Effects of therapies on cytokine patterns in psoriasis
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In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin

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Abstract
Keratinocytes are able to contribute to cutaneous immune responses by expression of cytokines. The heterodimer IL-23 is a newly defined IFN-γ-inducing cytokine, composed of a unique p19 subunit and a p40 subunit shared with IL-12. The IL-12/IL-23 p40 subunit is known to be expressed by keratinocytes. We investigated whether keratinocytes can express the IL-23 p19 subunit as well and are able to form the heterodimer IL-23, using skin biopsies from normal individuals and psoriatic patients. Cultured keratinocytes from normal and lesional psoriatic skin were found to express constitutively mRNA for both subunits of IL-23. In cell lysates and supernatants from stimulated keratinocytes the heterodimer IL-23 protein could be detected by immunoblotting and ELISA. Cytospin preparations of freshly isolated keratinocytes showed abundant expression of p19 and p40 subunits of IL-23, whereas IL-12 p70 was present in only few cells. Immunostaining of skin sections showed the expression of both subunits of IL-23 in the epidermis and dermis, being significantly higher in psoriatic lesional skin as compared to normal and psoriatic nonlesional skin. All these findings together prove that keratinocytes are capable of producing the p19 subunit and to form the heterodimer of IL-23. Double staining of cytospin preparations of epidermal and dermal cell suspensions revealed that IL-23 p19 subunit is also expressed by epidermal Langerhans cells, dermal dendritic cells and macrophages. Psoriasis is a chronic inflammatory skin disease mediated by IFN-γ expressing type 1 memory T cells. As IL-23 is important to activate memory T cells to produce IFN-γ, its augmented expression by keratinocytes and dermal antigen presenting cells may contribute to the chronic inflammation present in psoriatic skin.
**Introduction**

The skin is the main organ connecting the body to the potentially harmful environment. To achieve homeostasis and to defend the body against microbial invaders, it does not only serve as a physical barrier, but is also equipped with numerous immunological functions collectively called the skin immune system. In addition to the well-known immunocompetent cells, which traffic into and out of the skin (e.g. dendritic cells and T cells), keratinocytes as the main constituent of the epidermis also have an important contribution to the development of optimal cutaneous immune responses. Keratinocytes can, constitutively or after stimulation, produce many cytokines as reviewed elsewhere. Physical, chemical and pathogenic triggers induce keratinocytes to secrete proinflammatory cytokines, such as IL-1β and TNF-α, resulting in autoactivation of these cells to produce other inflammatory cytokines and chemokines. The proinflammatory cytokines may also activate other cells in the skin, such as dendritic cells, which start to mature and migrate to lymph nodes inducing a specific immune response.

Expression of type 1 cytokines is crucial in the defense against intracellular microorganisms and in the development of delayed type hypersensitivity reactions. The main representative of type 1 cytokines is IFN-γ, which is produced by type 1 T cells. The expression of IFN-γ is strictly regulated by other soluble factors. IL-12 was the first described inducer of IFN-γ expression. In less than a decade, several other cytokines were found to contribute to this regulation as well, namely IL-15, IL-18, IL-21, IL-23, and IL-27, which act separately or in combination to induce type 1 cytokine responses. In this group of IFN-γ-inducing cytokines, IL-23 and IL-27 exhibit structural and functional similarities to IL-12. However, they differ at the timing of their contribution to the type 1 responses. IL-27 is known to be important in the early expansion of naïve T cells and IFN-γ expression by these cells, while IL-23 can only act on memory T cells to induce IFN-γ expression, suggesting that IL-23 might be important in the maintenance of immune responses. The heterodimer IL-23 is formed by the combination of a p19 and a p40 subunit and possesses the most structural similarity to IL-12 as compared to the other IFN-γ-inducers. The p40 subunit of IL-23 is identical to the IL-12 p40 subunit, whereas the unique IL-23 p19 molecule has resemblance to the specific IL-12 p35 subunit. In addition to their structural similarity, IL-12 and IL-23 conduct their actions through specific receptors that share a common IL-12β1 chain. The two subunits of the heterodimer IL-12 can be produced by different cell types, including human keratinocytes, allowing these cells to favor type 1 T cell responses. However, it is as yet not known whether keratinocytes can express the IL-23 p19 subunit as well.
It has been suggested that the expression of IL-12 might play an important role in certain inflammatory skin diseases\(^9\,10\). One of those, psoriasis vulgaris, is a chronic inflammatory, type 1 cytokine-related, cutaneous disease, which is characterized by infiltrates of activated memory T cells that have a high IFN-γ expression\(^11\). An enhanced expression of IL-12 mRNA and protein was found in psoriatic lesional skin, as compared to nonlesional and normal skin, and a causative relationship to the high IFN-γ expression in lesional skin was suggested\(^12\). It has been demonstrated that memory T cells proliferate when they are stimulated with IL-23, but not with IL-12\(^7\). Indeed, part of the T cell population within psoriatic lesional skin was found to proliferate\(^13\). Having in mind that IL-23 preferentially activates memory T cells and the fact that activated memory T cells are abundantly present in psoriasis lesions, we consider that IL-23 rather than IL-12 would play a major role in the sustained inflammatory reaction taking place in the psoriasis plaques. This consideration prompted us to investigate the expression of IL-23 p19 in normal and lesional psoriatic skin. Special attention was paid to keratinocytes, because mutual interaction and activation of keratinocytes and T cells is thought to be responsible for the perpetuation of the inflammatory process in the psoriatic lesions\(^14\).

