# Identification and Induction of Human Keratinocyte-derived IL-12

**Gabriele Müller, Joachim Saloga, Tieno Germann,* Iris Bellinghausen, Mansour Mohamadzadeh, Jürgen Knop, and Alexander H. Enk**

*Clinical Research Unit, Universitäts-Hautklinik Mainz, and *Institut für Immunologie der Universität Mainz, D-55131 Mainz, Federal Republic of Germany*

## Abstract

**Interleukin 12 is a heterodimeric molecule that serves as a potent co-stimulator enhancing the development of Th1 cells. As one of the classical Th1 cell-mediated responses is contact sensitivity in skin, we wondered whether IL-12 might be produced by epidermal cells and serve as a mediator of this immune response. Using a sensitive, quantitative PCR technique we demonstrate that p35 chain mRNA of IL-12 is produced constitutively by human epidermal cells, whereas p40 chain mRNA can only be detected in epidermis treated with contact allergen, but not epidermis exposed to irritants or tolerogens. Time course studies showed a dramatic induction of IL-12 p40 mRNA 4 h after in vivo allergen treatment reaching peak strength after 6 h. In cell depletion assays we show that epidermal keratinocytes are the major source of this cytokine in the epidermis. This was further supported by analysis of mRNA derived from the human keratinocyte cell line HaCat expressing IL-12 p35 and p40 mRNA upon stimulation. The presence of bioactive IL-12 in supernatants derived from allergen-stimulated epidermal cells was demonstrated by IL-12–specific bioassay. Additional evidence for the functional importance of IL-12 in primary immune reactions in skin was obtained in allogeneic proliferation assays using human haptenated epidermal cells containing Langerhans cells as APC and allogeneic CD4⁺ T cells as responders. Anti–IL-12 mAb inhibited the proliferation of T cells by ~ 50%. In aggregate our data demonstrate that nonlymphoid keratinocytes are capable of producing functional IL-12 and provide evidence for the functional significance of IL-12 in primary immune responses in skin. (*J. Clin. Invest.* 1994. 94:1799–1805.) Key words: co-stimulation • Th1 differentiation • contact allergy • cytokine pattern**

## Introduction

Contact sensitivity has served as a classical model for Th1 cell-mediated primary immune responses in skin for a long time. Since the early studies by Landsteiner, Chase, and Macher (1–3) elucidated that contact allergy was a T cell–mediated response where the allergen had to be moved to the regional lymph node to initiate sensitization, a lot of knowledge has been accumulated about the cells and the molecules involved in the sensitization process. The current concept for the sensitization process is that haptens that serve as allergens when applied to skin bind to peptides attached to MHC molecules on epidermal Langerhans cells (LC)[1] as the APC of the epidermis (4). These cells belong to the dendritic cell system and are capable of inducing primary immune responses (5, 6). Aiba and Katz (7) demonstrated that after contact with allergens LC become functionally activated, upregulate their MHC class II molecules and become more potent accessory cells. Kripke et al. (8) demonstrated that these LC migrate to regional lymph nodes probably to activate naive T cells.

Our own group has recently defined the cytokines involved in the early induction phase of contact sensitivity that help LC to become more potent APC. We defined a cytokine profile specific for allergens that was not detected when irritants or tolerogens were applied to the skin (9). Furthermore we showed that LC-derived IL-1 $\beta$ not only was the first cytokine to be induced after hapten application but also was essential for the induction of a sensitization, as injection of an anti–IL-1$\beta$ mAb before application of allergen completely prevented epidermal sensitization (10). Since Mosmann and co-workers (11, 12) defined the existence of two distinct T helper cell subsets it is known that T lymphocytes which are required for both cell-mediated immune responses or the production of antibody by B lymphocytes, are composed of two distinct subsets—T helper 1 cells producing IL-2 and IFN-$\gamma$ execute cell-mediated immune reactions, whereas Th2 cells producing IL-4, IL-5, IL-6, and IL-10 assist in antibody production for humoral immunity. The mechanisms, however, by which a particular T cell lineage is steered down the path towards a Th1 or Th2 fate have remained unclear.

Lately, the novel cytokine IL-12 (originally called "natural killer cell stimulatory factor" or "cytotoxic lymphocyte maturation factor") has evolved as a major player in the regulation of this homeostasis (13, 14). IL-12 is a heterodimeric molecule composed of two covalently linked chains, p40 and p35 (15, 16). The two chains are linked to form a biologically active 70-kD dimer, whereas no IL-12–like biological activity is mediated by either recombinant chain separately (17). Whereas the p35 chain of IL-12 is expressed by numerous, including nonlymphoid, tissues, expression of p40 was thought to be restricted to the lymphoid cell system (16, 18–19). IL-12 was originally identified as a product of B lymphoblastoid cell lines enhancing specific cytolytic T cell responses and inducing NK cell cytotoxic activity (13, 14). In the meantime IL-12 was shown to be

Address correspondence to Alexander H. Enk, M.D., Universitäts-Hautklinik Mainz, Langenbeckstr. 1, D-55131 Mainz, Federal Republic of Germany.

