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Inhibition of Keratinocyte Apoptosis by IL-15: A New Parameter in the Pathogenesis of Psoriasis? 1

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Keratinocytes (KC) are important sources of targets for several cytokines. Although KC express IL-15 mRNA, the functional effects of IL-15 on these epithelial cells remain to be dissected. Investigating primary human foreskin KC and HaCaT cells, we show here by semiquantitative RT-PCR and flow cytometric analysis that both translate IL-15 and IL-15R mRNA and express IL-15 and IL-15Rα protein on the cell surface, suggesting that human KC can employ IL-15 for Juxtacrine signaling. While IL-15 exerted no significant effect on KC proliferation and IL-6 or IL-8 secretion, IL-15 inhibited both anti-Fas and methylcellulose-induced KC apoptosis in vitro. This is in line with the recognized potent anti-apoptotic effects of IL-15. IL-2, whose receptor shares two components with the IL-15R, failed to inhibit KC apoptosis. Together with the role of IL-15 in sustaining chronic immune reactions, this invited the question of whether a reduction of KC apoptosis by IL-15 may be involved in the pathogenesis of psoriasis, a chronic hyperproliferative inflammatory skin disease characterized by abnormally low KC apoptosis in the epidermis. Remarkably, compared with nonlesional psoriatic skin and skin of healthy volunteers, lesional psoriatic epidermis showed high IL-15 protein expression in the epidermis and enhanced binding activity for IL-15. Therefore, antagonizing the inhibitory effects of IL-15 on KC apoptosis deserves exploration as a novel therapeutic strategy in psoriasis management. The Journal of Immunology, 2000, 165: 2240–2250.

The proliferation and differentiation of keratinocytes (KC) ³ are controlled by a complex network of growth factors and cytokines (1), which are partially produced by the KC itself. This includes epidermal growth factor, insulin-like growth factor I, and TGF-α (2–4) as well as IL-1, IL-6, and TNF-α (1, 5, 6). The expression of several of these cytokines is up-regulated in inflammatory skin diseases, where these cytokines are thought to mediate skin inflammatory immune responses and leukocyte chemotaxis (e.g., keratinocyte cytokines, such as TNF-α, IL-8, and GM-CSF), are known to be responsible for the leukocyte infiltrate characteristic of the mature lesion of psoriasis (7, 8). Taken together, KC-derived cytokines are likely to play a critical role in various inflammatory skin diseases.

One parameter in the maintenance of epidermal homeostasis is the loss of KC adhesion leading to terminal differentiation to corneocytes or apoptosis. KC may undergo apoptosis by loss of cell-cell contact (9), after cross-linking of the Fas (CD95) molecule (10, 11), or by UV radiation (12, 13). KC apoptosis may be inhibited by different growth factors, such as epidermal growth factor (14, 15), KC growth factor (16), or nerve growth factor (17) in vitro. However, the molecular controls of KC apoptosis in vivo are still poorly understood. Therefore, in chronic inflammatory, hyperproliferative skin diseases such as psoriasis, where an abnormally low rate of apoptosis contributes to the development of epidermal hyperplasia (18, 19), it is important to gain a deeper understanding of the roles of specific cytokines and their cognate receptors in down-modulating the normal rate of KC apoptosis. In fact, it has been recently shown that psoriatic KC are abnormally resistant to apoptosis (9, 20). In addition, some of the proinflammatory cytokines that drive development of a psoriatic plaque may suppress KC apoptosis. Interestingly, in addition to its well-established immunosuppressive properties, recent data indicate that methotrexate, one of the best established and most effective anti-psoriatic agents, induces KC apoptosis (21).

In this context it is interesting to explore the role of IL-15 in KC apoptosis and the pathogenesis of psoriasis. IL-15 not only is implicated in the maintenance of several chronic inflammatory immune responses (21–23), but also is a very potent inhibitor of lymphocyte and hepatocyte apoptosis, both in vivo and in vitro (24). Since IL-15 mRNA is also expressed in KC and dendritic cells in the skin (25) and is a potent growth factor and chemoattractant for leukocytes (26, 27), it may play a role in KC biology and pathology. The aim of the present study was to further explore this as yet obscure functional role. Normal, primary foreskin KC were used to study the effects of IL-15 on native, untransformed cells. Moreover, the spontaneously transformed, immortalized HaCaT cells (28, 29) were used, not the least because this KC cell line displays a keratin expression pattern typically seen in psoriatic epidermis and has therefore been employed by some investigators for analyzing an easily handled epithelial cell population that shares some, although certainly not all, features of psoriatic epidermal KC.