In this study, we show that normal human keratinocytes constitutively express mRNA for the two subunits of IL-23 and demonstrate that these cells are able to express IL-23 heterodimer. Specific staining for IL-23 in cryostat sections confirmed the expression of this cytokine by keratinocytes in situ and furthermore, IL-23 expression appeared to be significantly stronger in psoriatic skin as compared to normal skin. Double staining experiments indicated that Langerhans cells, dermal dendritic cells and macrophages expressed IL-23 as well.

**Materials and Methods**

**Skin biopsies and isolation of keratinocytes.** Skin biopsies (5 mm) were obtained from patients with chronic plaque-type psoriasis (16 lesional and 10 nonlesional biopsies) and from the residual skin of normal individuals (n = 13) who were operated for abdominal or breast reduction. The study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam. These biopsies were either snap frozen for in situ stainings or prepared for in vitro experiments under sterile conditions. The frozen biopsies were cut into 5 mm sections and saved at -80 °C until use. The biopsies to be used for cell isolation were incubated overnight at 4 °C in 0.3 % dispase (Boehringer Mannheim, Mannheim, Germany) in PBS with 50 mg/ml gentamycin (Duchefa BV, Haarlem, The Netherlands). Next day, the dermis was removed and the epidermis was trypsinized (0.25 % trypsin; Life Technologies, Paisley, UK) for 5 min at 37 °C. After stopping the reaction by
adding an equal volume of fetal calf serum (HyClone, Logan, UT), the cells were filtered through a cell strainer, washed in Keratinocyte serum free medium (KGM; Gibco, Paisley, UK) and plated into Petri dishes (100 mm) to generate keratinocyte cultures or used to make cytospin preparations. The dermal cell suspension was prepared by means of collagenase digestion as described before\textsuperscript{15}.

**Stimulation of cells**. For Western blot analysis, normal human keratinocytes were cultured in one 100 mm Petri dish and when reaching 80-90 % confluency they were stimulated with 20 mg/ml synthetic dsRNA poly I:C (Sigma-Aldrich, St. Louise, MO) plus 20 U/ml IFN-γ (Roche Diagnostics, Mannheim, Germany) in 5 ml KGM with 50 mg/ml gentamycin. For the determination of the IL-23 heterodimer by ELISA, immature monocyte-derived dendritic cells (MoDCs; generated as described elsewhere\textsuperscript{16} were stimulated at 5x10\textsuperscript{4} cells/well in a flat-bottomed 96-wells plate (final volume 200 ml) with either 10 mg/ml peptidoglycan (PGN; Sigma-Aldrich), or 10 mg/ml poly I:C, or an equal number of CD40L-transfected J558 plasmyctoma cells (J558-CD40L, a gift of Dr. P. Lane, Birmingham, UK), or 10 ng/ml IL-1β (PBH, Hannover, Germany), or 10\textsuperscript{3} U/ml IFN-γ, or different combinations of these stimuli for 24 h. Supernatants and cell lysates of keratinocytes were obtained from subconfluent cultures maintained in Petri dishes and stimulated overnight with a cocktail of aforementioned stimuli in order to achieve a maximal response.

