Keratinocyte Interleukin-10 Expression Is Upregulated in Tape-Stripped Skin, Poison Ivy Dermatitis, and Sezary Syndrome, but Not in Psoriatic Plaques

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Despite the highly diverse reaction patterns of benign and malignant skin diseases involving T lymphocytes, polymerase chain reaction analysis of cytokine mRNAs present in biopsy samples has revealed that many cutaneous responses can be categorized into essentially two discrete groups. One group exemplified by psoriasis is characterized by consistently detectable mRNAs for IL-2, IFN-γ, and TNF-α, but not IL-4, IL-5, IL-10, thereby closely resembling the murine Th1-type cell-mediated response. The second group exemplified by tape-stripped skin, poison ivy dermatitis, and Sezary syndrome contains predominantly IL-4, IL-5, and IL-10 mRNAs resembling the Th2-type cytokine profile. Because of the growing interest in the immunoregulatory role of IL-10, which can suppress IFN-γ production and inhibit cell-mediated reactions, we produced a rabbit antiserum that was used to immunohistochemically localize IL-10 in a total of 27 biopsies. The results revealed that in Th2-type skin diseases, IL-10 was predominantly identified throughout all levels of epidermis in the cytoplasm of keratinocytes (KCs), with accentuation of their membranes in upper level cells. In Sezary syndrome, T cells were also immunoreactive for IL-10, which was confirmed using the HUT 78 T cell line derived from a Sezary syndrome patient. While normal skin was devoid of IL-10 expression, KCs began expressing it as early as 6 hr following tape stripping or application of poison ivy antigen and became strongly and diffusely positive by 18–24 hr. In contrast, psoriatic plaques contained no IL-10 immunoreactivity in either the parakeratotic scale or the epidermal KCs. These results confirm the earlier IL-10 mRNA analysis using whole skin samples and immunolocalize IL-10 to epidermal KCs in the Th2 diseases.   © 1994 Academic Press, Inc.

INTRODUCTION

Epidermal keratinocytes (KCs), after being neglected by immunologists for the past century, have become increasingly recognized for their ability to modulate immune reactions in skin. KCs can function as immunocytes actively participating in T cell-mediated diseases by directly binding lymphocytes via adhesion molecules on their surface or by producing immunoregulatory cytokines (1). The primary pair of adhesion molecules mediating T cell:KC interactions in a wide variety of skin disease are lymphocyte function associated-antigen-1 (LFA-1) expressed by T cells and intercellular adhesion molecule-1 (ICAM-1) which is rapidly induced on KCs (2). In contrast, the cytokine production repertoire of KCs is not restricted to one or two molecules, but includes numerous cytokines including: IL-1α, IL-1β, IL-3, IL-6, IL-7, IL-8, tumor necrosis factor-alpha (TNF-α), granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-10 (reviewed in (3)). In addition to this array of KC-derived cytokines, skin disease sites also contain T cell cytokines including IL-2, IL-3, IL-4, IL-5, IL-10, and interferon-γ (IFN-γ). However, despite these large numbers of cytokines produced by either KCs or T cells, it is becoming clear that overall cytokine profiles can be grouped into one of two types. These profiles follow the classification system initially discovered for murine T cell clones (4). So-called Th1-type cytokines, primarily IL-2 and IFN-γ, are important for cell-mediated reactions, whereas Th2 cytokines, IL-4, IL-5, and IL-10, provide B cell help in humoral responses. Moreover, not only are these cytokine profiles distinct and relatively nonoverlapping, they also tend to be self-reinforcing. That is, the Th1-type cytokine IFN-γ can inhibit Th2-type cytokine production, whereas the Th2-type cytokine IL-10 can inhibit IFN-γ production (5, 6). To gain further insight into the immunoregulatory pathways operative in various dermatological disorders, we and others have begun to characterize the cytokine networks in several different skin diseases (7, 8).

