Interleukin-15 mRNA Is Expressed by Human Keratinocytes, Langerhans Cells, and Blood-Derived Dendritic Cells and Is Downregulated by Ultraviolet B Radiation

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Interleukin (IL)-15 is a recently described cytokine that shares many functional activities with IL-2; however, unlike IL-2, IL-15 is produced by monocytes/macrophages, and not by lymphocytes. In this report, we assessed IL-15 mRNA expression by freshly isolated human epidermal cells, as well as by negatively selected keratinocytes and positively selected Langerhans cells, utilizing reverse transcription and polymerase chain reaction. In addition, cultured keratinocytes, immortalized keratinocytes (HaCaT cells), and dendritic cells expanded from adult peripheral blood in the presence of granulocyte/macrophage-colony stimulating factor and IL-4 were examined for IL-15 transcripts. Using cultured keratinocytes, we also studied the regulation of IL-15 mRNA expression by ultraviolet B radiation *in vitro*. Freshly isolated keratinocytes, HaCaT cells, and cultured keratinocytes all constitutively expressed IL-15 mRNA, and IL-15 expression was downregulated by ultraviolet B radiation in cultured keratinocytes in a time- and dose-dependent manner. In addition, IL-15 transcripts were constitutively expressed by freshly isolated Langerhans cells and by adult blood-derived dendritic cells. IL-15 produced by keratinocytes, Langerhans cells, and other tissue-specific dendritic cells may be important in attracting and activating antigen-specific Th1 T cells. Furthermore, ultraviolet B-induced downregulation of keratinocyte IL-15 production may contribute to the relative state of immunosuppression induced by sun exposure. ***Key words: cytokines/epidermis/Th1/RT-PCR. J Invest Dermatol 106: 1047–1052, 1996***

Interleukin (IL)-15 is a recently described 14- to 15-kDa glycoprotein produced at high levels in monocytes/macrophages (Mø), placenta, and skeletal muscle, as well as in low levels in many other tissues, including heart, lung, liver, and kidney (Grabstein *et al*, 1994). IL-15 is not synthesized by T and B cells (Grabstein *et al*, 1994). Functionally, IL-15 enhances the proliferation and activity of mitogen-stimulated T cells ($CD4^+$ and $CD8^+$) (Grabstein *et al*, 1994), antigen-stimulated Th1 T cell clones (Grabstein *et al*, 1994; Kennedy *et al*, 1994), anti-IgM-stimulated B cells (Armitage *et al*, 1995; Matthews *et al*, 1995), cytotoxic T lymphocytes (Grabstein *et al*, 1994), lymphokine-activated killer cells (Grabstein *et al*, 1994), and NK cells (Carson *et al*, 1994). In addition, IL-15 can act as a chemoattractant for T cells, but not for Mø, neutrophils, or B cells (Wilkinson and Liew, 1995). Thus, IL-15 has IL-2-like activity. Although it shares no sequence homology with IL-2, both cytokines initiate signal transduction through the β and γ chains of the IL-2R complex (Giri *et al*, 1994; Grabstein *et al*, 1994; Lin *et al*, 1995). Anti-IL-2R β-chain antibodies (Abs), but not anti-IL2R α-chain Abs, abrogate IL-15 activity (Carson *et al*, 1994; Giri *et al*, 1994; Grabstein *et al*, 1994; Armitage *et al*, 1995; Wilkinson and Liew, 1995). In addition, activated B cells isolated from patients with X-linked severe combined immunodeficiency that are IL-2R γ-chain deficient fail to proliferate and differentiate with the addition of IL-15 (Matthews *et al*, 1995).

Numerous cytokines are constitutively produced by epidermal cells (Luger and Schwarz, 1991; Schuler, 1991; Schwarz and Luger, 1992; Kupper, 1993). Mohamadzadeh *et al* (1995) have very recently reported that IL-15 mRNA expression could not be detected in normal nonirradiated epidermis, but could be induced in UVB-irradiated epidermis. Furthermore, they found that cultured keratinocytes constitutively expressed IL-15, and that expression was upregulated by UVB (Mohamadzadeh *et al*, 1995). Our current investigation of IL-15 mRNA expression by epidermal cells demonstrate contrasting results. Freshly isolated unseparated epidermal cells, as well as purified populations of negatively selected keratinocytes and positively selected Langerhans cells (LC), constitutively expressed IL-15 mRNA; expression by cultured keratinocytes was downregulated by UVB radiation in a time- and dose-dependent manner. Furthermore, we found that IL-15 mRNA was expressed by purified adult blood-derived dendritic cells (DC). These cells, which were generated from adherent peripheral blood mononuclear cells (PBMC) cultured in the presence of granulo-

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Abbreviations: DC, dendritic cell; LC, Langerhans cell; UVB, ultraviolet B; Mø, monocyte/macrophage; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells.