In addition, the expression of IL-15 cytokine and IL-15R in situ was analyzed in skin biopsies from psoriatic patients and compared with that in clinically uninvolved or normal skin and in skin...
biopsies from chronic eczema and lichen simplex, which are also characterized by acanthotic epidermis. For receptor demarcation by immunohistology, a recently generated IL-15-IgG2b fusion protein was employed.

The data reported here suggest a role for IL-15, which is expressed in a juxtaocular manner on the KC cell surface, in inhibiting KC apoptosis, probably via the IL-15R α-chain.

Materials and Methods

**KC cell culture**

Human epidermal cell suspensions were obtained from normal donors undergoing foreskin surgery. KCs were propagated in serum-free medium (KGM, BioWhittaker, Heidelberg, Germany) with the following supplements: 0.1 ng/ml epidermal growth factor, 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 7.5 mg/ml bovine pituitary extract, 50 μg/ml gentamicin, 50 ng/ml amphotericin B, and 0.15 mM calcium. KCs were passaged by dissociating the monolayer with 0.025% (w/v) trypsin/0.01% (w/v) EDTA. For the experiments performed, cells were derived from the third to the fifth passage and grown to subconfluence as a monolayer. The human KC cell line HaCaT, provided by N. Fusenig (29), was cultured in DMEM with 5% (v/v) FCS and antibiotics at 37°C with 5% CO2.

**Induction of apoptosis by anti-Fas**

Semisquamous keratinocyte cultures were incubated for 24 h in the presence or the absence of human recombinant IFN-γ at a concentration of 100,000 U/ml to promote Fas expression as previously described (30). The cells were washed twice with PBS and once with culture medium and were treated with 1 μg/ml anti-Fas Ab (clone SM1/1; Bender Med Systems, Boeblingen, Heidelberg, Germany) for 2 h at 37°C. After washing twice with PBS, the cells were treated with a cross-linking goat antimouse IgG Ab (Sigma, Deisenhofen, Germany) in the presence or the absence of human rIL-15 (10 and 50 ng/ml; Genzyme, Cambridge, MA) or rIL-2 (10 and 50 ng/ml PeproTech, Rocky Hill, NJ) and cultured for 24, 48, or 72 h at 37°C. As control, cells were incubated with an irrelevant, isotype-matched IgG2a Ab (anti-HIV p24) (31) or with the cross-linking Ab alone. Detached cells were recovered by collecting the supernatant, and adherent cells were harvested by trypsinization. All cells were then used for viability tests and apoptosis detection.

**Induction of apoptosis in methylcellulose (MC)**

Induction of apoptosis in semisolid medium was performed as previously described (9, 32).Briefly, sterile 1.68% MC (MG 4000, Serva, Heidelberg, Germany) was prepared by autoclaving 3.37 g MC and a magnetic stir bar in a 250-ml glass bottle. Keratinocyte basal medium (KBM) (100 ml) was heated to 60°C, added to the MC powder, and stirred at room temperature for 20 min. An additional 100 ml of serum-free KBM was added, and the mixture was stirred at 4°C for 1 h. Aliquots of the MC solution were centrifuged at 10,000 rpm for 90 min at 4°C. Freshly trypsinized HaCaT cells or human keratinocytes were resuspended in 1.68% MC solution at 10,000 cells/ml with or without the addition of human rIL-15 (20 ng/ml; Genzyme) or rIL-2 (20 ng/ml PeproTech, Rocky Hill, NJ) and cultured for 24, 48, or 72 h in a humidified 5% CO2 atmosphere. Cells were recovered from suspension by repeated dilution of the MC by KBM followed by centrifugation.

**Detection of apoptosis**

KC were harvested at the indicated time points, and cytopsinos of 106 cells were prepared, air-dried, fixed, and stained for apoptotic cell death (TUNEL assay) (24) with an apoptosis-detection kit (ApopTag, Oncor, Gaithersburg, MD) following the manufacturer’s instructions. Cell nuclei were counterstained with Hoechst 33342 dye (Sigma), and apoptotic and nonapoptotic cells were counted in 10 microscopic fields (¼ 700 cells). Cells were referred to as undergoing apoptosis based on two microscopic criteria: 1) positive TUNEL staining, and 2) an apoptotic phenotype of the cells, i.e., shrinkage and fragmentation of the nucleus, as observed in the Hoechst 33342 counterstaining. The percentage of apoptosis was calculated. Shown is the inhibition of apoptosis (percentage) by the added IL-15 or IL-2 compared with the apoptosis induced without IL-15 or IL-2 in the culture medium (without cytokines added, no inhibition). As proof, apoptosis was also detected by FACS staining of propidium iodide inclusion as previously described (33).