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1. *Abbreviations used in this paper:* DNTB, dinitrothiocyanobenzene; EC, epidermal cell; LC, Langerhans cells; PDB, phosho 12,13-dibutyrate; TNBS, trinitrobenzenesulfonate; TNCB, trinitrochlorobenzene.

produced by monocytes and B cells triggering the production of IFN-$\gamma$ by NK and T cells and thereby helping to select Th1-type immune responses and initiating cell-mediated immunity (20–25). Additionally IL-12 has been shown to augment proliferation of certain T lymphocytes (26, 27) and to have antitumoral activities in mice (28).

Goal of this study was to investigate whether skin cells are capable of producing IL-12 mRNA and protein. We demonstrate herein that nonlymphoid cells like human keratinocytes release IL-12 after stimulation with allergen and that IL-12 seems to be involved in T cell responses induced by human LC. The data demonstrate that IL-12 might also have a role in directing contact sensitivity reactions in skin.

## Methods

*Cells.* Epidermal cell (EC) suspensions were prepared from either normal human foreskin, or plastic surgery tissue, or from punch biopsies from normal healthy volunteers by treatment with 0.5% Dispase II (Boehringer Mannheim, Mannheim, FRG) at 4°C overnight or at 37°C for 1 h, followed by 0.5% Trypsin (United States Biochemical Corp. Bad Homburg, FRG) for 20 min. HaCat cells (kindly provided by Dr. N. Fusenig, Deutsches Krebsforschungszentrum Heidelberg, Heidelberg, FRG) were kept in culture using standard conditions.

*Chemical treatment.* Healthy normal human volunteers were treated epicutaneously with 3% trinitrochlorobenzene (TNCB, a contact allergen) (Polysciences Inc., Warrington, PA), or 2% dinitrothiocyanobenzene (DNTB, a tolerogen) (Eastman Kodak Co., Rochester, NY), or 20% sodium lauryl sulfate (SLS, an irritant) (Sigma Chemical Co., St. Louis, MO) for the time indicated. Afterwards skin samples were obtained by 6-mm punch biopsies. EC suspensions were prepared as described above and RNA was extracted by RNAzol B (Paesel und Lorei, Frankfurt, FRG) following the instructions of the manufacturer. For in vitro stimulation EC suspensions were prepared as described and haptenized with 0.1 M trinitrobenzenesulfonate (TNBS, a water soluble analog of TNCB) (Eastman Kodak Co.) as described (29) or treated with 0.2% SLS for 1 h and then cultured for 24 h. Afterwards supernatants were harvested and concentrated 25× by centrifuge concentrators (Amicon, Palo Alto, CA). For HaCat cells stimulation was performed with phosho 12,13-dibutyrate (PDB; Sigma Chemical Co.) at 25 ng/ml for 24 h, or with rmIL-1$\beta$ or TNF-$\alpha$ (R + D Systems, Inc., Minneapolis, MN) (50 ng/ml) or TNBS for 6 h. Each experiment was performed at least in triplicate.

*Cell depletion assays.* EC suspensions were prepared as described above and cells were incubated with mAb YE2/36HLK (for HLA-DR; American Type Culture Collection, Gaithersburg, MD), anti-CD4 (Becton Dickinson, Mountanview CA), and anti-CD8 (Becton Dickinson) for 30 min at 4°C. After washing low-tox-rabbit complement (Cedarlane, Ontario, Canada) was added for 30 min at 37°C. After washing aliquots were stained for HLA-DR, CD4, or CD8 again and analysed by FACScan. The remaining cells were subjected to RNA extraction by RNAzol B. Cell depletion was controlled by PCR using primers for CD-3$\epsilon$, CD18 (macrophages, Langerhans cells), and HLA-DR (macrophages, Langerhans-cells, B-cells), as well as tyrosinase (melanocytes) and tryptase (mast cells).