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Table I. Cell Surface Antigen Expression by Adult Blood-Derived DC using Flow Cytometry

High levels[a] of the following cell surface antigens:
MHC class II molecules (HLA-DR[b], -DP[b], -DQ[c]), MHC class I molecules (HLA-A, B, C[d]), invariant chain (CD74)[e], CD1a[f], CD1b[e], CD1c[e], ICAM-1 (CD54)[b], ICAM-2 (CD50)[e], CD40[e], B7-2 (CD86)[e], CD11b[e], integrins $\beta 1$ (CD29)[e] and $\beta 2$ (CD18)[e], CD58[e], CD13[b], CD33[b], CD44[e], CD45RO[e]

Low levels[a] of the following cell surface antigens:
CD4[b], CD11a[e], CD11c[e], FcεRI[g], FcεRII (CD23)[e], FcγRII (CD32)[e], B7-1 (CD80)[b]

No expression[a] of the following cell surface antigens:
CD3[e], CD5[e], CD8[e], CD25[e], CD26[h], CD28[b], CD14[b], CD36[e], FcγRI (CD64)[e], FCγRIII (CD16)[e], CD1d[e], CD19[b], CD24[e], CD34[b], CD56[e], CD57[b], E-cadherin[i]

[a] High levels: mean fluorescence intensity (MFI) > 10x MFI of isotype control mAb; low levels: MFI 3-10x MFI of isotype control mAb; no expression: MFI < 3x MFI of isotype control mAb.
[b] mAbs were purchased from Becton Dickinson, San Jose, CA.
[c] mAbs were purchased from PharMingen, San Diego, CA.
[d] mAb was purchased from INCSTAR Corp., Stillwater, MN.
[e] mAbs were purchased from BioSource International, Camarillo, CA.
[f] mAb was purchased from Ortho Diagnositic Systems Inc., Raritan, NJ.
[g] mAb was a kind gift from Dr. Jean-Pierre Kinet, NIAID, Rockville, MD.
[h] mAb was purchased from Coulter Corp., Hialeah, FL.
[i] mAb was purchased from Zymed Laboratories, Inc., S. San Francisco, CA.

cyte/macrophage-colony stimulating factor and IL-4, have many of the classic features of freshly isolated LC and other DC (Romani *et al*, 1994; Sallusto and Lanzavecchia, 1994) and therefore represent a useful tool in the study of DC biology.

MATERIALS AND METHODS

Preparation and Characterization of Cells This protocol was approved by the National Cancer Institute Institutional Review Board, and informed consent was obtained from each volunteer prior to entry into the study. Blisters were induced on clinically normal skin of the volar forearms or anterior thighs by vacuum suction and heat, and epidermal cell suspensions were prepared by limited trypsinization of blister roofs (epidermal sheets), as previously described (Cooper *et al*, 1986).

To negatively select keratinocytes, aliquots of epidermal cells were resuspended in a cocktail containing the following mouse anti-human monoclonal antibodies (mAbs) (each at 5 μg/ml): anti-HLA-DR, anti-CD1a, anti-CD3, and anti-CD14 (see **Table I** for source of Abs). Cells were then incubated for 30 min at 4°C, with gentle agitation every 10 min. During this incubation, magnetic beads (10 beads/epidermal cell) coated with sheep anti-mouse IgG Abs (Dynal Inc., Great Neck, NY) were washed using an MPC™-1 magnetic particle concentrator (Dynal Inc.) and resuspended in 1 ml of Hanks' balanced salt solution/10% fetal calf serum (wash medium). After the 30-min epidermal cell-cocktail incubation, cells were washed, mixed with the magnetic bead suspension, and incubated for 30 min at 4°C, with gentle agitation every 10 min. Cells coated with beads (nonkeratinocytes) were then separated from uncoated cells (predominantly keratinocytes) through a series of washes using the magnetic particle concentrator. This experiment was performed three times, using epidermal cells obtained from three different individuals.

In three additional volunteers, LC were positively selected from epidermal cell suspensions. For each individual, epidermal cells were incubated in wash medium containing anti-CD1a mAbs diluted to 10 μg/ml for 30 min at 4°C, with gentle agitation every 10 min. $CD1a^+$ and $CD1a^-$ (i.e., non-LC) cells were then separated using anti-mouse IgG Ab-coated magnetic beads as described above. Flow cytometric analysis of separated epidermal cell populations was performed as previously described (Blauvelt *et al*, 1995).