**PCR analysis.** Total RNA was purified from cultured unstimulated keratinocytes by using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) following the manufacturer’s instructions. First strand cDNA was generated with help of a synthesis kit for RT-PCR (MBI Fermentas, St Leon-Rot, Germany), using 9 ml of total RNA, 1 ml oligo(dT)\textsubscript{18} and 1 ml D(N)\textsubscript{6} and heating the mix at 94 °C for 5 minutes. The primers used were: IL-23 p19: forward, 5'-TCG GCA CGA GAA CAA CTG AG-3'; reverse, 5'-TGG GGA ACA TCA TTT GTA GTC T-3' defining a 353-bp product; IL-12 p35: forward, 5'-AAG AGA CCA GAG TCC CGG G-3'; reverse, GGA GCA TGT TGC TGA CGG C-3' defining a 311-bp product; IL-12 / IL-23 p40: forward, 5'-ATT GAG GTC ATG GTG GAT GC-3'; reverse, 5'-AAT GCT GGC ATT TTT GCG GC-3' defining a 297-bp product; b2 microglobulin: forward, 5'-AAG ATT CAG GTT TAC TCA CGT C-3'; reverse, 5'-TGA TGC TGC TTA CAT GTC TCG-3'. The PCR protocol was as follows: first a 3 min incubation at 94 °C, followed by 45 cycles of sequential incubations at 94 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 1 min. Analysis of the PCR products was done on a 1% agarose gel containing ethidium bromide. A 100-bp DNA ladder standard (MBI Fermentas) was used as a size marker.
Western blot analysis. The supernatant from stimulated keratinocytes was concentrated 20-fold by means of centrifugation (Millipore, Billerica, MA) whereas adherent keratinocytes (~2x10^6) were washed once with PBS before the cells were lysed in 150 ml PBS by freeze/thawing. After protein concentration measurement, 50 mg protein of the supernatant and cell lysate was loaded onto a 12% poly-acrylamide gel (Bio-Rad Laboratories, Veenendaal, The Netherlands) and separated by electrophoresis under reducing conditions. Next the separated proteins were transferred onto a nitrocellulose membrane (Schleicher&Schuell Bioscience, Dassel, Germany). Recombinant human IL-23 (R&D Systems, Abingdon, UK) was used as a positive control and recombinant prestained proteins of known molecular weight (Bio-Rad Laboratories) were included to enable the estimation of the molecular mass of our specific IL-23 signal. Before incubating the nitrocellulose membrane with polyclonal rabbit anti-human IL-23 p19 (a kind gift of Dr. J. Pirhonen, National Public Health Institute, Helsinki, Finland) for 18 h at 4 °C, the membrane was blocked with PBS containing 0.05% Tween 20 and 5% non-fat dried milk. After incubation with peroxidase-conjugated goat anti-rabbit Ig (Dako, Glostrup, Denmark) for 1 h at room temperature, specific protein bands in the filter were visualized by the ECL Plus Western Blotting Detection System (Amersham Biosciences, Roosendaal, The Netherlands) and detected by a fluorescence imager (Typhoon 9400; Amersham Biosciences).

Determination of IL-23 heterodimer by ELISA. The amount of IL-23 protein in the supernatants from keratinocytes and MoDCs and in keratinocytes cultures was determined by a solid-phase sandwich ELISA, using polyclonal goat anti-human IL-23 p19 (R&D Systems) as the coating antibody and biotin-conjugated monoclonal mouse anti-human IL-12/IL-23 p40 (BD Pharmingen, San Jose, CA) as the detecting antibody. Hence, this ELISA detects only the heterodimer form of IL-23, but not the separate subunits. Specificity of the ELISA was tested by using recombinant human IL-23 heterodimer (R&D Systems), IL-12 heterodimer (Strathman Biotech, Hannover, Germany), and IL-12 / IL-23 p40 subunit (Biosource, Camarillo, CA). Only the recombinant IL-23 heterodimer, but neither the IL-12 heterodimer nor the common p40 subunit, was detectable by this ELISA (data not shown), having a detection limit of 400 pg/ml.