In a series of recent studies, punch biopsies of normal and diseased skin were divided into several pieces and portions subjected to polymerase chain reaction (PCR)

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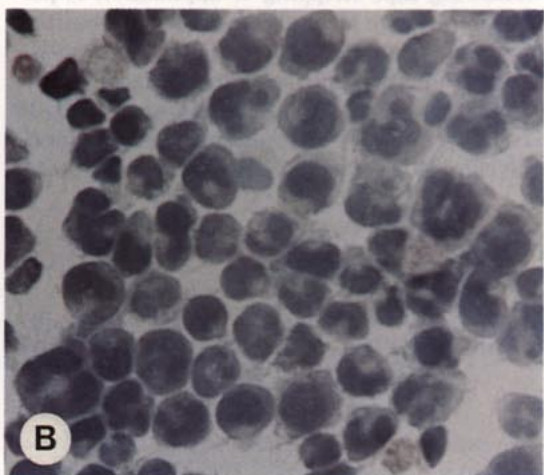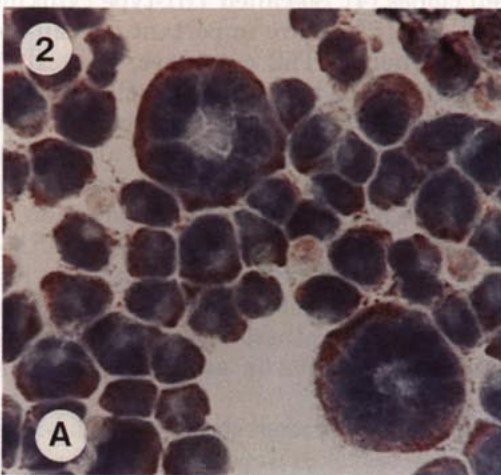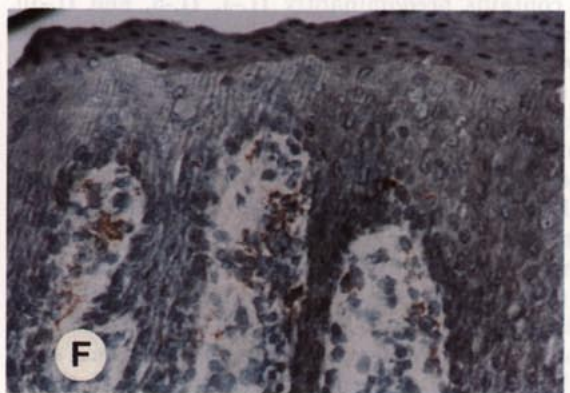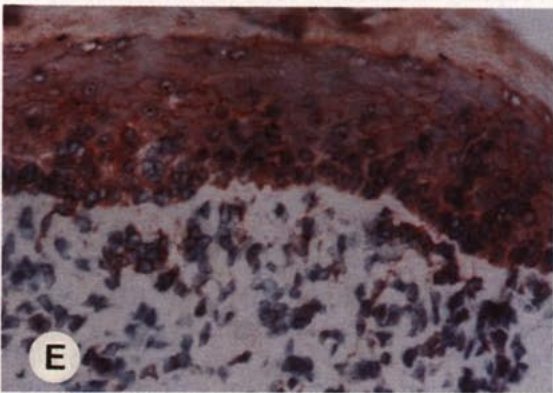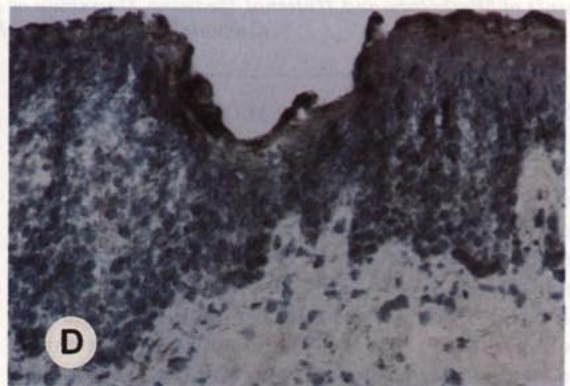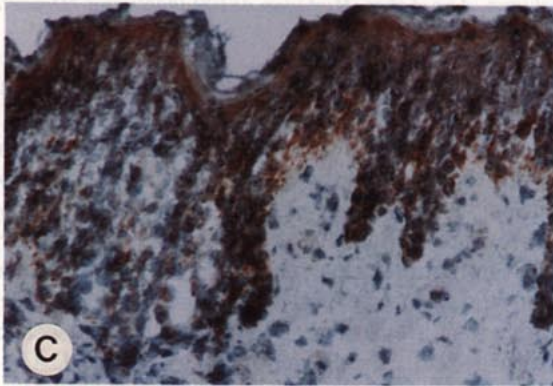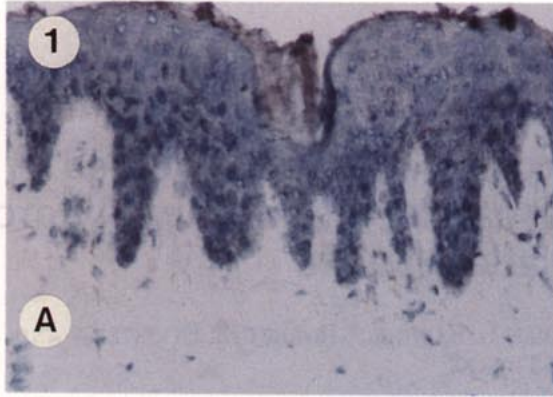
analysis using primers designed to detect a panel of cytokine and adhesion molecule mRNAs. We, and others, have observed that while most of the cytokine mRNAs studied were absent in normal human skin, discrete and reproducible profiles were present among different individuals with the same skin disease (reviewed in (8)). Psoriatic plaques consistently contain IFN-γ, IL-2, TNF-α, ICAM-1 mRNAs, but not IL-4, IL-5, or IL-10 mRNAs (9). Thus, we conclude the T cell activation in this common disease is predominantly characterized as a Th1-type response. In contrast, when normal skin is injured to abrogate the barrier function of skin by repeated tape stripping (10) or during allergic contact dermatitis (11), inflammatory sites are characterized by expression of the Th2-type cytokine IL-10. Similarly, in the skin of Sezary syndrome patients, there is a Th2-type response with detection of IL-10 mRNA (12).