Homogeneous cultures of both cryopreserved and fresh neonatal keratinocytes were obtained from three separate lots (Clonetics, San Diego, CA) and cultured in KGM™ supplemented with bovine pituitary extract (Clonetics) at 37°C in a moist atmosphere containing 5% CO_2, with a change of medium twice weekly. Subculture was performed according to Clonetics' guidelines, using a trypsin/EDTA and trypsin-neutralizing solution (both from Clonetics). HaCaT cells, a spontaneously immortalized keratinocyte cell line kindly provided by Dr. Norbert E. Fusenig (Deutsches Krebsforschungszentrum, Heidelberg, Germany) were cultured as previously described (Boukamp *et al*, 1988). mRNA was extracted from both cultured keratinocytes and HaCaT cells when cultures were approximately 80% confluent.

H9 cells (a $CD4^+$ T cell line) were a kind gift of Dr. Robert C. Gallo (National Cancer Institute, Bethesda, MD).

DC were propagated from human adult peripheral blood, using a protocol slightly modified from previous reports (Romani *et al*, 1994; Sallusto *et al*, 1994). PBMC were prepared from buffy coats of HIV^- blood donors using lymphocyte separation medium (Organon Teknika Corp., Durham, NC), washed, resuspended in RPMI 1640 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum, 100 U/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO), 2 mM L-glutamine (GIBCO), 10 mM HEPES (GIBCO), and 5×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) (complete medium) at $5\text{–}8 \times 10^6$ cells/ml, and placed in 35-mm tissue culture plates (Becton Dickinson, Mountain View, CA) for 2 h at 37°C. Nonadherent cells were then drawn off, using care not to disturb loosely adherent DC precursor cells. Fresh complete media were returned to culture wells, and each well was then supplemented with 1000 U/ml recombinant human granulocyte/macrophage-colony stimulating factor (Immunex Corporation, Seattle, WA) and 1000 U/ml rhIL-4 (R&D Systems, Minneapolis, MN). Half the total volume of cultures was replaced with fresh media and cytokines every other day.

At day 7, cells were harvested, washed, and resuspended in a cocktail containing the following mouse anti-human mAbs (each at 5 μg/ml): anti-CD3, anti-CD14, anti-CD16, and anti-CD19. Contaminating T cells, Mø, natural killer cells, and B cells were then separated from $CD3^-CD14^-CD16^-CD19^-$ cells (i.e., adult blood-derived DC) using anti-mouse IgG Ab-coated magnetic beads as described above. IL-15 mRNA was assessed in adult blood-derived DC propagated from three individuals on three separate occasions.

Prior to mRNA studies, phenotypic, morphologic, and functional properties of adult blood-derived DC isolated from numerous individuals on separate occasions were extensively characterized. The materials and methods used for this characterization are described in detail elsewhere (Blauvelt *et al*, submitted for publication).

UVB Irradiation of Cultured Human Keratinocytes In Vitro UVB irradiation *in vitro* was performed as previously described (Enk *et al*, 1995). Doses of 10, 50, 100, and 200 J/m^2 were used. After irradiation, cells were incubated for an additional 6, 12, or 24 h. Dishes were then washed in phosphate-buffered saline and stored at −70°C until mRNA was extracted directly from plastic adherent cells. Tumor necrosis factor α (TNF-α) and IL-10-specific primer-probe sets were used as positive polymerase chain reaction (PCR) controls in these experiments, because UVB radiation has been previously shown to upregulate gene expression for these cytokines (Kock *et al*, 1990; Enk *et al*, 1995). Dose-response and time-course experiments were performed two and five times, respectively.

Determination of IL-15 Protein Content in Culture Supernatants Cultured keratinocytes, HaCat cells, and adult blood-derived DC were assessed for their ability to secrete IL-15 protein. For keratinocytes and HaCat cells, supernatants were collected from both confluent and ~70% subconfluent cultures. For adult blood-derived DC, supernatants were collected 24 h following purification (as outlined above) and reculture of the cells. All supernatants were concentrated 10-fold using Centricon® concentrators (Amicon, Inc., Beverly, MA), according to the manufacturer's instructions. Concentrated supernatants were then assessed for IL-15 protein content by enzyme-linked immunosorbent assay in conjunction with Genzyme Corporation, Cambridge, MA, or by a commercially available enzyme-linked immunosorbent assay kit (BioSource International), according to the manufacturer's instructions. Experiments were performed on two separate occasions.