**RT-PCR**

RNA was extracted from cells by using the RNA Clean reagent (AGS, Heidelberg, Germany) according to the manufacturer’s instruction. A 5-μg aliquot of total cellular RNA was reverse transcribed using random hexamers as primers and the Superscript II preamplification kit (Life Technologies, Karlsruhe, Germany). cDNA was amplified in 50-μl PCR reaction mixtures containing 250 nM of each dNTP, 200 nM of primers, and 5 μl of 10-fold PCR buffer (Perkin-Elmer/Cetus, Emeryville, CA), and 2.5 U Taq DNA polymerase (AmpliTaq, Perkin-Elmer/Cetus). The primers used were (27): human IL-15Rα: sense, 5′-GCC AGC GCC ACC TTC CAC AGT AA-3′; antisense, 5′-ACC GAC GGA GGG GTA TGT CTT GAC C-3′; human IL-15: sense, 5′-GGC TTT GAG TAA TGA GAA TTT-3′; antisense, 5′-GAATT CCTCGGAAGATGGCCAGTCCCA-3′; anti-sense, 5′-GAATTCCGAGTTGAAATGATTGACACCAATG-3′; human IL-2Rβ: sense, 5′-GAATTT CCTGGGAGATGGCAGTCCCA-3′; antisense, 5′-TTA AAGCCGGTCGGAACACGAAA-3′; and human β-actin: sense, 5′-GTCGGGCGCCCCAGC CAC CA-3′; antisense, 5′-CTCCTAACTGTACGCGG CGATTTC-3′. All primers used were purchased from Tib Molsys (Berlin, Germany). Samples were amplified in a DNA thermocycler (Perkin-Elmer/Cetus) for 35 cycles. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 2 min. Aliquots of PCR products were then electrophoresed on 1.5% agarose gel and visualized by ethidium bromide staining. To evaluate mRNA expression semiquantitatively, in addition to the PCR product, 20 ng of primer 2 was PCR amplified from 25 cycles and the 25 cycles run simultaneously. β-Actin message expression was used to normalize the cDNA amount to be used. A mock PCR (without cDNA) was included to exclude contamination in all experiments.

**Immunohistology**

Skin biopsies of psoriatic plaques and of uninvolved skin were obtained from 12 patients. Moreover, three biopsies each from patients with chronic eczema and lichen simplex were used to analyze additional chronic hyperproliferative, inflammatory skin diseases that also displayed acanthotic epi- dermis. Control biopsies from 10 normal, healthy donors were obtained from surgical skin explants from patients. Biopsies were immediately snap-frozen in liquid nitrogen and stored at −80°C. Ten-micron cryostat sections were air-dried, fixed in acetone (−20°C, 10 min), and stained with mouse anti-human IL-15 (34) or biotinylated IL-15-IgG2b fusion protein (FP) following standard avidin-biotin complex technique as previously described (35). Controls were obtained by omission of the primary Ab or by using biotinylated murine IgG2b (clone 49.2; PharMingen, Hamburg, Germany). To verify the specificity of the IL-15-IgG2b binding to the IL-15R, additional staining was performed on Con A-activated human peripheral blood T cells after stimulation with IL-2 (10 ng/ml for 24 h) (27). IL-15 binding to these cells was blocked by preincubation of the FP with anti-IL-15 Ab (M111, Genzyme, Alzenau, Germany). Additional control staining was performed by preincubation of psoriatic skin sections with a 100-fold excess of IL-15 for 30 min. This completely blocked binding of the biotinylated IL-15-IgG2b FP to the cognate IL-15R in the skin sections.

**FACS staining**

Expression of the IL-15Rα or surface IL-15 cytokine on primary KC or HaCaT was analyzed on unstimulated or on cells treated with IFN-γ (100 U/ml) for 24 h. LPS-activated (24-h incubation) PBMC, which express surface IL-15 as well as the IL-15Rα (36, 37), were stained as a positive control. The IL-15Rα was detected with mouse anti-huIL-15Rα (M116, provided by Immunex, Seattle, WA), and IL-15 surface expression was detected with a mouse anti-human IL-15 Ab (M111, Genzyme), both followed by FITC-labeled goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Isotype-matched Abs were used as controls, and the fluorescence was analyzed with a FACSscan (Becton Dickinson, Hei- delberg, Germany).

