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Katia Boniface; ... et. al

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IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes[1]

Katia Boniface,* François-Xavier Bernard,[‡] Martine Garcia,* Austin L. Gurney,[§] Jean-Claude Lecron,*[†] and Franck Morel[2]*

IL-22 belongs to a family of cytokines structurally related to IL-10, including IL-19, IL-20, IL-24, and IL-26. In contrast to IL-10, IL-22 has proinflammatory activities. IL-22 signals through a class II cytokine receptor composed of an IL-22-binding chain, IL-22RA1, and the IL-10RB subunit, which is shared with the IL-10R. In the present study, we show that short-term cultured human epidermal keratinocytes express a functional IL-22R but no IL-10R. Accordingly, IL-22 but not IL-10 induces STAT3 activation in keratinocytes. Using a cDNA array screening approach, real-time RT-PCR, and Western blot analysis, we demonstrate that IL-22 up-regulates, in a dose-dependent manner, the expression of S100A7, S100A8, S100A9, a group of proinflammatory molecules belonging to the S100 family of calcium-binding proteins, as well as the matrix metalloproteinase 3, the platelet-derived growth factor A, and the CXCL5 chemokine. In addition, IL-22 induces keratinocyte migration in an in vitro injury model and down-regulates the expression of at least seven genes associated with keratinocyte differentiation. Finally, we show that IL-22 strongly induces hyperplasia of reconstituted human epidermis. Taken together, these results suggest that IL-22 plays an important role in skin inflammatory processes and wound healing. ***The Journal of Immunology,*** **2005, 174: 3695–3702.**

Interleukin-22 belongs to a family of cytokines structurally related to IL-10, together with IL-19, IL-20, IL-24, and IL-26 (1). Initially, IL-22 had been identified as an IL-10-related, T cell-derived inducible factor produced by IL-9-activated murine T cells, as well as by activated human Th cells and mast cells (2). IL-22 up-regulates the production of acute-phase proteins in hepatoma cells (3) and pancreatitis-associated protein 1 in pancreatic acinar cells (4), suggesting its involvement in the inflammatory response. IL-22 also inhibits, albeit weakly, IL-4 production by Th2 cells (5). IL-22 signals through a class II cytokine receptor composed of two chains, the IL-22RA1 subunit and the IL-10RB chain (5, 6). The latter is also known as a component of the IL-10R in association with the IL-10RA-specific subunit (7). As with IL-10, binding of IL-22 to its receptor activates JAK1 and Tyk2 tyrosine kinases, leading to the phosphorylation of STAT3 and, to a lesser extent, of STAT1 and STAT5. IL-22 was also found to activate the ERK, JNK, and p38 MAPK in the rat hepatoma cell line H4IIE (8). A soluble IL-22-binding protein, IL-22RA2, encoded by a distinct gene has been identified. This soluble receptor, which has 34% amino acid identity to the extracellular domain of the IL-22RA1, binds IL-22 and antagonizes its functional activities (9–11).

With respect to its anti-inflammatory and immunosuppressive properties, the regulatory action of IL-10 in the skin has been reported in numerous studies. High levels of IL-10 mRNA are detected in skin lesions of patients with atopic dermatitis, cutaneous T cell lymphoma, or melanoma (12). In contrast, a relative deficiency is found in psoriasis, a cutaneous inflammatory disease characterized by a Th1 cytokine pattern (12, 13). IL-10 has been reported to be effective clinically in psoriasis despite the absence of the IL-10RA chain on keratinocytes (14, 15). Contrasting with the effects of IL-10 in the skin, IL-20 promotes hyperproliferation of keratinocytes and has proinflammatory effects on skin. Its overexpression in transgenic mice causes neonatal lethality with skin abnormalities resembling the clinical features of human psoriatic skin (16, 17). Very recently, Wolk et al. (18) reported that skin is a target for IL-22, which enhanced β-defensin 2 and β-defensin 3 expression. In addition, high IL-22 expression was detected in the skin of patients with T cell-mediated dermatoses (18). Relationships between members of the IL-10 family and skin led us to further investigate the potential effects of IL-22 on human keratinocytes.

We show that cultured normal human epidermal keratinocytes (NHEK)[3] express a functional receptor for IL-22 but not for IL-10. IL-22 up-regulates the expression of proinflammatory genes in these cells. We also demonstrate that IL-22 enhances keratinocyte migration, increases the thickness of reconstituted human epidermis (RHE), and down-regulates a set of differentiation-related Ags. Taken together, these results suggest that IL-22 plays an important role in skin inflammatory processes and wound healing.