mRNA Extraction, RT-PCR, and Liquid Hybridization mRNA was extracted from cells using superparamagnetic polystyrene Dynabeads $Oligo(dT)_{25}$ (Dynal Inc.) and reverse-transcribed by specific priming to first strand cDNA using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT), and cDNA was amplified by PCR; all procedures were as previously described (Enk and Katz, 1994; Enk *et al*, 1995). Denaturation of cDNA was performed for 1 min at 94°C, annealing for 1.5 min at a variety of temperatures **(Table II)**, and extension for 2 min at 72°C. Cycle numbers **(Table II)** were titrated to establish standard curves which demonstrated linearity. This allowed for semiquantitative analysis of signal strength. Primers (**Table II**; Genosys Biotechnologies, Inc., The Woodlands, TX) were designed according to published Genbank sequences, except G3PDH, TNF-α, and IL-10 primers which were purchased from Clontech (Palo Alto, CA).

Table II. Oligonucleotide Primers and PCR Conditions

Gene	Primer	Sequence	Annealing Temp. (°C)	No. of Cycles
IL-15	5′	5′-CAAGTTATTTCACTTGAGTCCGGAG	63	30
	3′	5′-TTCTAAGAGTTCATCTGATCCAAGG		
CD1a	5′	5′-TGAGAGACCAGCAGCCCAAG	63	26
	3′	5′-AATGTGGCGGGAGTTCAGACC		
K5	5′	5′-GGGGTAGCAGCTCCAGCGTC	63	28
	3′	5′-TCAGCAGGGGCCATGCTGTG		
CD14	5′	5′-TCAGAGTGCTCGATCTCAGC	63	19
	3′	5′-AAGCCAAGGCAGTTTGAGTC		
IL-10	5′	5′-AAGCTGAGAACCAAGACCCAGACATCAAGGCG	63	28
	3′	5′-AGCTATCCCAGAGCCCCAGATCCGATTTTGG		
TNF-α	5′	5′-GAGTGACAAGCCTGTAGCCCATGTTGTAGCA	63	28
	3′	5′-GCAATGATCCCAAAGTAGACCTGCCCAGACT		
G3PDH	5′	5′-TGAAGGTCGGAGTCAACGGATTTGGT	55	18
	3′	5′-CATGTGGGCCATGAGGTCCACCAC		

Probes (TNF-α and IL-10 purchased from Clontech, all others from Genosys) were designed to be complementary to internal sequences of the specific PCR products. Probe sequences were as follows:

IL-15 probe: 5′-GACAAACTGTTGTTTGCTAGGATGATCAGA
CD1a probe: 5′-ACTGCTTCCCACTTGTAAGTCAGGGAAGGC
K5 probe: 5′-CTGAAGGCTGATTTGAAGCAGAATA
CD14 probe: 5′-CATTATTCTGTCTTGGATCTTAGGCAAAGC
IL-10 probe: 5′-GGCTTTGTAGATGCCTTTCTCTTGGAGCTT
TNF-α probe: 5′-ATCTCTCAGCTCCACGCCATTGGCCAGGAG
G3PDH probe: 5′-GAGTGGGTGTCGCTGTTGAAGTCAGAGGAG

As described (Enk *et al*, 1994), 5 μl of amplified PCR product were hybridized to an excess of ^{32}P-end-labeled probe, electrophoresed on 4% polyacrylamide gels, and dried, and signal intensity was determined by autoradiography (exposure time 2–24 h using Kodak BIO-MAX films and intensifying screens).

RESULTS

Freshly Isolated Human Keratinocytes, Cultured Keratinocytes, and Immortalized Keratinocytes Constitutively Express IL-15 mRNA Freshly isolated human keratinocytes, obtained from suction blister roofs and purified by negative selection, constitutively expressed IL-15 mRNA **(Fig 1)**. To determine the efficiency of our negative selection procedure, we assessed selected cell populations for the presence of LC by 1) anti-CD1a mAb labeling/flow cytometry and 2) RT-PCR using CD1a-specific primers. LC were not detected in negatively selected populations using flow cytometry (not shown), whereas only faint CD1a-specific mRNA bands (compared with G3PDH- and IL-15-specific mRNA) were detected using RT-PCR **(Fig 1)**. To control for genomic DNA contamination, reactions were regularly performed in the absence of reverse transcriptase and were always negative (not shown). As additional controls, PBMC had readily detectable IL-15 transcripts, whereas H9 cells did not express IL-15 mRNA **(Fig 1)**.