**Statistical analysis**

Results are presented as the mean ± SD, unless stated otherwise. Mann-Whitney U test was used to determine the significance of difference between treatment groups or between patients and healthy donors (p < 0.05 was considered significant). In the vitreous experiments were repeated at least three times, with highly comparable results.
IL-15 and IL-15Rα expression was analyzed on primary foreskin KC from different donors and on the HaCaT cell line. mRNA transcription was analyzed in KC treated with IL-15 (10 ng/ml) or IFN-γ (10^3 U/ml) or left untreated for 24 h. Incubation with IL-15 was performed, since this reportedly down-regulates its own high affinity receptor chain (IL-15Rα) on T cells (38). IFN-γ was studied in this assay, because it is overexpressed in psoriatic lesions (39), stimulates KC expression of activation markers (HLA-DR) (40) and cytokine receptors (IL-1R) (41), and enhances the expression of IL-15 in epithelial cells of human fetal retina (42).

By RT-PCR, unstimulated primary KC showed expression of IL-15 cytokine and receptor mRNA (Fig. 1). Steady state levels of the receptor transcripts were slightly down-regulated by incubation with IL-15. In contrast, IFN-γ seems to up-regulate mRNA levels for both IL-15 cytokine and receptor (Fig. 1). HaCaT cells showed substantially higher baseline mRNA levels for IL-15 and IL-15Rα. However, neither IL-15 nor IFN-γ stimulation altered significantly IL-15 cytokine or receptor steady state levels (Fig. 1).

Next, protein levels were analyzed. Primary KC from different donors and HaCaT cells were stained for surface IL-15Rα and surface IL-15 cytokine expression as determined by FACS analysis. Cell surface expression was studied, since it has been shown that IL-15 secretion in the supernatant is very tightly controlled and that most cells that transcribe IL-15 mRNA do not secrete detectable amounts of this cytokine (43). Therefore, biological effects may be mediated by membrane-bound IL-15 (in fact, we had previously detected membrane-bound IL-15 on monocytes (37)).

As shown in Fig. 2, primary KC and HaCaT cells both express IL-15 cytokine as well as IL-15Rα protein on their cell surface. Stimulation for 24 h with IFN-γ did not up-regulate the surface expression of IL-15 cytokine or its receptor at the protein level (not shown). Since monocytes express IL-15 and IL-15Rα on their surface (37) and are a major source of IL-15 (36), we stained LPS-activated monocytes and analyzed them by FACS as positive control for IL-15 and IL-15Rα surface expression. As shown in Fig. 2, E and F, expression was higher on monocytes compared with KC (Fig. 2, A–D).

These experiments provide the first evidence that in vitro normal and transformed human KC not only transcribe the IL-15 gene, but also translate it and express both surface IL-15 cytokine and its cognate high affinity receptor.

**KC express IL-2Rβ and IL-2Rγ mRNA**

The IL-15R consists of three components: the high affinity IL-15Rα-chain, the IL-2Rβ, and the common γ-chain (IL-2Rγ) (22). We have recently shown that inhibition of apoptosis in fibroblasts is mediated by the IL-15R α-chain (44). However, IL-15 can activate cells (e.g., NK cells) by binding and signaling through the IL-2Rβ and γ-chain (45). Therefore, we used PCR to detect the IL-2R on primary KC and HaCaT cells. As shown in Fig. 3, both primary KC and HaCaT cells express mRNA for IL-2Rβ and γ-chain, but fail to express the high affinity IL-2R α-chain. This shows that KC express all components of the IL-15R, namely, the high affinity IL-15R α-chain as well as the IL-2Rβ and γ-chain.

**IL-15 inhibits MC- and anti-Fas-induced KC apoptosis in vitro**

We and others have previously demonstrated that IL-15 is a very potent inhibitor of apoptosis of various cell types in vitro and/or in vivo, including on epithelial cell population (24). In a fibroblast cell line, we were able to demonstrate that the IL-15R α-chain probably mediates these anti-apoptotic effects by blocking the recruitment of adaptor proteins, thereby inhibiting apoptosis induction at a very early stage of the apoptotic signaling cascade (44). Since the above experiments had shown that IL-15 and IL-15Rα proteins are expressed by KC itself, we tested whether IL-15 also inhibits human KC apoptosis in vitro.

