skin of unmanipulated transgenic mice (data not shown), correlating with the absence of recognizable cutaneous lesions in mice followed for periods of up to 1 year.

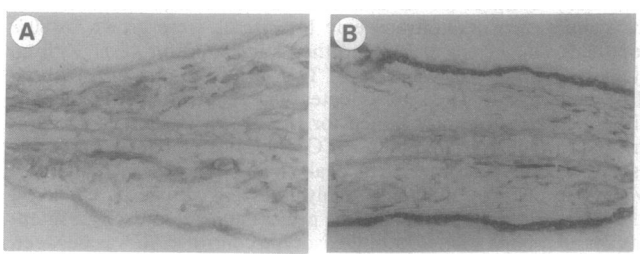

FIG. 2. Localization of cells in skin expressing the ICAM-1 transgene. Frozen sections of normal FVB (*A*) or I12 transgenic (*B*) ear skin were stained for ICAM-1.

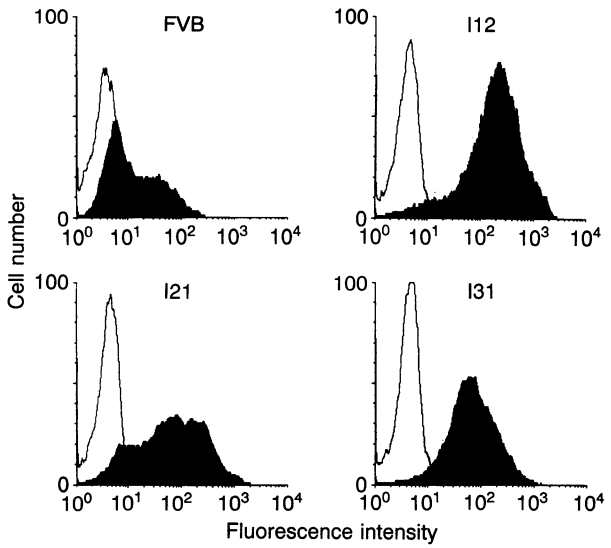

FIG. 3. Flow cytometric analysis of keratinocyte ICAM-1 expression in K14/ICAM-1 transgenic mice. Epidermal cell suspensions were stained with the YN1 mAb to ICAM-1 (shaded histogram) or no primary antibody (unshaded histogram) followed by FITC-conjugated goat anti-rat immunoglobulin.

To probe for more subtle changes in the composition of the epidermis in the I12 mice, two-color flow cytometric analysis of epidermal cells was done with mAbs specific for CD45 and I-A^q. This dual-color staining permitted determination of the percentage of leukocytes (CD45$^+$ cells), Langerhans cells (I-A^{q+} cells), and keratinocytes (negative for CD45 and I-A^q) within the epidermal compartment. As a positive control for this analysis, epidermal cells were prepared from the ears of mice treated with 10 μg of phorbol 12-myristate 13-acetate (PMA) on each ear 24 hr previously, a well-characterized inflammatory stimulus. While ICAM-1 was induced on epidermal cells from PMA-treated mice, the constitutive level of ICAM-1 on I12 keratinocytes was significantly higher (Fig. 4). Despite the high level of surface ICAM-1 on I12 keratinocytes, no increase in CD45$^+$ cells was seen compared with nontransgenic mice. In contrast, a substantial expansion (from 2.4% to 9.6%) occurred in the number of CD45$^+$, I-A^{q-} cells in the epidermis of PMA-treated mice. This result confirmed the histological impression that constitutive high-level ICAM-1 expression failed to significantly alter leukocyte recruitment or retention in epidermis.

Table 1. Cytokine effects on ICAM-1 expression by cultured normal and K14/ICAM-1 transgenic keratinocytes

Source of keratinocytes	Cytokine stimulation	Peak channel fluorescence intensity
Nontransgenic	None	81*
	IFN-γ	202
	TNF-α	109
	IFN-γ + TNF-α	300
Transgenic	None	340
	IFN-γ	604
	TNF-α	433
	IFN-γ + TNF-α	1046

Primary cultures of keratinocytes isolated from neonatal I12 transgenic mice or nontransgenic littermates were grown to 80% confluence in 60-mm dishes and then stimulated for 48 hr with no cytokine, IFN-γ (1000 units/ml), TNF-α (1000 units/ml), or both IFN-γ and TNF-α. The keratinocytes were removed from the culture dishes with trypsin and stained for ICAM-1 with biotinylated 3E2 mAb followed by streptavidin-FITC. Biotinylated hamster IgG was used as an isotype control. The peak channel fluorescence intensity was determined by statistical analysis of histograms plotted on a four-decade logarithmic scale. The peak channel fluorescence intensity for all groups stained with the isotype control mAb was between 4 and 6.