Because epidermal cell suspensions were not depleted of contaminating melanocytes, it was theoretically possible that melanocytes, and not keratinocytes, were the epidermal cells expressing IL-15 mRNA. To address this issue, we assessed for and found IL-15 transcripts in pure populations of cultured keratinocytes and HaCaT cells, an immortalized keratinocyte cell line **(Fig 1)**. Taken together, these data conclusively demonstrate that keratinocytes express IL-15 mRNA. Concentrated HaCat cell supernatants from confluent cultures contained low levels of IL-15 protein (26 pg/ml), whereas no protein was detectable in concentrated supernatants of subconfluent cultures nor in any of the cultured keratinocyte supernatants.

Expression of IL-15 mRNA by Cultured Keratinocytes Is Downregulated by UVB Irradiation In Vitro UVB is a potent inducer of gene transcription for many keratinocyte-derived cytokines (Kupper *et al*, 1987; Kock *et al*, 1990; Kirnbauer *et al*, 1991; Kondo *et al*, 1993; Enk *et al*, 1995). We found, however, that expression of IL-15 mRNA was progressively decreased at time intervals of 6, 12, and 24 h following UVB irradiation **(Fig 2*a*,*b*)**. In contrast, IL-10- **(Fig 2*a*)** and TNF-α-specific **(Fig 2*a*,*b*)** mRNAs were upregulated with UVB radiation compared to sham controls. IL-15 transcripts were also decreased in a dose-dependent manner in the range of 10 to 100 J/m^2 **(Fig 2*b*)**; 200 J/m^2 was too toxic to cells and did not yield sufficient mRNA for study.

Freshly Isolated Human Epidermal LC Constitutively Express IL-15 mRNA Freshly isolated human epidermal LC, ob-

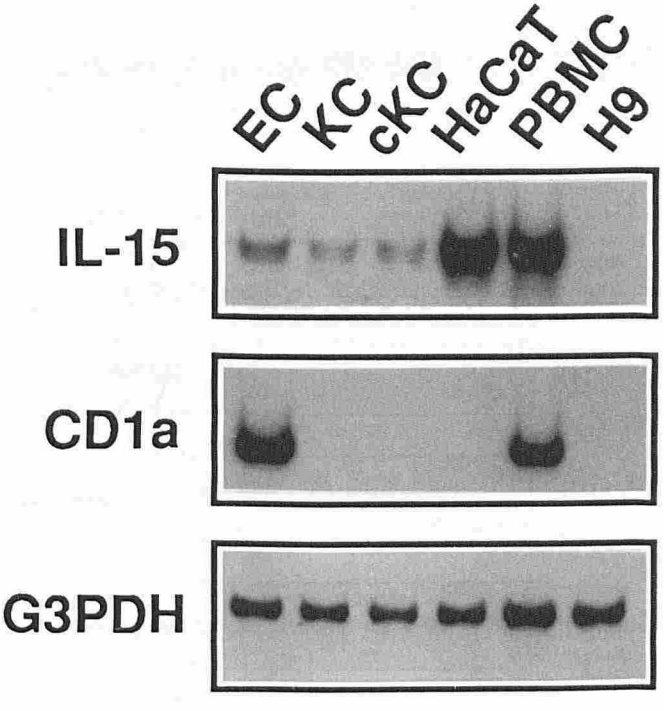

Figure 1. Human keratinocytes constitutively express IL-15 mRNA. mRNA was extracted from freshly isolated epidermal cells (*EC*) and negatively selected keratinocytes, homogeneous cultured (*c*) keratinocytes (*KC*), HaCaT cells (a spontaneously immortalized keratinocyte line), PBMC (positive control), or H9 cells (negative control) using oligo(dT)-coated magnetic beads. mRNA was then reverse-transcribed by specific priming to single-stranded cDNA, and cDNA was amplified by PCR. PCR products were visualized by ^{32}P-labeled binding of specific internal probes and autoradiography. CD1a-specific primers/probe were used to detect contaminating LC in freshly isolated keratinocyte populations. IL-15- and CD1a-specific signals were adjusted according to expression of the housekeeping gene G3PDH. Representative results from a single experiment are shown.