Primary KC and HaCaT cells were incubated in semisolid MC medium, which induces apoptosis by inhibiting cell-cell contact (9). After 24 h ~10% of the KC were TUNEL positive, i.e., had undergone apoptosis; after 48 h 30% and after 72 h in culture >60% of the KC undergo programmed cell death, comparable to the previously published values (Fig. 4A) (9). Inhibition of apoptosis was calculated as the percent inhibition to be able to compare the effects of IL-15 on MC- or anti-Fas-induced apoptosis (MC or anti-Fas without IL-15 was set equal to 0% inhibition).

The addition of IL-15 (20 ng/ml) to the MC medium significantly inhibited apoptosis, with the most prominent effects seen after 24 h (75% inhibition in primary KC and 50% in HaCaT, compared with MC alone (=0%)). After 72 h 35% inhibition was still seen in primary KC, whereas no significant effect of IL-15 was observed after this prolonged incubation period in HaCaT cells compared with culture in MC without IL-15 (Fig. 4A). Therefore, inhibition of apoptosis by IL-15 (not apoptosis itself) is highest after 24-h culture, while the total percentage of apoptosis increases over time, as stated above. Thus, apoptosis itself is not absent after 72 h, but the effect of the added IL-15 is absent (added at time zero, i.e., at the time point apoptosis is induced). The failure of IL-15 to inhibit apoptosis is probably due to degradation of the cytokine. In addition, IL-15 could not rescue those cells that are primed to undergo apoptosis; rather, it seems to inhibit the induction of apoptosis at an early time point (i.e., it rescues cells before apoptosis could be induced).

To address whether these apoptosis inhibitory effects of IL-15 reflected general down-modulatory properties of IL-15 on human KC apoptosis, we used a second, receptor-mediated method to induce apoptosis via an mAb to Fas that is expressed by KC (30, 46) (this was confirmed in the present study by FACS analysis; not
shown) and whose cross-linking induces apoptosis by oligomerization of this membrane-bound receptor. KC were treated for 24 h with IFN-\(\gamma\) to maximize Fas-mediated apoptosis as previously described (30). Then, KC were incubated with an anti-Fas Ab, which thereafter was cross-linked by a second Ab. This technique induced apoptosis in 10% KC after 24 h and 60% KC after 72 h of culture. Simultaneous addition of IL-15 in two different concentrations to the cell culture significantly suppressed Fas-induced apoptosis (Fig. 4B). The effect was most potent in primary KC (after 48 h), where addition of IL-15 (10 ng/ml) suppressed apoptosis by 75% compared with that in controls (anti-Fas alone). Addition of a 5-fold higher dose did not further increase this anti-apoptosis effect. In HaCaT cells, apoptosis was maximally inhibited after 72 h (60%) by 10 ng/ml IL-15. In HaCaT cells, higher concentrations of IL-15 (50 ng/ml) markedly inhibited apoptosis by 75% compared with that in controls (anti-Fas alone). Addition of a 5-fold higher dose did not further increase this anti-apoptosis effect. In HaCaT cells, apoptosis was maximally inhibited after 72 h (60%) by 10 ng/ml IL-15. In HaCaT cells, higher concentrations of IL-15 (50 ng/ml) exhibited stronger anti-apoptotic effects. After 24 h 60% apoptosis inhibition by IL-15 was seen in HaCaT cells, but it was not significantly modified at 48 or 72 h (Fig. 4B). This inhibition again seems to represent the rescue from apoptosis at an early time point, before or shortly after apoptosis is induced by Fas cross-linking.

Although the TUNEL method is a reliable and specific method for the detection of apoptotic KC cell death in vitro (9), terminally differentiating KC in vivo may also show a positive TUNEL reaction (18, 47). Therefore, a second method was applied, which detects apoptotic cell death by flow cytometric propidium iodide staining (33). This method confirmed the results obtained with the TUNEL method (data not shown). In addition to TUNEL and propidium iodide staining, morphological signs of anti-Fas-induced apoptosis were analyzed by light microscopy and were photodocumented. In primary KC grown in a confluent monolayer (Fig. 5A) addition of the anti-Fas and the cross-linking Abs induced marked morphological alterations consistent with the morphological features of apoptosis, such as cell condensation and shrinkage, appearance of apoptotic bodies, and loss of adhesion (Fig. 5A). Incubation with an isotype-matched, irrelevant control Ab did not generate these phenomena. Coincubation with anti-Fas and IL-15 (50 ng/ml) markedly inhibited apoptosis-associated cell detachment and shrinkage, as shown in Fig. 5A. The same effects were seen in HaCaT cells (Fig. 5B).