*Mouse keratinocytes cultured with medium containing 0.05 mM Ca^{2+} express low to moderate levels of ICAM-1 constitutively (I.R.W., unpublished observations).

Cultured Transgenic Keratinocytes Expressing ICAM-1 Support LFA-1-Dependent Leukocyte Adhesion. We used an *in vitro* adhesion assay to establish that the absence of spontaneous inflammatory changes in the K14/ICAM-1 mice was occurring despite keratinocyte surface expression of a fully functional transgene-encoded ICAM-1 molecule. I12 keratinocytes bound fluorescently labeled YAC-1 cells much more strongly than FVB keratinocytes did (Fig. 5), and this increase in adhesion was completely inhibited by a blocking anti-LFA-1 mAb.

CHS Responses in K14/ICAM-1 Mice. Application of contact sensitizers such as TNCB and DNFB to epidermis stimulates a T-cell-mediated immune response that can be assessed by subsequent challenge with the same hapten. We predicted that constitutive expression of ICAM-1 by keratinocytes would enhance at least one parameter of the CHS response. Surprisingly, the dose–response curves obtained by hapten challenge of groups of control or transgenic mice sensitized with graded concentrations of TNCB or DNFB were similar (Fig. 6), indicating that keratinocyte ICAM-1 expression did not improve the efficiency of the afferent limb of the CHS response. The kinetics of the efferent limb of the response was also unaltered; both control and transgenic mice exhibited a similar decline in ear swelling between 24 and 72 hr after challenge.

Serum Levels of Soluble ICAM-1 Are Not Elevated in K14/ICAM-1 Mice. Although ICAM-1 is a transmembrane protein, a soluble form of the molecule has been detected in biological fluids (22). Soluble ICAM-1 has been proposed to exert a regulatory effect on ICAM-1-mediated adhesion,

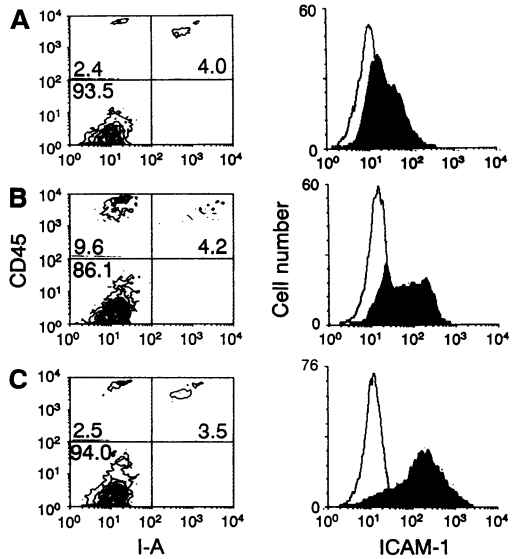

FIG. 4. Constitutive overexpression of ICAM-1 in I12 transgenic line does not increase the percentage of CD45$^+$ cells in epidermis. Epidermal cell suspensions were prepared from untreated FVB mice (*A*), FVB mice pretreated with PMA (10 μg diluted in ethanol on each ear 24 hr previously) (*B*), or untreated I12 mice (*C*). (*Left*) Contour plots of dual-color staining with phycoerythrin-conjugated anti-CD45 and biotinylated anti-I-A^q plus streptavidin-FITC. Internal numbers indicate percent cells in each quadrant. (*Right*) Fluorescence histograms of the same cell suspensions stained with biotinylated 3E2 (shaded) or biotinylated hamster immunoglobulin (unshaded) plus streptavidin-FITC.

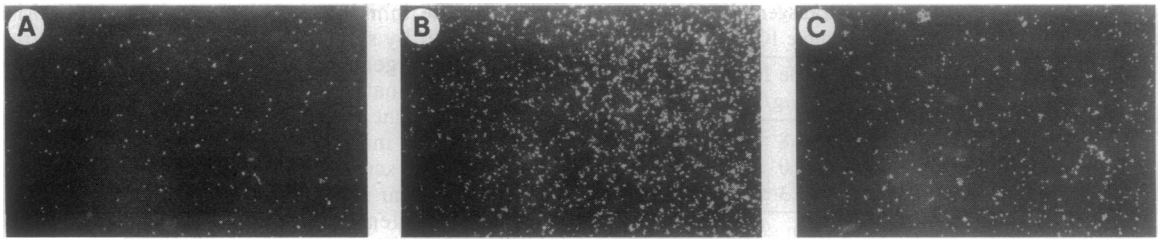

FIG. 5. Overexpression of ICAM-1 on cultured transgenic keratinocytes is associated with enhanced adhesion of T cells. Fluorescently labeled YAC-1 cells activated for binding of ICAM-1 by addition of 2 mM $MgCl_2$ were allowed to adhere to wells containing cultured keratinocytes. After 45 min, nonadherent cells were removed by washing and the residual T cells bound to keratinocytes were visualized by fluorescence microscopy. Representative fields were photographed to show the extent of T cell binding to FVB keratinocytes (*A*), I12 keratinocytes (*B*), and I12 keratinocytes to which blocking anti-LFA-1 mAb (1:200 dilution of ascites) was added before the addition of the YAC-1 cells (*C*). (×18.)