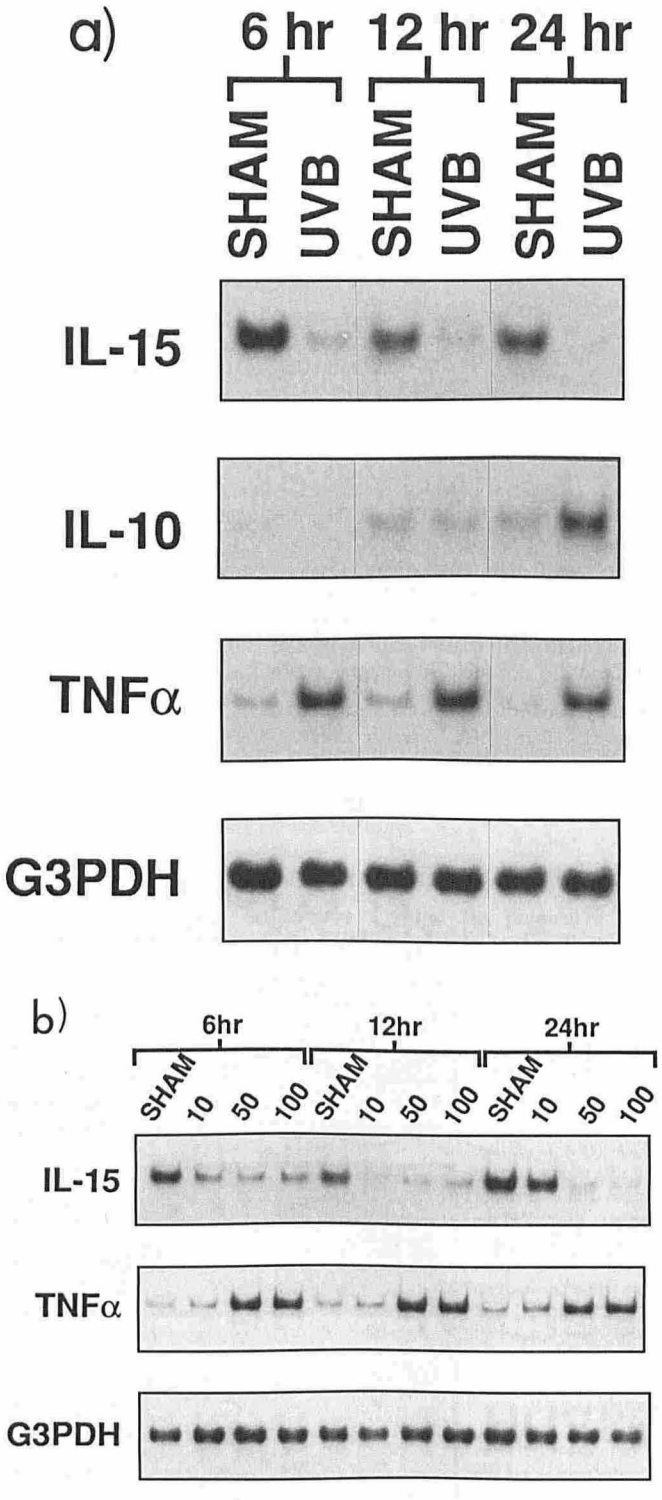

Figure 2. Expression of IL-15 mRNA by cultured keratinocytes is downregulated by UVB radiation *in vitro* in a time- and dose-dependent manner. After media was replaced with phosphate-buffered saline, cultured keratinocytes were either sham-irradiated or irradiated with UVB (100 J/m^2 for *a*; 10, 50, or 100 J/m^2 for *b*). Media was returned to cultures and cells were incubated for an additional 6, 12, or 24 h. At these times, mRNA was extracted from cells and examined for specific gene expression as described in *Materials and Methods*. Primers/probes specific for IL-10 (*a*) and TNF-α (*a*, *b*), cytokines known to be upregulated by UVB radiation, were used as positive controls. Signals were adjusted according to expression of the housekeeping gene G3PDH. Representative results from two separate experiments are shown.

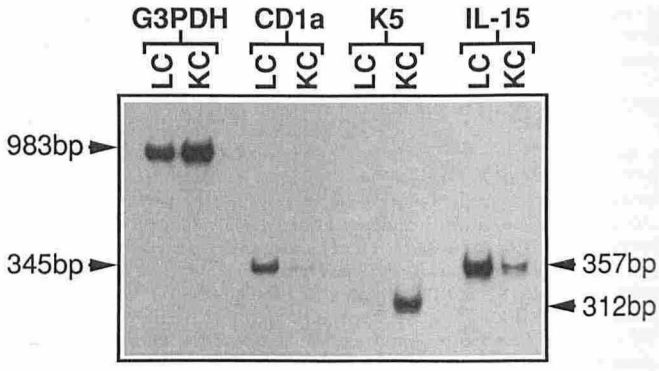

Figure 3. Human LC constitutively express IL-15 mRNA. mRNA was extracted from cells and examined for specific gene expression as described in *Materials and Methods*. CD1a-specific primers/probe were used to detect contaminating LC in selected keratinocyte populations, and K5-specific primers/probe were used to detect contaminating keratinocytes in selected LC populations. Signals were adjusted according to expression of the housekeeping gene G3PDH. Representative results from a single experiment are shown.

tained from suction blister roofs and purified by positive selection, constitutively expressed IL-15 mRNA **(Fig 3)**. To determine the efficiency of our positive selection procedure, we assessed selected cell populations for the presence of keratinocytes by RT-PCR using a K5-specific primer-probe set. In positively selected LC, only faint K5-specific mRNA bands (compared with G3PDH-, CD1a-, and IL-15-specific mRNA) were detected using RT-PCR **(Fig 3)**. Similar to keratinocytes, these findings strongly suggest that epidermal LC also express IL-15 mRNA.