**IL-2 does not inhibit KC apoptosis in vitro**

To further clarify the anti-apoptotic effects of IL-15 on KC we used IL-2 in the same apoptosis assay. IL-15 can bind to KC via the IL-15R\(\alpha\)-chain as well as via the IL-2R\(\beta\) and \(\gamma\)-chain, whereas IL-2 binds and signals exclusively via the IL-2R\(\beta\) and \(\gamma\)-chain. In addition, IL-2 can signal via the high affinity IL-2R\(\alpha\)-chain, which is not expressed on KC (see Fig. 3), but fails to transmit a signal via the IL-15R\(\alpha\)-chain (22). As shown in Fig. 6 induction of apoptosis by methylcellulose (Fig. 6A) and by anti-Fas Ab (Fig. 6B) in HaCaT is not suppressed by IL-2. In fact, the anti-Fas pathway in contrast is, rather, increased by 50% (Fig. 6B). Similar results were obtained using primary KC (not shown). Since both cytokines bind to the IL-2R\(\beta\) and \(\gamma\)-chain, and since IL-2 fails to inhibit KC apoptosis, this suggests that inhibition of apoptosis by IL-15 in KC is mediated by the IL-15R\(\alpha\)-chain, as previously shown for fibroblasts (44).
IL-15 failed to stimulate IL-6 and IL-8 production and proliferation of KC

In vitro IL-15 is not a growth factor for KC, since incubation of HaCaT and primary KC with IL-15 could not induce significant proliferation, as assessed by [3H]thymidine incorporation (not shown). Also, incubation of both, primary KC and HaCaT with IL-15 or IL-2 failed to induce significant IL-6 and IL-8 release into the supernatant (not shown). Taken together, IL-15 itself is not a growth factor for KC and cannot induce KC to secrete this two proinflammatory cytokines.

IL-15 cytokine and IL-15 binding sites are overexpressed in lesional psoriatic epidermis

Since these experiments had revealed that IL-15 inhibits human KC apoptosis in vitro, it was interesting to begin to explore whether IL-15 might play a role in hyperproliferative inflammatory skin diseases such as psoriasis or other dermatoses such as chronic eczema and lichen simplex. IL-15 mRNA and protein are produced by human KC (25), but nothing was known about cutaneous expression of the IL-15 cytokine and its receptor. To analyze the distribution of IL-15 cytokine and IL-15 binding sites, immunohistologic staining was performed on skin sections from normal, healthy donors as well as on sections derived from lesional and nonlesional skin of patients with psoriasis vulgaris as well as from patients with chronic eczema or lichen simplex. Staining of psoriatic plaques for IL-15 with an mAb revealed high expression throughout the entire epidermis (from the stratum basale, which contains epidermal stem cells, to the stratum granulosum, which is characterized by KC that undergo programmed cell death by terminal differentiation and cornification) (19) as well as in the dermis directly adjacent to the epidermal basal membrane. Skin sections from chronic eczema/lichen simplex with acanthotic epidermis showed IL-15 expression mainly restricted to the basal layer of the epidermis and the subepidermal compartment, comparable to healthy skin. In addition, single cells in the dermis stained positively for IL-15, probably representing hemopoietic cells of the inflammatory infiltrate that is characteristic of these dermatoses (48).
FIGURE 5. Morphological signs of anti-Fas-induced cell death are blocked by IL-15. Representative photomicrographs were taken at $\times 100$ magnification after 24-h culture of primary KC (A) or HaCaT (B) in medium, medium plus an irrelevant, isotype-matched control Ab (mouse IgG2a, anti HIV p24), anti-Fas, or anti-Fas plus IL-15 (50 ng/ml).
IL-2 fails to inhibit KC apoptosis in vitro. IL-2 was used in the apoptosis assay instead of IL-15 to determine which receptor chain is responsible for inhibition of apoptosis. Apoptosis was induced in HaCaT cells by MC (A); addition of IL-2 could not rescue the cells from apoptosis, which, induced by anti-Fas Ab (B) is, rather, enhanced by IL-2. Negative values represent an increase in apoptotic cells.

By immunohistology, in normal skin from healthy donors only subepidermal expression of IL-15 was seen, and no IL-15 immunoreactivity could be detected in the epidermal KC (Fig. 7A). However, a few IL-15-positive cells were detected in the epidermal and subepidermal layers, probably representing macrophages or dendritic cells. As positive control we used skin biopsies from hypertrophic scars, since they overexpress IL-15 (49), as confirmed here (Fig. 7A). Omission of the anti-IL-15 Ab as a negative control showed the specificity of the staining (Fig. 7A).