although recombinant monomeric molecules of soluble ICAM-1 are actually very poor inhibitors of *in vitro* responses that are readily inhibited by antibodies to ICAM-1 (23). Using an ELISA, we found that the mean serum levels of soluble ICAM-1 in I12 transgenic and control mice were nearly identical (Table 2). FVB mice pretreated with lipopolysaccharide had increased levels of serum ICAM-1, demonstrating that the assay used was able to detect changes in circulating levels of soluble ICAM-1 in response to an inflammatory stimulus.

DISCUSSION

The relative importance of different adhesion molecules and cytokines expressed concurrently at inflammatory sites to the recruitment and maintenance of a leukocytic infiltrate has been difficult to assess. One approach to this problem is to express such proinflammatory molecules individually in transgenic mice under the control of tissue-specific promoters. The present study was initiated to determine whether elevated expression of ICAM-1 by keratinocytes, as an isolated event, influenced the pattern of leukocyte trafficking to skin and epidermis. Transgenic mice were generated in which constitutive expression of high levels of functional ICAM-1 was targeted to basal keratinocytes using the promoter/enhancer of the human K14 gene. To our knowledge, this report is the first description of transgenic animals in which a leukocyte adhesion molecule has been constitutively overexpressed by means of a tissue-specific promoter. The most striking characteristic of these mice is the lack of a spontaneous inflammatory infiltrate at the site of ICAM-1 overexpression, as assessed by microscopic and macroscopic criteria, despite the integration of up to ≈150 copies of the transgene per genome (in homozygous I12 mice) and concomitant expression of 36-fold more functional cell surface ICAM-1 on keratinocytes compared with nontransgenic mice. These results provide a new perspective on the function of ICAM-1 expression by epithelial tissues that must ultimately influence the interpretation of the well-documented association between ICAM-1 expression by keratinocytes and epidermal localization of leukocytes in inflammatory skin disease.

Initial studies on the tissue distribution of human ICAM-1 showed that ICAM-1 expression was not detected on several types of normal epithelial cells by immunostaining procedures but was inducible in epithelial cells including keratinocytes following a local immune response (6, 24). This finding prompted several investigators to examine ICAM-1 expression by keratinocytes in a variety of inflammatory skin diseases. Elevated levels of keratinocyte ICAM-1 expression were found to be spatially and temporally associated with leukocytic infiltration of epidermis in spontaneously occurring inflammatory diseases of skin (7, 9) and experimentally induced cutaneous inflammation (10). In the poison-ivy dermatitis model, ICAM-1 expression was reported to precede epidermal infiltration with T lymphocytes (10). Proper interpretation of these results with regard to the function of ICAM-1 hinges on the issue of the cause-and-effect relationship between ICAM-1 expression and localization of inflammatory cells at a specific epithelial site.

Two models can account for the association of keratinocyte ICAM-1 expression and inflammatory lesions. The first model postulates that ICAM-1 induction on kerati-

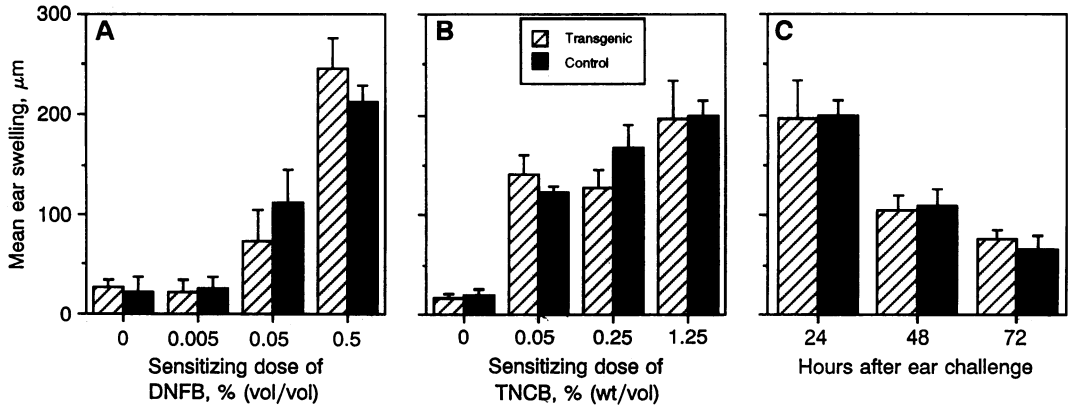

FIG. 6. Overexpression of ICAM-1 on keratinocytes does not enhance CHS responses. I31 transgenic mice (hatched bars) or nontransgenic littermates (solid bars) were sensitized to DNFB (*A*) or TNCB (*B*) by application of graded concentrations of these haptens to shaved abdominal skin. Five days later, one ear was challenged with 0.2% DNFB (*A*) or 0.5% TNCB (*B*), and ear swelling was measured 24 hr later. The kinetics of the swelling response was evaluated in the transgenic and control groups sensitized with 1.25% TNCB by measuring ear swelling at 24, 48, and 72 hr after challenge (*C*). All swelling measurements are reported as the mean ear swelling and SEM. None of the differences between the control and transgenic groups were statistically significant ($P > 0.05$ by Student's t test).