IL-10 is a cytokine that was originally found in T cells and monocytes (13, 14). Recently, KCs have also been shown to produce IL-10 (15, 16). Therefore, we have now considered the possibility that the IL-10 detected by PCR might be at least partially derived from KCs, and not from T cells, as originally assumed. In this report, we produced a rabbit antiserum which can immunolocalize IL-10 in cryostat sections of skin biopsies. We discovered the immunostaining profile of IL-10 protein was entirely consistent with the aforementioned mRNA results. Furthermore, we localize IL-10 protein primarily to the epidermal KCs in those Th2-type diseases, such as tape-stripped skin, poison ivy dermatitis, and Sezary syndrome, but it was absent within KCs in the Th1-type disease psoriasis. In this report, we present our staining results and integrate our findings that highlight the immunoregulatory role of epidermal KCs.

MATERIALS AND METHODS

Skin Samples

Punch biopsies (6 mm) were obtained from normal and untreated diseased skin after informed consent and approval of the University of Michigan and Henry Ford Hospital Human Subjects Committees. When possible, cryostat sections were cut from the unused portions of tissue samples obtained from our earlier PCR-based reports. Additionally, three fresh samples were obtained of normal skin and psoriatic plaques to verify that any lack of IL-10 expression in stored samples was not due to a technical artifact from cryopreservation. The final total number of samples evaluated for each group of individuals was: normal skin (N = 8), tape stripped skin (N = 5), poison ivy dermatitis (N = 5), Sezary syndrome skin (N = 3), and psoriatic plaques (N = 6). For the tape-stripped skin and poison ivy dermatitis reactions, biopsies were analyzed at both 6 and 18–24 hr after initiation of cutaneous inflammation.

Cells

HUT 78 T cells represent a malignant CD4$^+$ T cell line initially obtained from a Sezary syndrome patient (18). These cells were purchased from American Type Culture Collection (Rockville, MD) and maintained in RPMI plus 10% FCS (Gibco, Grand Island, NY) at 37°C in a humidified incubator containing 5% CO_2 as previously described (19).

Immunohistochemical Staining Procedure and Reagents

Five-micrometer-thick cryostat sections were air dried and fixed for 10 min in cold (4°C) acetone and immunostained using a sensitive avidin–biotin peroxidase technique (Vectastain Kit, Vector Laboratory, Burlingame, CA) as previously described (17). Cytospin preparations of HUT 78 T cells were prepared using a Shannon centrifuge (Shandon, PA) and fixed with a 50:50 mixture of acetone:ethanol for 10 min at room temperature. After immunostaining, tissue sections and cytospin pellets were incubated with the chromogen 3-amino-4-ethylcarbazole (Aldrich Chemical Co., Milwaukee, WI) producing a positive red reaction product and counterstained with hematoxylin.