Adult Blood-Derived DC Morphologically, Phenotypically, and Functionally Resemble Freshly Isolated Blood DC and Constitutively Express IL-15 mRNA Prior to mRNA studies, the adult blood-derived DC used in our studies were morphologically, phenotypically, and functionally characterized and are described in detail elsewhere (Blauvelt *et al*, submitted for publication). Briefly, adult blood-derived DC were large in size and exhibited prominent cell surface extensions and veils upon light microscopy and ultrastructural analysis. Of note, no Birbeck granules were detected within these cells. By flow cytometric analysis, adult blood-derived DC were >99% pure. Adult blood-derived DC expressed high levels of cell surface CD1a, major histocompatibility class I and II molecules, and accessory molecules (e.g., CD40, CD50, CD54, CD86), but did not express lineage specific antigens (e.g., CD3, CD14, CD19). **Table I** summarizes the expression of cell surface antigens by adult blood-derived DC. Functionally, adult blood-derived DC served as potent antigen-presenting cells in allogeneic mixed lymphocyte reactions and for recall antigen-stimulated autologous CD4$^+$ T cells. Thus, adult blood-derived DC resembled freshly isolated DC (Schuler, 1991; Steinman, 1991) in all aspects studied.

After detecting IL-15 mRNA in freshly isolated LC, members of the DC family, we used adult blood-derived DC to determine whether IL-15 expression was a general feature of DC populations. Adult blood-derived DC constitutively expressed IL-15 mRNA **(Fig 4)**. Because it is known that Mø produce IL-15, it was important to rule out the possibility that IL-15 mRNA detected in adult blood-derived DC was not derived from contaminating Mø. In purified populations of adult blood-derived DC, CD14$^+$ cells (i.e., Mø) were not detected by mAb staining and flow cytometry (not shown), nor was CD14-specific mRNA detected by RT-PCR **(Fig 4)**. IL-15 protein was not detected in concentrated culture supernatants of adult blood-derived DC 24 h following purification.

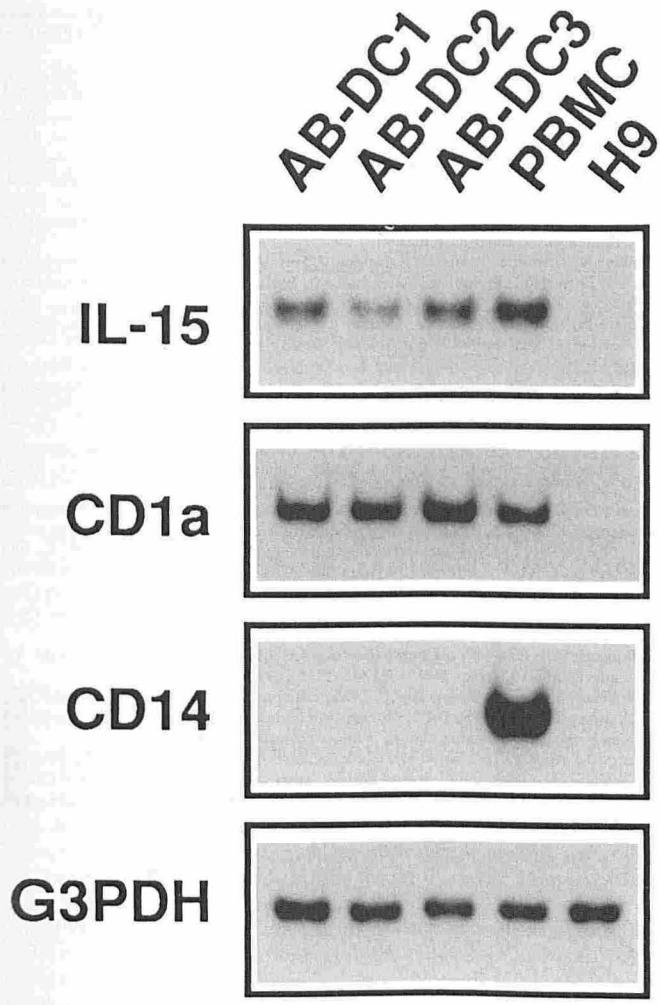

Figure 4. Purified adult blood-derived DC constitutively express IL-15 mRNA. Adult blood-derived DC were propagated from cultures of plastic adherent adult blood PBMC grown in the presence of granulocyte/macrophage-colony stimulating factor and IL-4 for 1 week. Contaminating CD3, CD14, CD16, and $CD19^+$ cells were depleted from cultures by immunomagnetic bead separation. mRNA was extracted from cells and examined for specific gene expression as described in *Materials and Methods*. CD14-specific primers/probe were used to detect contaminating Mø in selected populations of adult blood-derived DC. Signals were adjusted according to expression of the housekeeping gene G3PDH. Results from three separate individuals are shown.