Immunohistochemical staining. Immunohistochemical staining was performed to determine the expressions of the p19 subunit of IL-23, the common p40 subunit of both IL-23 and IL-12, and the p70 heterodimer of IL-12 in 5 mm skin sections, cultured keratinocytes
on object glass and cytospin preparations from epidermal and dermal cell suspensions. Primary antibodies were polyclonal rabbit anti-human IL-23 p19 (a kind gift of Dr. J. Pirhonen), biotin-conjugated monoclonal mouse anti-human IL-12/IL-23 p40 (BD Pharmingen) and monoclonal mouse anti-human IL-12 p70 (R&D Systems). After fixation with acetone at 4 °C for 10 min, sections were incubated sequentially with 10 % normal goat serum (Dako) at room temperature for 15 min, primary antibody at room temperature for 1 h or overnight at 4 °C, biotin-conjugated goat anti-mouse (Dako) at room temperature for 30 min and avidine peroxidase (Dako) at room temperature for 30 min (Dako). In case of p19 staining, biotin-conjugated goat anti-rabbit (Dako) was used as the second step. After each incubation step, except for the incubation with normal goat serum, the sections were washed three times with Tris-buffered saline. Peroxidase activity was detected as red color using the chromogen 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich). Hematoxilin was used to perform nuclear staining. Mouse anti-human IgG1 (Dako) was used as isotype control for both p40 and p70 antibodies and polyclonal rabbit anti-human factor XIIIa antibody (detecting dermal dendritic cells; Biogenex, San Ramon, CA) was used as a control for the p19 staining. In addition, a recently available polyclonal goat anti-human IL-23 p19 (R&D Systems) was used to stain the same skin sections to evaluate whether it shows the same staining pattern as polyclonal rabbit anti-human IL-23 p19.

In situ expression of the IL-23 p19 subunit was assessed blindly in three sections per biopsy, scoring separately suprabasal epidermis, basal epidermis, papillary dermis and superficial dermis as indicated below: 0, no staining; 1, very weak staining and/or occasional single or few cells (1-5 cells/section); 2, weak staining and/or scattered cells and small groups of cells (5-30 cells/section); 3, moderate staining and/or relatively big groups of cells (30-100 cells/section); 4, strong staining and/or many cells in big groups (100-400 cells/section); 5, very strong staining and/or very large groups of cells (>400 cells/section). Epidermal expression was defined as the sum of basal and suprabasal scores; while dermal expression was the sum of papillary and superficial dermal scores.

To identify the cells expressing IL-23 p19, dermal and epidermal cell cytospin preparations were double-stained with p19 and one of the following cell markers: CD1a (Langerhans cells; BD Pharmingen), HMB45 (melanocytes; Dako), CD1c (dendritic cells; BD Pharmingen), CD3 (T cells; BD Pharmingen), CD83 (mature dendritic cells; BD Pharmingen), CD36 (macrophages; Beckman Coulter, Fullerton, CA), HLA-DR (antigen presenting cells; BD Pharmingen) and CD15 (polymorphonuclear leukocytes; Dako). After performing the IL-23 p19 staining as described above, the sections were sequentially incubated with the
second primary antibody, goat anti-mouse (Dako) and alkaline phosphatase-anti-alkaline phosphatase complex (Dako). The color development was achieved by using naphtol-AS-MX-phosphate (Sigma) for blue and AEC for red.

**Statistical analysis.** The scoring of the in situ expression of IL-23 p19 was expressed as mean values ± SD. Data were analyzed for the statistical significance either with independent samples t test to compare the expression in normal skin versus lesional and nonlesional psoriatic skin or with paired sample t test to compare the expression in nonlesional versus lesional psoriatic skin. P < 0.05 was considered as the level of significance.