*PCR and liquid hybridization.* PCR and liquid hybridization were performed as described previously (9). Briefly, total cellular RNA was extracted by RNAzol B following the instructions of the manufacturer. Extraction was followed by spectrophotometric quantitation and 25 cycles RT-PCR as described, using the Perkin Elmer RT-PCR kit (Perkin Elmer, Überlingen, FRG) and specific primers. Primer sequences were as follows:
p40 5′TCTAAGCGATTCGCTCCTGC,
p40 3′AAGCTGCTGGTGTAGTTTTG;
p35 5′TGTCCCTGCAGTGCC,
p35 3′TAGGATCCATCAGAAGG,
p40 int 5′GCCTGCCCAGCTGCTCAGGAGAGTC,
p35 int 5′CAAAACATAAAACCAGCACAGTGGACG,
CD18 5′ GACTCCATTCGCTGCGACAC,
CD18 3′ CCTTCGTGGACAAGACCGTG,
CD18 int 5′ CTTGATGACCTCAGGAATGTCAAGAAGCTAG,
HLA-DR 5′ GTGCTCACGAACAGCCCTCTG,
HLA-DR 3′ CTCTGGGAGAGGGCTTGGAGC,
HLA-DR int GAGGACGTTTACTACTGCAGGGTGCAC,
CD-3 5′ GATATATCTGTGTAATCCGAC,
CD-3 3′ GGTATCTTCACGATCTCGAAG,
CD-3 int CATGAGACCGCAGGGAGAGATGCTC,
tyr 5′ ATCGTATGCTACAGGGTA,
tyr 3′ ATGGTCCTGGGGAAATGAC,
tyr int CATGTGTGTACGTTAAACCTGA,
tryp 5′ AGTTGATGATGCTGGCCT,
tryp 3′ GGTCCCTAACGTCCAGT,
tryp int GGCCAATGTACCTGGGTAACCTG.
The linear range of signal strength for each cytokine and control mRNA was determined by performing titrations for RNA, primer concentrations, and cycle number. Each individual signal can therefore be compared only with its own baseline. A liquid hybridization technique was used for quantitation where a specific internal $^{32}$P-endlabeled probe added in excess was hybridized to a defined amount of PCR product. Hybridization products were resolved by 4% PAGE, dried, and detected by autoradiography.

*IL-12 bioassay.* Bioactive IL-12 protein was measured in a modified capture bioassay as described elsewhere (30). Briefly, the anti–human IL-12 mAb 2-4A1 (kindly provided by Dr. Maurice Gately, Hofmann-LaRoche Inc., Nutley, NJ) was absorbed (2,5 $\mu$g/ml, 100 $\mu$l/well, in sodium carbonate buffer, pH 9.6) overnight at 4°C to sterile 96-well ELISA plates (Maxisorp, Nunc-Immunoplate, Nunc GmbH, Wiesbaden, Germany). The plates were shook out and blocked with 0.5% BSA in PBS (200 $\mu$l, 1 h, room temperature). After three washes with distilled water, dilutions of rhIL-12 standard (kindly provided by Dr. Maurice Gately, Hofmann-LaRoche Inc.) or supernatant fluids to be tested were added (100 $\mu$l/well, 3 h, room temperature). The plates were washed five times again and human PHA stimulated lymphoblasts (PBMC stimulated with PHA for 3 d and rhIL-2 for 1 d) were added ($2 \times 10^4$/well, 100 $\mu$l/well). After 48 h incubation (37°C, 5% $CO_2$), proliferation was measured by incorporation of [$^3$H]Thymidine (1 $\mu$Ci/well for 6 h). A standard curve was plotted and IL-12 contents of samples was calculated. In some experiments samples were preincubated with rat anti–human anti–IL-12 mAb 2-4A1 at 10 $\mu$g/ml (kindly provided by Dr. M. K. Gately).

*Allogeneic proliferation assay.* EC suspensions were prepared as described above and either stimulated with TNBS or left unstimulated. Allogeneic CD4$^+$ T cells were prepared from blood of healthy normal volunteers by Ficoll density centrifugation. Afterwards interface cells were stained with anti-CD4 mAb (Becton Dickinson) and positively enriched by anti–mouse IgG-coated paramagnetic beads (Dynal, Hamburg, FRG). As assessed by inverted phase immunofluorescence microscopy, cells were > 95% pure CD4$^+$. Afterwards T cells were cocultured with EC for 4 d in the presence or absence of anti–IL-12 mAb or control mAb. [$^3$H]Thymidine was added at 1 $\mu$Ci for the last 12 h.

## Results

*Induction of IL-12 mRNA in human epidermis.* 3% TNCB was applied to skin of human healthy volunteers after informed consent. Skin punch biopsies were obtained after various time points (0–48 h) after application of the allergen. Afterwards EC suspensions were prepared as described and total epidermal RNA was extracted by RNAzol B immediately. RNA was quantitated by spectrometric analysis and subjected to 25 cycles of RT-PCR followed by liquid hybridization. Signals for p35, p40, and $\beta$-actin control were detected by autoradiography (Fig. 1).