For the detection of IL-15 binding sites, a recently generated FP was used, consisting of functional human IL-15 and murine IgG2b (24). This allows easy and specific demonstration of the murine IgG2b as a tracer for IL-15 binding sites in human tissue. As a positive control, activated human PBMC were employed, which highly express IL-15Rα (27). In vitro, preincubation of the IL-15-IgG2b FP with an anti-IL-15 Ab served as a negative control (no staining with the biotinylated FP was observed under these conditions; Fig. 7B). As an additional control, it was demonstrated that human cells did not bind the murine IgG2b tail of the FP, as determined by staining with biotinylated murine IgG2b. The specificity of the staining was further demonstrated by preincubation of psoriatic skin with a 100-fold excess of IL-15 cytokine for 30 min, which completely blocked binding of the IL-15-IgG2b fusion protein (Fig. 7B).

Nonlesional skin from psoriatic patients and normal skin from healthy donors were found to constitutively express binding sites for IL-15 on epidermal KC at very low levels (Fig. 7B). In contrast, staining of skin sections from lesional psoriatic skin revealed a striking overexpression of IL-15 binding sites on epidermal KC (Fig. 7B). Acanthotic epidermis in chronic eczema/lichen simplex did not show this strong epidermal overexpression of IL-15 binding sites. The staining in the two disease settings was restricted to the basal layer of the epidermal KC and to the subepidermal dermis. Single IL-15-binding cells could be detected in the dermis, probably representing cells of the inflammatory infiltrate associated with chronic eczema/lichen simplex.

**Discussion**

We have shown here that primary human foreskin KC as well as the human HaCaT cell line transcribe mRNA for IL-15 and for the IL-15Rα, IL-2Rβ, and γ-chain. In addition, we provide evidence that human KC express the cytokine as well as the cognate high affinity receptor on the cell surface, which suggests that juxtacrine signaling of KC is mediated via surface-bound IL-15. Furthermore, we show that in vitro IL-15, but not IL-2, strongly inhibits MC- and anti-Fas-induced KC apoptosis. Since epidermal hyperplasia in chronic inflammatory hyperproliferative skin diseases such as psoriasis may at least in part be due to abnormally reduced or delayed apoptosis (9), it was intriguing to note that immunohistology revealed an altered distribution pattern of IL-15 cytokine, with IL-15 also detected on epidermal KC above the basal membrane, rather than being restricted to the dermis, compared with uninvolved or normal human skin. Additional staining of acanthotic epidermis from chronic eczema/lichen simplex showed expression of IL-15 and its specific binding sites to be restricted to the basal epidermal layer and to inflammatory cells in the upper dermis, while no overexpression could be detected in the epidermis as seen in psoriasis.

Loss of cell-cell contact promotes KC differentiation and apoptosis in normal epidermis (50, 51). Inhibition of this physiological control process of KC turnover by IL-15 supports a role for IL-15 in the promotion of epidermal hyperplasia in psoriasis. In addition, KC express the Fas receptor (30), which induces apoptosis after receptor cross-linking (10). As demonstrated here, Fas-induced apoptosis is potently inhibited by IL-15. If confirmed, e.g., in skin organ culture assays of psoriatic vs healthy human skin during administration of IL-15Rα antagonists, IL-15R-mediated signaling may well become a promising target in the management of chronic inflammatory, hyperproliferative skin diseases. Pharmacological blockade or down-regulation of IL-15 release, IL-15Rα overexpression, and/or IL-15Rα-mediated signal transduction pathways might represent useful targets for adjuvant therapy in the management of skin diseases such as psoriasis.

Interestingly, psoriatic KC are now recognized to display abnormal resistance to apoptosis (9, 20). Also, the induction of apoptosis in KC represents one mode of action of the well-established and highly effective anti-psoriatic agent methotrexate (21). This encourages us the pursuit of adjuvant treatment modalities in psoriasis that counteract KC strategies to rescue them from apoptosis, such as overexpression of anti-apoptotic IL-15 and IL-15Rα.