**Results**

**Constitutive expression of IL-23 p19 mRNA by human keratinocytes.** To investigate whether human keratinocytes are able to express the heterodimer IL-23, we extracted total RNA from cultured keratinocytes and assessed the presence of the two subunits of this cytokine by means of specific RT-PCR. As shown in Figure 1, nonstimulated keratinocytes from both normal and psoriatic lesional skin displayed mRNA for IL-23 p19 and IL-12/IL-23 p40, indicating that these cells constitutively express the two subunits of IL-23. In addition, we also demonstrated the constitutive presence of IL-12 p35 mRNA, confirming earlier studies.12,17,18

**Normal human keratinocytes express the IL-23 heterodimer.** The RT-PCR analysis clearly revealed that keratinocytes can synthesize the mRNA for both subunits of IL-23. As next step we wanted to determine whether these cells are able to produce these subunits at protein level and to combine them to heterodimer IL-23. The cell lysate and

**FIGURE 1.** Keratinocytes express mRNA for the p19 and p40 subunits of the IL-23 heterodimer. Total RNA was isolated and reverse transcribed and after 45 cycles of amplification, the products were analysed. The mRNA integrity was controlled by amplification of b2 microglobulin. Both normal and psoriatic lesional keratinocytes were found to express mRNA for the IL-23 specific p19 subunit, in addition to the known expression of the IL-12 p35 and IL-12/IL-23 p40 subunits. This result is representative of keratinocytes from 4 normal and 4 lesional psoriasis patients.
supernatant of stimulated normal keratinocytes were subjected to Western blot analysis, using a polyclonal rabbit antibody which is specific for IL-23 p19. In these samples we detected a protein with a molecular weight of ~60 kDa (Fig. 2), which likely represents a complex composed of the p19 and p40 subunits of IL-23 and is in line with an earlier study using the same antibody. In the recombinant IL-23 sample, the 19 kDa monomeric subunit was also detected, but this subunit was not perceptible in the cell lysate and supernatant samples (data not shown).

Immature MoDCs, being a potential rich source of IL-23, were stimulated with different stimuli, either alone or in combination, in order to determine which stimulus is most powerful to provoke IL-23 production. For this purpose we designed a specific ELISA that detects merely IL-23 heterodimer molecules. As shown in Fig. 3A, no IL-23 could be detected in supernatants of unstimulated cells. Triggering via the CD40 molecule present on the cell surface appeared to be effective to induce IL-23 whereas PGN and poly I:C were not. Costimulation with IL-1β resulted in an enhanced IL-23 secretion by immature MoDCs. The highest expression was achieved with a combination of J558-CD40L cells, PGN, poly I:C, IFN-γ, and IL-1β (Fig. 3A). Because of this, the same cocktail of stimuli was used to stimulate keratinocytes in the following experiment. IL-23 heterodimer was detected in the keratinocyte lysates (Fig. 3B), but not in the supernatants of these cells, even if the supernatant was 20 times concentrated. The expression of IL-23 was appeared to be ~2-fold higher in the lysate samples from stimulated keratinocyte than that in the unstimulated samples.

Expression of p19 and p40 subunits of IL-23 at protein level in keratinocytes.
To further substantiate the evidence that human keratinocytes are able to express IL-23, cultured keratinocytes from normal (n = 3), lesional psoriatic skin (n = 3) and nonlesional psoriatic skin (n = 3) were stained with antibodies recognizing the unique p19 subunit of IL-23 or the common p40 subunit shared by IL-23 and IL-12. For reasons of comparison, we stained the cells for the presence of the p70 heterodimer of IL-12 as well. The IL-23
Monocyte derived Dendritic cells IL-23

FIGURE 3. Analysis of the expression of the IL-23 heterodimer in MoDCs and keratinocytes. (a) Immature MoDCs from normal individuals were stimulated 24 h with different stimuli as indicated in the Figure. The highest induction of IL-23 heterodimer was found in case J558-CD40L cells were used as stimulus. Mo DCs were derived from 3 different donors. (b) The cocktail of all stimuli, causing the highest production of IL-23 in the MoDC supernatants, was used to stimulate normal keratinocytes. The cell lysate of stimulated keratinocyte contained a higher expression of IL-23 as compared to unstimulated cells (representative data of keratinocytes from 2 different donors).