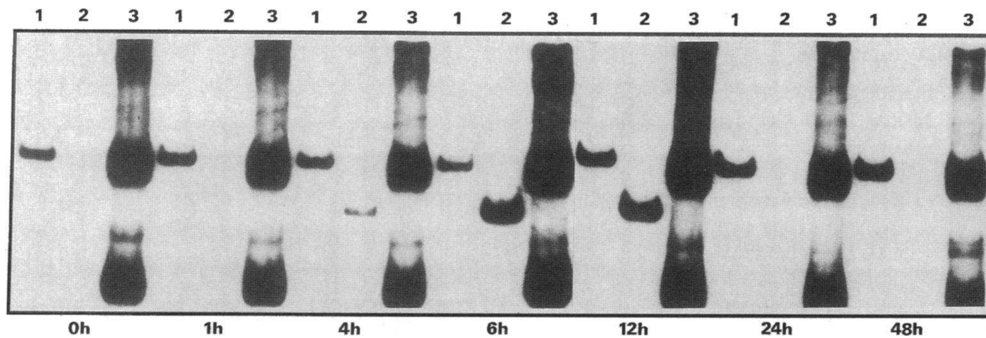


*Figure 1.* Time course of IL-12 mRNA upregulation after hapten treatment. Healthy normal volunteers were treated with 3% TNCB and punch biopsies were obtained after time points indicated. Epidermal RNA was extracted as described and subjected to 25 cycles RT-PCR followed by liquid hybridization. Lane *1*, p35 (471bp); lane *2*, p40 (383bp), and lane *3*, $\beta$-actin (472bp). All experiments were performed in triplicate.

Whereas signals for p35 chains were expressed constitutively in human epidermis, p40 chain signals were only found after treatment with allergen. Time course studies showed that p40 was found within 4 h of hapten application reaching peak strength after 6 h and being downregulated after 24 h. $\beta$-actin control signals remained unchanged during treatment with allergen. These data indicate that epidermal cells after stimulation are capable of producing IL-12 p35 and p40 chain mRNA's.

*Induction of IL-12 p40 chain is allergen-specific.* 3% TNCB (an allergen), 2% DNTB (a tolerogen), 20% SLS (an irritant), or nothing was applied to skin of healthy normal volunteers. 6 h later punch biopsies were obtained and EC suspensions were prepared as described. After RNA extraction RT-PCR was performed with 25 cycles and signals for p35, p40, and $\beta$-actin control were sought by liquid hybridization. Whereas the p35 chain of IL-12 was again found to be expressed constitutively and did not change during activation, p40 chain mRNA signals could only be detected after stimulation with contact allergen, not after stimulation with tolerogen, or irritant (Fig. 2). These data indicate that IL-12 p40 chain is selectively induced by contact allergen in our test system.

*Cell depletion assays.* EC suspensions were prepared as described in Methods and stimulated with TNBS (a water-soluble analogue of TNCB) as described. Afterwards EC suspensions were treated with either anti-HLA-DR mAb or CD4/CD8 mAb or an unrelated control mAb followed by treatment with low tox complement to deplete LC or T cells, respectively. Afterwards aliquots were stained again with the respective mAb and subjected to FACScan analysis to control for cell depletion. The remaining cells were lysed immediately by RNAzol B for RNA extraction and subjected to PCR analysis for expression of p35, p40, and $\beta$-actin (Fig. 3). To exclude the presence of contaminating (phagocytic) cells, depletion was controlled by PCR (data not shown) using primers specific for CD-3$\epsilon$, CD-18 (macrophages, Langerhans cells), and HLA-DR (macrophages, Langerhans cells, B-cells), as well as tyrosinase (tyr—melanocytes) and tryptase (tryp—mast cells). As human epidermis only contains keratinocytes, LC, some T cells, very few melanocytes, and under inflammatory conditions occasionally some macrophages, the purity of our keratinocytes was 100% as controlled for by PCR. None of the lytic cocktails could deplete the cells responsible for the signals for p35 or p40 indicating that human keratinocytes are the major sources of this cytokine in skin.

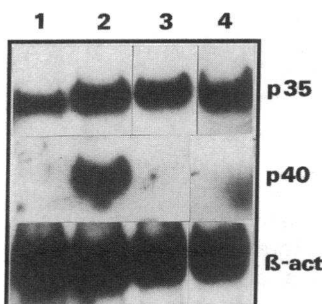


*Figure 2.* Induction of p40 mRNA in human keratinocytes is allergen-specific. Nothing (lane *1*), 3% TNCB (lane *2*), 20% SLS (lane *3*), or 2% DNTB (lane *4*) were applied to skin of healthy volunteers. Punch biopsies were obtained 6 h later and epidermal RNA was extracted as described. Signals for p35, p40, and $\beta$-actin were sought by RT-PCR and liquid hybridization.