*Laboratoire Cytokines et Inflammation, UPRES EA 3806, and [†]Laboratoire Protéines et Inflammation, CHU de Poitiers, Pôle Biologie Santé, Université de Poitiers, Poitiers, France; [‡]BIOalternatives, Gençay, France; and [§]Department of Molecular Biology and Immunology, Genentech, South San Francisco, CA 94080

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[2] Address correspondence and reprint requests to Dr. Franck Morel, Laboratoire Cytokines et Inflammation, UPRES EA 3806, Pôle Biologie Santé, Université de Poitiers, 40 avenue du recteur Pineau, 86022 Poitiers Cedex, France. E-mail address: f.morel@univ-poitiers.fr

[3] Abbreviations used in this paper: NHEK, normal human epidermal keratinocyte; RHE, reconstituted human epidermis; SFM, serum-free medium; EGF, epidermal growth factor; PBGD, porphobilinogen deaminase; MMP-3, matrix metalloproteinase 3; PDGF, platelet-derived growth factor; Hsp, heat shock protein; CLSP, calmodulin-like skin protein.

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Materials and Methods

Cell cultures, cytokines, and reagents

NHEK were obtained from surgical samples of healthy breast skin. The use of these samples for research studies was approved by the Ethical Committee of the Poitiers Hospital. Skin samples were incubated overnight at 4°C in a dispase solution (25 U/ml; Invitrogen Life Technologies). Epidermal sheets were removed from the dermis, and NHEK were dissociated by trypsin digestion (trypsin-EDTA; Invitrogen Life Technologies) for 15 min at 37°C. Cells were cultured in keratinocyte serum-free medium (SFM) supplemented with bovine pituitary extract (25 μg/ml) and recombinant epidermal growth factor (EGF) (0.25 ng/ml; all purchased from Invitrogen Life Technologies). NHEK were starved for 24 or 48 h in keratinocyte SFM without the addition of growth factors before stimulation. The keratinocyte cell line SVK14, the hepatoma cell line HepG2, the colorectal cell line HT29, and the human PBMC (obtained from healthy donors by Ficoll hypaque density centrifugation) were grown in RPMI 1640 medium supplemented with Glutamax I (Invitrogen Life Technologies), penicillin (100 U/ml)/streptomycin (100 μg/ml), and 10% FCS (Sigma-Aldrich). Recombinant human IL-10 was provided generously by F. Brière (Schering-Plough, Dardilly, France).

Microarray analysis

RNA was isolated from healthy human tissue samples and used to perform microarray analysis by GeneLogic. Briefly, tissues were snap frozen in liquid nitrogen, then milled to powder, and RNA was extracted using TRIzol reagent (Invitrogen Life Technologies) and RNeasy columns (Qiagen). Probes were then prepared and hybridized to the Affymetrix U-133 GeneChip as recommended by the manufacturer. Data for IL-22RA1 were obtained by examining relative signal strength for probe set 220056_at. Tissue samples were considered normal and without obvious pathology.

RT-PCR and real-time RT-PCR analysis

Total cellular RNA was isolated using TRIzol reagent and treated with DNase I (0.05 U/μl; Clontech). Four micrograms of total RNA were reverse transcribed, using 10 U/μl Superscript II RNase H^- Reverse Transcriptase (Invitrogen Life Technologies) and 160 ng/μl $pd(N)_6$ random hexamer (Amersham Biosciences), according to the manufacturer's instructions (Invitrogen Life Technologies). cDNA samples were amplified for 35 PCR cycles (30 s at 94°C, 30 s at 55°C, and 44 s at 72°C), using AmpliTaq Gold (0.06 U/μl; Applied Biosystems) and specific primers for porphobilinogen deaminase (PBGD), abelson kinase, β_2-microglobulin (19), IL-10RA (20), IL-22RA2 (11), IL-22RA1 (forward, 5′-CCCCACT GGGACACTTTCTA-3′, and reverse, 5′-TGGCCCTTTAGGTACTGT GG-3′), and IL-10RB (forward, 5′-AGGGCTGAATTTGCAGATGA-3′, and reverse, 5′-CCGTTTTTCCAGTATTGCAC-3′; designed with Primer3 Output software). PCR products were analyzed on 1.8% agarose gels containing 0.5 μg/ml ethidium bromide.