p19 subunit was expressed in normal, lesional and nonlesional psoriatic keratinocytes (Fig. 4A, 4B and 4C). The staining was stronger in small and round cells than in big cells with abundant cytoplasm, suggesting that the p19 expression was more prominent in the undifferentiated keratinocytes. Staining with the p40 antibody revealed a similar picture as the staining with the IL-23 p19 antibody (Fig. 4D, 4E and 4F). However, IL-12 p70 expression which represents the biologically active form of IL-12 was seen only in a few cells both in normal and in lesional psoriatic keratinocytes (Fig. 4G, 4H and 4I). Polyclonal rabbit factor XIIIa antibody, which is used as a control for the polyclonal rabbit p19 antibody, did not show any staining in the keratinocytes (data not shown).
In situ expression of the IL-23 p19 subunit in skin. In order to confirm the expression and localization of IL-23 in situ, we stained normal (n = 7), nonlesional (n = 5) and lesional (n = 10) psoriatic skin sections with the IL-23 p19-specific antibody. The expression of the p19 subunit in the epidermis of psoriatic lesional skin was diffuse and very strong, while in the dermis, perivascular cells abundantly expressed this molecule (Fig. 5A). In contrast, in normal human skin the IL-23 p19 expression in the epidermis was much weaker and in the dermis this expression was present in only a limited number of cells, mainly around the capillaries (Fig. 5C). The control stainings with Factor XIIIa, known to be specifically expressed by dermal dendritic cells, revealed a completely different staining pattern, showing positive cells in the papillary and superficial dermis and no positive cells in the epidermis.
In addition, another IL-23 p19 specific polyclonal goat antibody showed the same distribution pattern and intensity of IL-23 p19 immunostaining (data not shown). The expression of IL-23 p19 in normal, nonlesional and lesional psoriatic skin was scored in arbitrary units and was summarized in Figure 6. Statistical analysis of these values indicates that lesional psoriatic skin had a significantly higher expression of IL-23 than normal skin and nonlesional psoriatic skin. The expression of IL-23 p19 in normal versus nonlesional psoriatic skin did not differ significantly. These results are different from the staining pattern seen in the cytopsin preparations from isolated and cultured keratinocytes showing a similar level of p19 expression in lesional, nonlesional and normal skin. This discrepancy might be caused by the induction of IL-23 p19 expression due to in vitro processing and culturing of keratinocytes.

**FIGURE 5.** Expression of IL-23 p19 in normal and psoriatic lesional skin in situ. Immunohistochemical staining of skin sections for IL-23 p19 subunit showed a diffuse positive staining pattern in epidermis. The epidermis stained markedly stronger in psoriatic lesional skin than in normal skin and the number of the positive dermal cells were higher in the psoriatic than that in the normal dermal skin (100x magnification) (see page 193).
Identification of IL-23 p19 expressing epidermal and dermal cells. In the following experiments, we wanted to determine which cell types are responsible for the in situ expression of IL-23 p19 in epidermis and dermis. Cytospin preparations of freshly isolated epidermal cells from normal (n = 3) and lesional psoriatic (n = 3) skin and of a fresh normal dermal cell suspension (n = 1) were double stained with IL-23 p19 and different cell markers to identify the cell types expressing this subunit. Concerning the epidermal compartment, double staining with p19 and CD1a revealed that Langerhans cells could express IL-23 p19. However, most of the cells expressing IL-23 p19 were negative for CD1a showing that Langerhans cells were not the major population expressing IL-23 p19 subunit in epidermal compartment (Fig. 7A). Double staining with p19 and HMB45 indicated that melanocytes are not able to produce IL-23 (Fig. 7B). In the dermal cell suspensions from normal skin, ~60% of CD1c (Fig. 7C), 60% of CD36 (Fig. 7D), 80% of HLA-DR (Fig. 7E), and 20% of CD3 (Fig. 7F) expressed IL-23 p19. CD83+ cells were quite low in number, but 78% of these cells did express IL-23 p19 (Fig. 7G). There were no CD15+ polymorphonuclear cells present in the dermal cell suspension (data not shown).