To further support this finding, the immortalized human keratinocyte cell line HaCat was cultured as described and treated with PDB (a phorbolester). After RNA extraction and PCR analysis, p35, p40, and $\beta$-actin signals were sought. Again p35 chain mRNA signals were expressed constitutively, whereas only PDB-treated HaCat cells expressed mRNA signals for p40 (Fig. 4). $\beta$-actin controls remained unaffected. Our data indicate that human keratinocytes are capable of producing IL-12 mRNA. It should be noted however that stimulation of HaCat cells with the soluble allergen TNBS alone did not result in induction of p40 chain mRNA. p40 chain could be induced in those cells by addition of IL-1$\beta$ at 50 ng/ml (but not TNF-$\alpha$) for 6 h (Fig. 5) as has been shown for most other allergen-specific cytokines before (10). As IL-1$\beta$ is usually derived from LC during the early induction phase of contact sensitivity this suggests that LC are essential for induction of IL-12 under physiological circumstances.

*Identification of keratinocyte-derived IL-12 protein.* EC suspensions were prepared as described and either left untreated or stimulated with 0.2% SLS, or 1 mM TNBS as described. After 24 h of culture, supernatants were harvested and concentrated by centrifuge concentrators about 25×. These concentrates were then analyzed by a sensitive capture bioassay for IL-12 (Fig. 6). Only concentrated supernatant from allergen-stimulated EC suspensions, not control-treated concentrates contained measurable amounts of IL-12 protein. Similar results were obtained from concentrated supernatants of HaCat cells. Whereas PDB-stimulated HaCat cell supernatant showed production of IL-12 protein, nonstimulated cells did not show IL-12 protein production. Preincubation of samples with neutralizing anti-IL-12 mAb (2-4A1 rat anti-human at 10 $\mu$g/ml) resulted in complete disappearance of IL-12 protein signals (data not shown). Our data indicate that human keratinocytes are capable of producing functionally active IL-12 protein after allergen stimulation.

*Anti-IL-12 mAb inhibits proliferation of allogeneic T cells induced by haptenated LC.* EC suspensions were prepared as described and either left untreated or haptenated with TNBS. Blood from healthy human volunteers was enriched for $CD4^+$ T

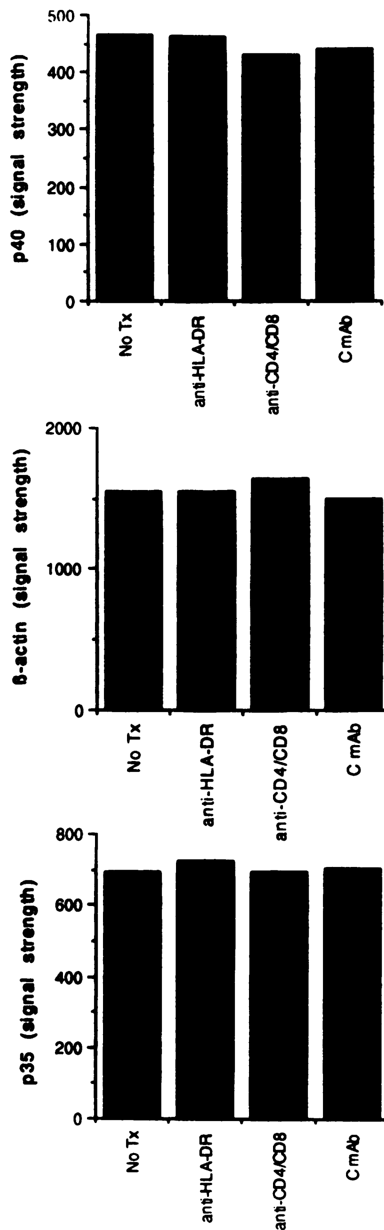


*Figure 3.* Epidermal keratinocytes are the source of IL-12 mRNA in the epidermis. EC suspensions from human foreskin were prepared and haptenated as described in the methods section. Afterwards cells were treated with the mAb indicated and exposed to low tox complement. Cell depletion was assessed by FACScan. *No Tx,* TNBS, but no mAb or complement treatment; *anti-HLA-DR,* TNBS plus anti-HLA-DR plus C′; *anti-CD-4/CD-8,* TNBS plus anti CD-4 and CD-8 plus C′; *C mAb,* TNBS plus nonspecific control mAb plus C′. After treatment, cells were subjected to PCR analysis for p35, p40, and $\beta$-actin, as well as control primers CD-18, CD-3$\epsilon$, and HLA-DR (not shown). PCR products were hybridized and resolved by PAGE, and dried. After autoradiography, gels were scanned and analyzed by optometric scanner. Y-achses show integrated signal strength in arbitrary units. Whereas signal strength for p35 and $\beta$-actin remained unchanged compared to untreated control skin, p40 chain was undetectable before treatment with contact allergen (not shown). No signals were detected in HLA-DR depleted fractions for CD-18 and HLA-DR or for CD4/8-depleted fractions for CD-3$\epsilon$ (not shown).