DISCUSSION

IL-15 is a recently described cytokine with IL-2-like functional properties (Armitage *et al*, 1995; Carson *et al*, 1994; Giri *et al*, 1994; Grabstein *et al*, 1994; Kennedy *et al*, 1994; Lin *et al*, 1995; Matthews *et al*, 1995; Wilkinson and Liew, 1995). We have shown that freshly isolated populations of purified human keratinocytes and LC, as well as cultured keratinocytes and blood-derived cultured DC, express IL-15 mRNA. In addition, we found that UVB irradiation of cultured keratinocytes downregulated expression of IL-15 transcripts in a time- and dose-dependent manner, unlike most other keratinocyte-derived cytokines which are upregulated by UVB (Kupper *et al*, 1987; Kock *et al*, 1990; Kirnbauer *et al*, 1991; Kondo *et al*, 1993; Enk *et al*, 1995). We could detect IL-15 protein only in concentrated HaCat cell supernatants, suggesting that there is post-transcriptional regulation of IL-15 secretion.

Our findings contrast markedly to those of Mohamadzadeh *et al*, who were unable to detect IL-15 mRNA in freshly isolated epidermal sheets (Mohamadzadeh *et al*, 1995). The reason(s) for the disparate findings are not easily identifiable. It should be noted that our results are in keeping with preliminary findings by other investigators (Barbulescu K, Hemmerlein-Kraus M, Mohamadzadeh M, Enk A, Knop J, Lohman S: Identification of human keratinocyte-derived IL-15. *J Invest Dermatol* 105:480, 1995, abstr.; Sorel M, Cherel M, Dreno B, Bouyge I, Guilbert J, Dubois S, Minvielle S, Yannick J: Production of interleukin-15 by human keratinocytes. *J Invest Dermatol* 105:463, 1995, abstr.). Our results showing downregulation of keratinocyte IL-15 mRNA expression upon UVB treatment also differ form those by Mohamadzadeh *et al* (1995), who showed upregulation of IL-15 mRNA by UVB. Unfortunately, they did not compare their IL-15 findings with other well-studied epidermally derived cytokines, such as we did with TNF-α and IL-10. Further study is required to investigate these disparate results.

We propose that keratinocyte-derived IL-15 may be important during the generation of skin inflammatory and immune responses by 1) initially attracting T cells to epidermis and by 2) subsequently enhancing the activation of Th1 T cells within epidermis. Also, decreased IL-15 production by keratinocytes may contribute to immunosuppression induced by UVB radiation (Yoshikawa *et al*, 1990; Hersey *et al*, 1983; Kripke, 1994; Rivas and Ullrich, 1994). It would be interesting to determine whether exogenous IL-15 could overcome the induction and activity of UVB-induced suppressor T cells, as has been recently shown for IL-12 (Schmitt *et al*, 1995), another cytokine that promotes type 1 cytokine immune responses. Further study is required to test these hypotheses.

Constitutive and inducible expression of costimulatory and adhesion molecules by LC, as well as other DC, play an important role in antigen-specific activation of T cells (Steinman and Young, 1991; Young *et al*, 1992). Whether cytokines produced by LC and other DC, in addition, contribute to T-cell activation, has been less clear (Romani *et al*, 1990; Steinman, 1991). Determining cytokine production by isolated populations of DC has been confounded by the presence of contaminating cells, because DC numerically represent small sub-populations within skin, blood, and other tissues. Recently, however, Macatonia *et al* (1995) demonstrated that DC produce IL-12, and that this production was important in the development of Th1 T cells. Using purified epidermal LC, isolated by immunomagnetic beads and shown to be relatively free from contaminating keratinocytes, we show in this report that DC are also capable of producing IL-15. In addition, we show that cytokine-generated adult blood-derived DC, devoid of contaminating Mø, constitutively express IL-15 transcripts. Like IL-12, IL-15 produced by LC, and by DC in other tissues, may be important in the activation and development of Th1 T cells.

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