The constitutive expression of IL-15Rα, IL-2Rβ, and γ-chain as well as of IL-15 on the protein level demonstrated here on normal KC and on HaCaT cells in vitro suggests a physiological role of IL-15 in KC biology. Staining of skin sections for IL-15 binding sites also revealed low level expression of IL-15 binding sites in the epidermis in normal skin from healthy donors. Furthermore, the transcription of mRNA for IL-15 by KC in vitro demonstrated here confirms previously published results (25, 42).
The secretion of IL-15 is tightly controlled, and expression of mRNA does not necessarily correlate with cytokine secretion into the supernatant (43). However, membrane-bound IL-15 on the KC surface may serve as a juxtacrine signal for directly adjacent cells; directly neighboring KC that express IL-15R may be stimulated by IL-15 expression on the cell surface of KC in their immediate vicinity. Since the IL-15Rα-chain shows a very high affinity for IL-15 (Kₐ = 10¹¹ M⁻¹) (53), functional significance (e.g., cytokine production by macrophages) was reached at extremely low concentrations (picomolar to attomolar range) (23). All three chains of the IL-15R are expressed by KC, and IL-15 as well as IL-2 may signal through the β- and γ-chains of the IL-2R. However, suppression of apoptosis in the in vitro model employed here seems to be mediated by the IL-15Rα, since IL-2 did not reduce the rate of KC apoptosis.

Taken together, expression of membrane-bound IL-15 may affect epidermal homeostasis primarily by enhancing the survival of those KC that express cognate receptors. The suppression of KC apoptosis by IL-15 in vitro demonstrated here suggests one scenario for how this cytokine may inhibit the normal pattern of KC cell death and terminal differentiation of KC in the upper layers of the epidermis, thus leading to epidermal hyperplasia (acanthosis) in psoriasis, but not in other chronic inflammatory diseases such as lichen simplex cronica and chronic eczema. As one of the most common hyperproliferative skin diseases, psoriasis vulgaris is characterized by KC hyperproliferation as well as profound changes in KC maturation and turnover rate (54, 55). In addition, an abnormal resistance of psoriatic KC to apoptosis (9, 20) may contribute to the epidermal hyperplasia. However, little is known about the underlying mechanisms of this defect in the apoptosis.

FIGURE 7. IL-15 cytokine and IL-15 binding sites are expressed in lesional psoriatic plaques. Immunohistologic staining for IL-15 and IL-15 binding sites was performed on skin biopsies from psoriatic patients as well as patients with chronic eczema and lichen simplex and from healthy controls. Psoriatic skin showed strong IL-15 expression in the epidermis as well as in the dermal stratum papillare, whereas in normal skin only dermal expression was observed (A). Hypertrophic scar tissue served as a positive control (34). As a negative control, the primary Ab was omitted. Immunohistologic studies for detection of IL-15 binding sites were performed with the IL-15-IgG2b FP (B). Control stainings were performed on PBMC, which express the IL-15R after activation. Specificity was shown by blocking the IL-15 tail, which completely prevented binding of the FP (upper row) as well as by preincubation of the skin sections with a 100-fold excess of IL-15 cytokine, which completely blocked binding of the IL-15-IgG2b FP. Control sections were stained with murine IgG2b. Shown are representative staining results derived from a total of 10 biopsies from healthy controls, from 12 psoriatic patients, and from patients with chronic eczema and lichen simplex at ×100 magnification.
control machinery of psoriatic KC. Psoriatic KC have abundant amounts of the cell survival protein Bcl-xL (20). Whether this overexpression correlates with enhanced survival and how Bcl-xL is up-regulated in the cells are as yet unclear. We are currently testing whether IL-15 is involved in the regulation of Bcl-xL in KC, since IL-15 has been shown to up-regulate Bcl-2 in T cells (56).

Our findings provide the first indicator that delayed apoptosis of psoriatic KC (9) may be mediated at least in part by KC-derived IL-15. Epithelial cell production of IL-15 was not only seen in normal KC, but also in HaCaT cells, which are often advocated as an in vitro model for studying hyperproliferative skin diseases (57–59). The observed dramatic up-regulation of IL-15 binding to KC and the expression of IL-15Ra mRNA in psoriatic plaques are in line with the concept that IL-15 may inhibit apoptosis in psoriatic epidermis, which overexpresses IL-15 cytokine and the IL-15R. This overexpression suggests a correlation of the IL-15 cytokine and receptor expression and the dramatic overaccumulation of KC in psoriatic epidermis. However, acanthotic epidermis, as such, is not necessarily correlated to IL-15/IL-15R overexpression, since in chronic eczema and lichen simplex neither IL-15 nor...
IL-15 binding sites were found to be overexpressed above the basal layer of the epidermis.

The putative role of IL-15 in the pathogenesis of psoriasis may go beyond that of an inhibitor of KC apoptosis. Given that psoriasis increasingly surfaces as a T cell-mediated immune disease (61), it is particularly interesting to note that IL-15 is now accepted to go beyond that of an inhibitor of KC apoptosis. Given that psoriasis is induced by anti-Fas antibody alone in cultured human keratinocytes. Arch. Dermatol. 111:238.

References