The rabbit antiserum for IL-10 was produced by immunizing with recombinant human IL-10. The sensitivity and specificity of the purified antiserum was confirmed by ELISA using recombinant human IL-10, as well as a panel of 20 other cytokines. There was no cross-reactivity with the following tested proteins: IL-1β, IL-4, IL-6, IL-8, IL-1 receptor antagonist protein, monocyte chemotactic peptide, TNF-α, platelet factor 4, RANTES, and interferon-γ inducible protein-10 (IP-10). Nonspecific staining was checked by staining several cryostat sections in parallel with preimmune rabbit serum used at the identical dilution (1:1000). This same antiserum was also used to immunolocalize IL-10

FIG. 1. Expression of IL-10 in normal and diseased skin samples. (A) Normal skin is devoid of IL-10 expression. Mag. ×60. (B) Epidermal KCs in skin 18 hr after tape stripping are IL-10 positive; note the focal accentuation of KC cytoplasmic membranes (arrow). Mag. ×80. (C) KCs in skin 24 hr after exposure to poison ivy antigen are positive for IL-10. Mag. ×80. (D) Serial section of poison ivy dermatitis stained with preimmune rabbit serum is negative. Mag. ×80. (E) Sezary syndrome skin contains prominent IL-10 immunoreactivity in KCs with a "chicken-wire" appearance of intercellular spaces, as well as infiltrating lymphocytes in dermis. Mag. ×150. (F) Psoriatic lesional KCs are devoid of IL-10 immunoreactivity in epidermis, with only focal staining of occasional macrophage-like cells in dermis. Mag. ×125.

FIG. 2. Expression of IL-10 in HUT 78 T cells. (A) There is strong and diffuse IL-10 immunoreactivity within the cytoplasm of this T cell line. Mag. ×450. (B) Control staining using preimmune serum is negative. Mag. ×400.

to malignant epithelial cells contained within bronchogenic squamous cell carcinomas as recently described (20).

RESULTS

Within cryostat sections of normal human skin samples there was no detectable IL-10 immunoreactivity in the epidermal or dermal compartments (Fig. 1A). Occasional faint reactivity was observed in the stratum corneum for two samples, but this was judged to be nonspecific as the control preimmune rabbit serum produced similar results.

In contrast to normal skin, within 5 hr following tape stripping sufficient to significantly increase transepidermal water loss (10), epidermal KCs became focally IL-10 positive, and by 24 hr, there was diffuse and intense IL-10 immunoreactivity within the cytoplasm of keratinocytes throughout all layers of the epidermis (Fig. 1B). In the mid and upper epidermis, there was focal accentuation of the KC cytoplasmic membranes. Within the dermis, only rare lymphoid cells stained positive but this was not consistently seen in the tape-stripped skin samples. The endothelial cells and other dermal cell types were not stained.

After application of the poison ivy antigen to the skin of previously sensitized individuals, 6 hr time-point samples contained an immunoreactive profile similar to the aforementioned tape-stripping results. After 24 hr, there was diffuse keratinocyte IL-10 reactivity within the spongiotic epidermis (Fig. 1C). Once again, in upper-level KCs, the IL-10 staining accentuated the cytoplasmic membrane zones. Most of the dermis was negatively stained except for occasional lymphocytes. Using the preimmune rabbit serum, no immunoreactivity in the epidermis or dermis was seen on a serial section of the poison ivy dermatitis tissue sample (Fig. 1D).

All Sezary skin samples were characterized by diffuse and intense positive KC cytoplasmic reactivity with some zones possessing a "chicken-wire" appearance because of cytoplasmic membrane accentuation (Fig. 1E). Within the dermis, collections of lymphocytes also contained discrete cytoplasmic IL-10 reactivity, but the adjacent dendritic cells and endothelial cells were negative.

Biopsies of active, untreated psoriatic plaques stained for IL-10 revealed a different reaction pattern compared to those of the tape-stripped skin, poison ivy dermatitis, or Sezary syndrome skin. No IL-10 immunoreactivity was observed for KCs in the epidermis or in the parakeratotic scale. The lack of KC IL-10 in the psoriatic specimens included both recently obtained as well as stored tissue samples (Fig. 1F). Within the dermis from two samples, macrophage-like cells appeared to be focally positive, but not the lymphocytes, endothelial cells, or dermal dendritic cells.

Because of the IL-10 immunoreactivity of dermal lymphocytes in the Sezary syndrome patients, we further investigated the HUT 78 T cell line in vitro. Cytospins of HUT 78 T cells revealed uniformly positive IL-10 immunoreactivity in the cytoplasm of the cells with focal accumulation just beneath the cytoplasmic membrane (Fig. 2A). Using the preimmune rabbit serum, no immunoreactivity was detected in the HUT 78 T cells (Fig. 2B).