cells by sequential treatment with Ficoll gradient centrifugation followed by positive enrichment with paramagnetic beads as described in the methods section. Cells were > 95% pure CD4$^+$ T cells. Afterwards cells were cocultured with haptenated or

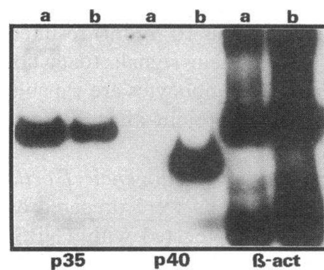


*Figure 4.* HaCat cells express p40 chain mRNA after stimulation. HaCat cells were kindly provided by Dr. N. Fusenig and stimulated with either nothing (*a*) or 25 $\mu$g/ml PDB (*b*) for 12 h. After RNA extraction, signals for p35, p40, and $\beta$-actin were sought by PCR and liquid hybridizyation.

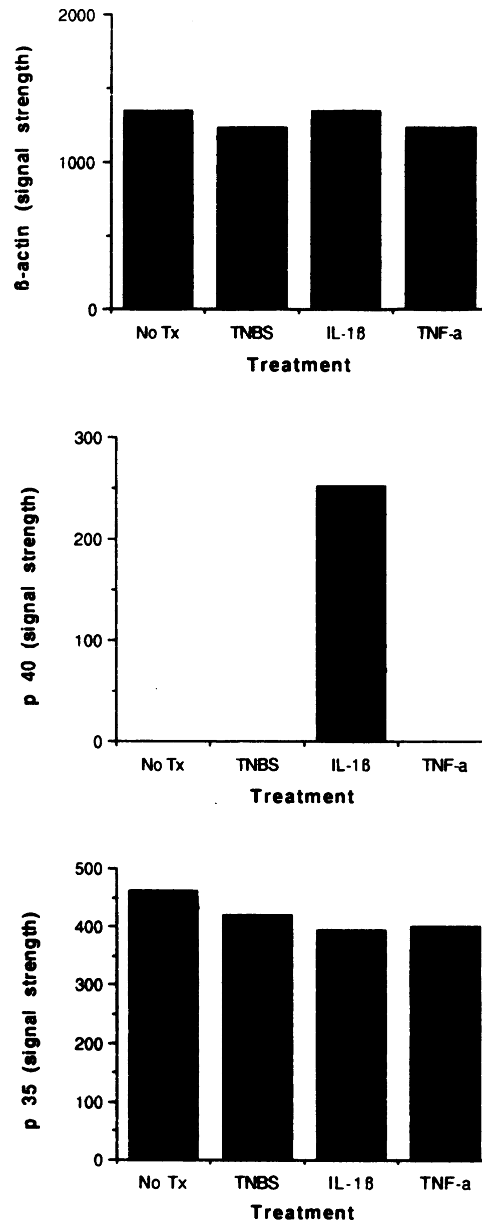


*Figure 5.* HaCat cells do not produce IL-12 after allergen challenge in vitro. HaCat cells were stimulated with either nothing (*No Tx*), TNBS, IL-1$\beta$ (50 ng/ml), or TNF-$\alpha$ (50 ng/ml) for 6 h. After RNA extraction signals for p35, p40, and $\beta$-actin were sought by PCR and afterwards analyzed by densitometric scanning. Three experiments were performed with identical results.

non-haptenated EC containing ~ 3% LC as APC for 4 d in the presence or absence of 25 $\mu$g/ml anti–IL-12 mAb. [$^3$H]-Thymidine at 1 $\mu$Ci/well was added for the last 12 h of culture to measure proliferation. Addition of anti–IL-12 mAb to cocultures of T cells and haptenated EC inhibited proliferation by ~ 50%, whereas control mAb or treatment of non-haptenated EC only showed minimal inhibition (Fig. 7). Our data indicate that IL-12 is of functional importance for the induction of human T cell proliferation by epidermal LC in the induction of primary immune responses and that the enhanced proliferation of allogeneic T cells towards haptenated EC may be due to release of IL-12 by keratinocytes after hapten application.