Discussion
In this study we demonstrated at the RNA and protein level that human keratinocytes constitutively express both IL-23 p19 and p40 subunits and can combine them to a heterodimer molecule of ~60 kDa, strongly indicating that these cells are able to produce the biologically active form of IL-23. This result not only supports the view that keratinocytes contribute to the cutaneous inflammation, but also indicate that they can skew the
FIGURE 7. Identification of IL-23 p19 expressing cells. Cytospin preparations of fresh epidermal and dermal cell suspensions were stained for IL-23 p19 (red) and several different cell markers (blue). The staining of the epidermal cells revealed that beside keratinocytes, the major group of epidermal cells expressing IL-23 p19, CD1a+ Langerhans cells expressed this subunit as well (a). HMB45+ melanocytes did not show a double staining with IL-23p19 subunit (b). Most IL-23 p19 subunit expression dermal cells were dendritic cells and macrophages as identified by the expression of surface markers CD1c (c), CD36 (d) and HLA-DR (e). Approximately one-fifth of CD3+ (f) T cells expressed IL-23 p19 as well. The few CD83+ (g) dendritic cells in cytospin preparations of dermal cells were mostly IL-23 p19 positive (thick arrows double positive cells and thin arrows single positive cells; 400x magnification) (§ page 193).

inflammatory process towards a type 1 immune response through the expression of IL-23. The presence of the IL-23 heterodimer was detected in cell lysates of stimulated keratinocytes by means of Western blot and ELISA techniques, whereas in the supernatants of these cells, IL-23 could only be detected by Western blot analysis, but not by ELISA. This discrepancy is likely due to a difference in the sensitivity of these two techniques. It should be noted that the detection limit of the ELISA used in this study was quite high (400 pg/ml).
Unfortunately, no other antibodies against IL-23 p19 are available at present to develop a more sensitive ELISA. Nevertheless, the ELISA we developed for this study is convenient to assess the production of the IL-23 heterodimer in high producers, such as immature MoDCs, and was also sensitive enough to detect IL-23 in keratinocyte lysates. From these results we conclude that human keratinocytes can secrete low but significant levels of IL-23.

We found that a powerful stimulation was provided via CD40 triggering for IL-23 production by MoDCs and this effect was enhanced by IFN-γ and in particular IL-1β. This kind of stimulation is biologically relevant, because dendritic cells and keratinocytes express CD40 and can interact with CD40L expressing T cells. IL-1β and IFN-γ are known to induce CD40 expression of dendritic cells which enable them to interact with CD40L+ cells, while IFN-γ, but not IL-1b, increases the expression of CD40 by keratinocytes and IFN-γ also stimulates the expression of IL-1β by keratinocytes. Although it is clear that triggering by CD40L, IFN-γ, and IL-1β are appropriate stimuli for immature MoDCs to produce IL-23, it cannot be excluded that other kind of co-stimuli are needed to boost IL-23 production by keratinocytes. On the other hand, one can imagine that keratinocytes are just low producers of IL-23, because a too robust secretion by the epidermal layer would be harmful, as will be discussed below.