Table 2. Expression of the K14/ICAM-1 transgene is not associated with elevated serum levels of soluble ICAM-1

Group of mice	Soluble ICAM-1, μg/ml
FVB/N	14.8 ± 1.9
Endotoxin-treated FVB/N	35.0 ± 3.4
I12 transgenic	14.3 ± 0.6

Individual serum samples were collected from groups of three to four mice and analyzed by ELISA. Nontransgenic FVB mice in the endotoxin-treated group were injected i.p. with 25 μg of *E. coli* O26:B6 lipopolysaccharide in 0.2 ml of phosphate-buffered saline 24 hr before the sample was collected. The results are expressed as the mean serum concentration ± SEM. The mean serum level of soluble ICAM-1 in normal mice reported by the ELISA kit manufacturer (Endogen, Cambridge, MA) is 13.1 μg/ml.

nocytes, by virtue of the capacity of ICAM-1 to serve as a ligand for LFA-1 and Mac-1 on leukocytes, is a pivotal signal that can independently regulate the influx and retention of inflammatory cells in the epidermis. The alternative model is that keratinocyte ICAM-1 expression facilitates localization and persistence of inflammatory cells in epidermis, but such processes cannot occur in the absence of additional changes (e.g., induction of various chemotactic cytokines and additional adhesion molecules) that typically occur at the same time. Conventional experimental manipulations cannot distinguish between these possibilities, since stimuli that induce ICAM-1 expression invariably produce concurrent changes in cytokine production and adhesion-molecule expression by keratinocytes and other cells in the cutaneous microenvironment. One of the major advantages of using a transgenic approach to analyze a complex *in vivo* biological response such as cutaneous inflammation is the ability to vary the expression of a target protein independently of other changes in a subset of cells. The absence of a constitutive inflammatory process in the K14/ICAM-1 mice demonstrates convincingly that ICAM-1 expression by keratinocytes, as a single isolated event, is not sufficient to initiate the influx of leukocytes into epidermis.

What are the events involved in leukocyte recruitment to epidermis that might be absent in unperturbed K14/ICAM-1 transgenic mice? The process by which circulating leukocytes exit the bloodstream to reach an epidermal site of localized inflammation has been subdivided into a series of discrete cell/cell or cell/matrix interactions (25). To migrate from the bloodstream toward the epidermis and physically interact with keratinocytes, leukocytes must successfully negotiate multiple and distinct obstacles. In their typical order of occurrence, these steps include selectin-mediated rolling on vascular endothelium, integrin-mediated tight adhesion to endothelial cells, transendothelial migration, directed migration through dermal connective tissue in response to chemotactic factors, and penetration of a second basement membrane underlying the basal layer of keratinocytes. The actions of locally generated cytokines and chemokines are undoubtedly critical to this process; cytokines can induce many of the adhesion molecules involved in the required cell/cell interactions, and chemokines provide important chemotactic and activating signals for leukocytes. In the absence of induction of endothelial adhesion molecules and release of chemotactic molecules, it is likely that most leukocytes in K14/ICAM-1 transgenic mice will fail to initiate the first stages of this migratory process. This will effectively prevent them from ever encountering the ICAM-1-expressing keratinocytes, despite expression by transgenic keratinocytes of maximal levels of this adhesion molecule.

CHS to epicutaneous application of haptens such as TNCB and DNFB is a T-cell-dependent immune response in skin that can be inhibited by blocking LFA-1/ICAM-1 interactions, either by systemic administration of blocking antibodies (16) or by generating ICAM-1 deficient mice by homologous recombination in embryonic stem cells (26, 27). We anticipated that overexpression of ICAM-1 on transgenic keratinocytes might alter CHS responses by permitting enhanced T-cell sensitization to suboptimal doses of hapten or, more likely, ear swelling responses that would persist longer following challenge. Neither of these changes was seen in our experiments. These results, in combination with the studies cited above, suggest that the level of ICAM-1 on keratinocytes is not a major influence on the development and maintenance of CHS. The inhibitory effects of anti-ICAM-1 antibody are most likely focused on endothelial cells, which bind the majority of administered antibodies (16).

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