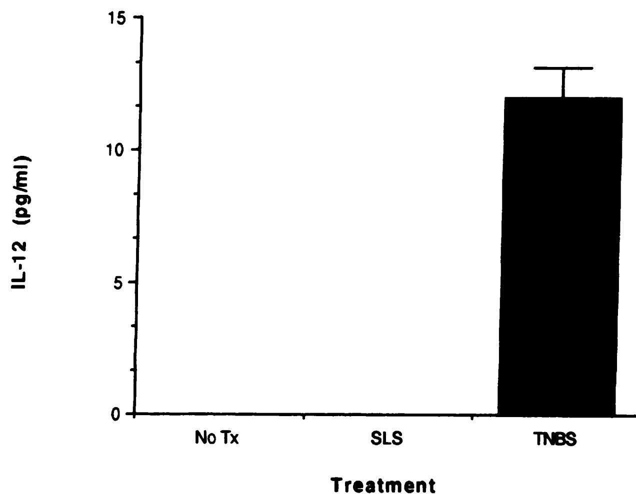


*Figure 6.* Epidermal cells are capable of producing IL-12 protein. Primary EC cultures were obtained from plastic surgery as described above. After trypsinization EC cultures were stimulated with either 0.2% SLS or TNBS, or nothing as described. Supernatants were harvested 24 h later and concentrated 25×, and analyzed by capture bioassay as described. Cell viability was not affected as assessed by propidiumiodine staining. Assay background was ~ 15,000 cpm with values for TNBS ranging from 40,000–45,000 cpm.

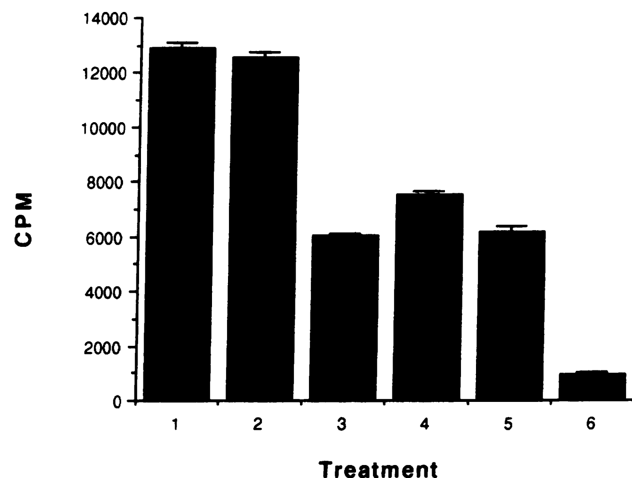


*Figure 7.* Anti–IL-12 mAb inhibits proliferation of allogeneic T cells induced by haptenated LC. EC cultures were obtained from plastic surgery as described. $1 \times 10^6$ EC were then cocultured with $2 \times 10^5$ $CD4^+$ T cells for 4 d with 1 $\mu$Ci/well [$^3$H]Thymidine added for the last 12–16 h. Anti–IL-12 or control mAb (C mAb) were added at 30 $\mu$g/ml final concentration on day 0. *1,* haptenated EC left untreated; *2,* haptenated EC plus C mAb; *3,* haptenated EC plus anti–IL-12 mAb; *4,* non-haptenated EC untreated; *5,* non-haptenated EC plus anti–IL-12 mAb; *6,* T cell background proliferation. Background proliferation for EC alone was about 1,000 cpm. Similar results were obtained in three consecutive experiments.

## Discussion

Interleukin 12 has been characterized as a key mediator of Th1 cell development by different investigators. In an elegant and very recent study, Hsieh and colleagues (24) demonstrated in a transgenic OVA-specific TCR-mouse model that macrophages activated with Listeria monocytogenes to produce IL-12–induced differentiation of Th1 cells from uncommited, naive T cells. However, using the same transgenic mouse model, the same investigators provided evidence that macrophages with antigen by themselves were incapable of inducing the proliferation of naive T cells (31). In contrast, dendritic cells as APC together with Ag induced T cell proliferation, but failed to induce T cell differentiation in the absence of added cytokines (31). Remarkably, addition of IL-12 was capable of twisting DC-induced naive T cell development to a Th1-phenotype. Addition of IL-12 was necessary for the induction process, as DC themselves did not seem to produce IL-12 (31). Thus for promoting Th1 development DC's and a bystander cell producing IL-12 are required.

Our own laboratory has recently been involved in characterizing the early molecular events in the induction phase of contact sensitivity (9). In prior studies we showed that the early induction phase of this primary immune reaction in skin is characterized by a distinct and specific pattern of epidermal cytokines that only occurs after application of allergen, not tolerogen or irritant (9). The earliest cytokine to be induced by allergen in this cytokine cascade is LC-derived IL-1$\beta$. The signal for this cytokine is found to be expressed within 15 min after application of allergen. Further functional studies demonstrated that IL-1$\beta$ injection into skin mimicks the changes in LC morphology and function usually caused by allergen, as well as inducing a cytokine pattern identical to the one caused by epicutaneous application of allergen (10). In addition, injection of anti–IL-1$\beta$–specific mAb before application of allergen completely prevented epicutaneous sensitization (10). Taken together our data indicate that LC-derived IL-1$\beta$ seems to be the essential cytokine in contact sensitivity.