Keratinocytes in lesional psoriatic skin in situ displayed a markedly stronger expression of IL-23 as compared to keratinocytes in normal skin. In a separate study we found that after successful narrow-band UVB therapy, a common treatment for moderate to severe psoriasis, the elevated levels of IL-23 were reduced to levels found in normal skin or in nonlesional skin (unpublished data). The increased expression of this cytokine in the epidermis of psoriatic lesions is not an intrinsic aberration of the keratinocytes, because cultured keratinocytes from normal skin and from nonlesional and lesional psoriatic skin display similar levels of IL-23 protein. The enhanced expression of IL-23 in psoriatic lesional keratinocytes in situ is likely induced by cells in their neighbourhood, perhaps the activated memory T cells within the infiltrate. A key role of cutaneous T cells in the induction of psoriatic lesions is underlined in a recent study in which nonlesional skin of psoriasis patients was grafted onto immunodeficient mice lacking T cells, B cells and natural killer cells on the nonlesional skin grafts from psoriatic patients. Due to the local proliferation of donor T cells in the transplanted skin the graft transformed into lesional psoriatic skin. The importance of IL-23 in immune responses in vivo has been clearly demonstrated in mice. IL-23-deficient mice display severely compromised T cell-dependent humoral immunity and strongly impaired delayed type hypersensitivity responses. The lack of IL-
23 could apparently not be compensated by endogenous IL-12, indicating that IL-23 is critical for memory T cell responses in vivo. On the other hand, T cell priming is not impaired in these animals and normal levels of memory T cells are present. Interestingly, IL-23-deficient mice show a clear reduction in IL-17 production, which matches with the finding that IL-23, but not IL-12, is a potent inducer of the proinflammatory cytokine IL-17 in both CD4+ and CD8+ T cells. In addition, the IL-23-deficient animals phenotypically resemble IL-17-deficient mice. In contrast to IL-23 deficiency, engineered systemic overexpression of IL-23, as present in transgenic p19 mice, causes multiorgan inflammation and premature death. In a more sophisticated tissue-specific model, using the keratinocyte-specific keratin 14 promotor, transgenic mice were generated that constitutively express the IL-12/IL-23 p40 subunit in the basal layer of the epidermis. These mice have an enhanced and constitutive production of IL-23, but not IL-12, and spontaneously develop an inflammatory skin disease, characterized by elevated numbers of Langerhans cells with marked upregulated expression of costimulatory molecules and a dermal infiltrate with increased numbers of T cells, macrophages, eosinophils, neutrophils, and mast cells. Repeated injections with recombinant IL-23 in wild-type littermate mice causes a similar inflammatory skin response. Altogether, these experiments in mice illustrate that IL-23 is indispensable for memory immunity, but in case this cytokine is excessively produced an overreaction of the immune system will take place.

In humans however, a possible role of IL-23 in cutaneous inflammation is not thoroughly studied yet. Psoriasis vulgaris may be a good model for such studies because it is considered to be a type 1 cytokine-mediated disease in which memory T cells play a dominant role. Considering the findings in mice that a local excess of IL-23 in the skin causes cutaneous inflammation and our observation that IL-23 is present at enhanced levels in psoriatic lesions, we assume that this cytokine may participate in the perpetuation of this disease. Although our in vitro experiments indicate a low secretion of IL-23 by keratinocytes, it can be speculated that continuous production of this cytokine by all epidermal keratinocytes still can result in the activation of cells in the close proximity, amongst others memory T cells. In response, these cells are stimulated to express proinflammatory cytokines like IFN-γ and IL-17, which in turn enhance the proinflammatory cytokine production by keratinocytes. Studies in mice indicate that IL-23 can also act on myeloid dendritic cells by inducing IL-12 production and, interestingly, promoting in vivo T cell responses to an otherwise tolerogenic peptide. In connection to this, we (Piskin et al, manuscript submitted) and others have observed a marked enhanced expression of IL-12 p70 in dermal dendritic
cells and macrophages in psoriatic lesions. Comparative in situ analysis of psoriatic lesional skin versus nonlesional skin and normal human skin (Fig. 5 and 6) revealed that apart from keratinocytes, the IL-23 expression in dermal antigen presenting cells in the psoriatic lesions was increased as well. This observation is supported by a recent publication, in which an increased expression of mRNA for p19 and p40 was found in dermal dendritic cells and macrophages from psoriatic lesional skin\textsuperscript{17}.

All the findings mentioned in the discussion emphasize that a complex relation exists between dendritic cells, keratinocytes and T cells in the psoriatic lesional inflammation. While dendritic cells and keratinocytes stimulate T cells to express IFN-γ by their expression of the IL-23 heterodimer, IFN-γ production by CD40L\textsuperscript{+} T cells might enhance the expression of CD40, IL-1β and IL-23 locally by keratinocytes and dendritic cells. This process may be further reinforced by CD40-CD40L interaction. In the absence of sufficient suppressive potential, this mutual activation of the activated cells may develop into a self-amplifying loop that ends in a chronic inflammation as in psoriatic lesions. Because the increased expression of IL-23 in the psoriatic lesional skin contributes to the maintenance of the chronic inflammatory process, it may be an interesting target for pharmacological intervention.

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References