DISCUSSION

These IL-10 immunostaining results reveal that KCs in normal and psoriatic epidermis are devoid of this important immunoregulatory cytokine, whereas KCs in tape-stripping skin, poison ivy dermatitis, and Sezary syndrome are IL-10$^+$. Both the positive and negative staining results are in good agreement with the previous mRNA analysis for IL-10 as performed by PCR. However, the earlier PCR analysis could not determine whether the source of IL-10 was T cells, monocytes, or the KCs themselves. Given the current immunolocalization results, it appears that KCs are the primary source of the positive PCR signal for IL-10 mRNA observed in tape-stripped skin and poison ivy dermatitis skin samples. The relative temporal appearance for IL-10 protein in the tape-stripped skin as well as the allergic contact dermatitis skin samples between 6 and 18–24 hr is also in good agreement with previous PCR-based results (10, 11). In one murine study, epidermal KCs exposed to a different contact allergen were shown to produce IL-10 (15). Thus, besides contact allergens, phorbol ester (15), and ultraviolet (uv) irradiation (16), which have been shown to induce KC IL-10, repeated tape stripping of human skin with perturbation of the barrier function of skin also induces this cytokine. The rapid induction of KC IL-10 in these different settings may explain the predominance of Th2-type cytokines over Th1 types in the responder T cell populations (21). Alternatively, certain stimuli may lead to the release of factors that also directly stimulate T cell IL-10 production independent of early KC participation.

Based on the existing in vitro and in vivo evidence regarding IL-10, we can suggest that the rapid and diffuse production of IL-10 by KCs in response to many epicutaneous stimuli (uv light, tumor promoter, contact allergens, tape stripping) may imbue the KC with a strategic and critically important immunological role in the genesis and propagation of cutaneous T cell reactions. Earlier, we proposed that KCs could initiate nonspecific cutaneous inflammation following a diverse array of environmental perturbations via TNF-α release (22). Based on these current results, it appears that they can also function to terminate or limit the extent of a T cell-mediated immune reaction via IL-10 production (1, 22). Indeed, in normal healthy individ-

uals the skin inflammatory response to these stimuli is relatively transient with spontaneous resolution. This implies an intrinsic, self-regulatory process in which KC-derived IL-10 could inhibit the proliferation and cytokine production of newly recruited Th1-type lymphocytes, or perhaps even the ability of Th0 cells to mature into TH1 cells (reviewed in (23)).

The absence of KC IL-10 in psoriatic plaques is also of considerable immunological interest. In contrast to the relatively transient and mild T cell-mediated reactions mentioned above in which KCs express IL-10, psoriatic plaques are persistent (often lasting for months/years without improvement) and contain a prominent and ongoing local activation of T cells. These T cells belong to the memory Th1 subset and can be induced in symptomless skin by intradermal injection of IFN-γ (24). The psoriatic process has been suggested to arise from early dermal dendritic cell:T cell activation (25), and we have recently observed that autologous combinations of peripheral blood T cells and lesional dermal dendritic cells produce high levels of IFN-γ (26). The early and prominent accumulation of IFN-γ may be responsible for suppressing KC IL-10 production given the generic ability of Th1-type cytokines to suppress Th2-type cytokines in other cell types. In any event, the lack of KC IL-10 expression may be very important in contributing to an overall highly permissive microenvironmental mileu in which T cell-mediated reactions in the dermis are allowed to proceed unchecked. Such a scenario in which psoriatic KCs fail to control an immune reaction has been previously suggested by Streilein (27). In preliminary studies, incubating psoriatic lesional dermal dendritic cells with IL-10 markedly reduces their capacity to stimulate T cells (R. S. Mitra et al., unpublished observations).

With respect to Sezary syndrome, yet another scenario appears to have developed besides those previously described for the reactive, nonneoplastic T cell mediated skin diseases. In Sezary syndrome, we previously reported malignant T cells could not produce IFN-γ (12, 28), and others have also indicated that they belong to the Th2 subset (29). As these IL-10-producing T cells infiltrate the skin, they would tend to have the impact on local skin cells (including epidermal cells) of reinforcing a Th2-type cytokine network. Thus, the striking increase in KC IL-10 in Sezary syndrome would actually be contributing to the ongoing proliferation of these neoplastic cells and tend to diminish the host response of reactive Th1-type cells, thereby contributing to its poor prognosis. In lung and skin cancers, locally produced IL-10 has been suggested to play a role in downmodulating an effective local T cell-mediated immune response to the neoplastic cells (20, 30).

Taken together, these results highlight the potential immunoregulatory role of epidermal KCs within skin by the presence or absence of their IL-10 production (1, 23). Further studies of the regulation of KC IL-10 expression are indicated to determine if new therapeutic approaches targeted at this cytokine will have beneficial effects in a dermatological setting.

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