Further studies demonstrated that as a second important cytokine IL-10 seemed to be involved in controlling the inflammatory process of allergic contact dermatitis (32). Whereas IL-1$\beta$ was produced within 15 min of hapten application, IL-10 was produced by epidermal keratinocytes late in the induction phase of contact sensitivity with signals reaching peak strength 12 h after hapten painting (32). Functional studies showed that IL-10 not only inhibited IFN-$\gamma$ and IL-2 production in LC-induced proliferation assays, but also inhibited LC accessory cell function by preventing the induction of membrane-bound costimulatory molecules on LC (33). Treatment of LC with IL-10 therefore converted those cells from potent immunostimulatory to tolerizing APC (33).

Since IL-1$\beta$ and especially IL-10 are generally considered as Th2-associated cytokines we wondered whether other mediators supporting Th1 immune reactions such as IL-12 might also be produced by EC. Here we demonstrate that epidermal cells are indeed capable of producing IL-12. In cell depletion assays as well as by using immortalized keratinocyte cell lines, we show that keratinocytes are the major sources of this cytokine in the epidermis. We demonstrate by using an IL-12 capture assay that keratinocytes are able to release bioactive IL-12 protein after stimulation with contact allergen. We furthermore provide experimental data that IL-1 $\beta$, but not TNF-$\alpha$ can substitute for allergen in LC-deprived HaCat cultures in terms of IL-12 induction, supporting the already documented essential role for LC and LC-derived IL-1$\beta$ in the induction of primary immune responses in skin (10). In a LC-induced proliferation assay we demonstrate that IL-12 might also be involved in the induction of T cell proliferation by LC, as a monoclonal anti–

IL-12 mAb inhibited T cell proliferation by 50% in an allogeneic proliferation system. In aggregate our data indicate that functional IL-12 is produced by epidermal cells. We furthermore provide evidence that this cytokine might be involved in balancing the intricate homeostasis of the early induction phase of contact sensitivity by helping direct T cell responses induced by LC.

The finding that IL-12 is produced by human keratinocytes after stimulation with allergen is especially interesting with regard to the data demonstrating that IL-12 seems to be a powerful mediator for a Th1 type differentiation of T helper cells in vitro and in vivo. The influence of IL-12 on T cell development is believed to be exerted on the level of the T cell directly (24, 25). However it is believed that LC/T cell interactions take place in the regional lymph node (8). At first glance it appears to be a contradiction that IL-12 produced in skin can influence an immune response taking place in the lymph node. There are several possible explanations. The easiest possibility is that keratinocyte-derived IL-12 is being released and washed away by lymph fluids to the draining regional lymph node during the induction phase of contact sensitivity, where it could directly influence the priming of naive T cells by, for example, LC. Especially for a very potent cytokine such as IL-12 that has been shown to be effective in picogram amounts this mechanism is very feasible.

Another possibility is that there might be an (indirect) effect of IL-12 on APC such as LC. In fact Seder et al. (34) showed recently that IL-12 in an APC-independent system using anti-CD3 mAb as a stimulus with naive $CD4^+$ T cells as responders enhanced Th1 development—an effect that was blocked by simultaneous addition of anti-IFN$\gamma$ mAb. However, in an APC-dependent assay using splenic accessory cells and naive $CD4^+$ T cells as responders, anti-IFN$\gamma$ mAb did not significantly inhibit IL-12 triggered Th1 differentiation (34). These data suggest that either IL-12 together with IFN-$\gamma$ or IL-12 and an APC-derived additional signal induce Th1 differentiation. One could speculate that under circumstances where IL-12 is produced in the presence of an APC, this APC is (indirectly) modified by IL-12 to a phenotype promoting Th1 differentiation. In skin this might mean that keratinocyte-derived IL-12 ''primes'' LC towards a Th1-inducing APC.

Additionally although most immunodermatologists believe that the priming of T cells by LC takes place in the regional lymph node, nobody knows whether this is the exclusive mechanism. It is known for some time that even in normal epidermis there are small amounts of $CD4^+$ T cells that would be a possible target for IL-12 action. Finally, during inflammatory reactions of the skin numerous T cells are attracted to the epidermis where they might be influenced by keratinocyte-derived IL-12 to differentiate towards a Th1 phenotype.

Of further interest is also the seemingly intricate balance of cytokine production in the epidermis. Apart from the essential inducing function of LC-derived IL-1$\beta$, production of IL-12 during earlier phases of primary immune responses might be controlled by the release of IL-10 during later phases, as has been demonstrated in other systems. This effect could subserve the function of a balancing mechanism avoiding dominance of a particular type of immune reaction that might be harmful to the organism. Furthermore, it cannot be ruled out that production of IL-12 p40 chain (that was shown to inhibit IL-12 activity) (35) may be so dominant at later phases of the immune reaction as to suppress the biologic effects of IL-12 heterodimer. Further studies are needed to exactly define the induction and mechanisms of directing primary immune responses in skin.

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