Quantitative real-time PCR was conducted using the LightCycler-FastStart DNA Master SYBR Green I kit (Roche). The reaction components were 1× FastStart DNA Master SYBR Green I, 3 mM $MgCl_2$, and 0.5 μM of forward and reverse primers for S100A7 (forward, 5′-GCATGATCGA CATGTTTCACAAATACAC-3′, and reverse, 5′- TGGTAGTCTGTGGC TATGTCTCCC-3′), S100A8 (21), S100A9 (forward, 5′-GCTCCTCG GCTTTGACAGAGTGCAAG-3′, and reverse, 5′-GCATTTGTGTCCAG GTCCTCCATGATGTGT-3′), matrix metalloproteinase 3 (MMP-3) (forward, 5′-TGGCATTCAGTCCCTCTATGG-3′, and reverse, 5′-AGGACA AAGCAGGATCACAGTT-3′), and PBGD as a housekeeping gene (22). After cDNA fluorescent quantification using propidium iodide, 250, 25, and 2.5 ng of cDNA were added as a PCR template in the LightCycler glass capillaries. The cycling conditions comprised a 10-min polymerase activation at 95°C and 40 cycles at 95°C for 10 s, 60°C or 64°C for 5 s, and 72°C for 18 s with a single fluorescence measurement. Melting curve analysis, obtained by increasing the temperature from 60°C to 95°C with a heating rate of 0.1°C/s and a continuous fluorescence measurement, revealed a single, narrow peak of suspected fusion temperature. A mathematical model was used to determine the relative quantification of target genes compared with the PBGD reference gene (23).

Gene expression profiling using cDNA macroarrays

Total RNA was isolated as described for PCR studies. DNase treatment, poly(A)$^+$ RNA enrichment, ^{33}P-labeled cDNA probe synthesis, and purification and hybridization to custom Atlas array membranes (24) were performed according to Clontech's recommendations (Clontech). Membranes were exposed for 4 days to a Molecular Dynamics Storm storage screen and scanned using a phosphorimager scanner (Molecular Dynamics Storm analyser; Amersham Biosciences). After local background substraction, an average signal intensity from duplicate spots was normalized for differences in probe labeling using the values obtained for housekeeping genes (24). For each gene, the IL-22-induced modulation was expressed as the relative expression value for stimulated vs control sample. Arbitrarily, only modulation >2 was considered significant for confirmation using real-time RT-PCR assay.

Western blot analysis

Abs used in this study were rabbit anti-phospho-STAT3 (Tyr^{705}) Ab (Cell Signaling), rabbit anti-IL-22RA1 Ab (ProSci), mouse anti-S100A7 mAb (Imgenex), rabbit anti-STAT3 sc-482 Ab, goat anti-S100A8 sc-8112 Ab, and goat anti-S100A9 sc-8114 Ab (Santa Cruz Biotechnology). Cell lysis was performed according to the manufacturer's recommendations. After separation by SDS-PAGE, proteins were transferred to nitrocellulose membranes (Amersham Biosciences). Proteins were detected with the relevant primary Ab and peroxidase-conjugated Ab (Sigma-Aldrich, Amersham Biosciences). Bound Abs were revealed using chemiluminescence reaction (Amersham Biosciences). Ponceau red staining was used to control loading homogeneity.

For blocking experiments, NHEK were preincubated for 1 h with mouse anti-human IL-10RB mAb (50 μg/ml; R&D Systems) or rabbit anti-human IL-22RA1 Ab (10 μg/ml) or with the appropriate isotype controls before stimulation with IL-22 (5 ng/ml). IL-22RA2 (1.5 μg/ml; R&D Systems) was incubated for 1 h together with IL-22 (5 ng/ml) prior to NHEK stimulation. The percentage of IL-22-induced STAT3 phosphorylation was expressed as the ratio of P-STAT3 vs STAT3 quantified after densitometric analysis using the Photo-captMw software.

In vitro keratinocyte migration assay

Keratinocytes were cultured in wells precoated with type I collagen (200 μg/ml; Institut Jacques Boy) until they reached 80% confluency. Cells were starved for 48 h in keratinocyte SFM and then treated with 10 μg/ml mitomycin C (Sigma-Aldrich) for 2 h to prevent cell proliferation. A cell-free area was created by scraping the keratinocyte monolayer with a plastic pipette tip. Keratinocyte migration to the cell-free area was evaluated after 48 h of culture in the absence or presence of 20 ng/ml IL-22, IL-10, or EGF, using an inverted phase contrast microscope. The number of migrating keratinocytes was counted in four nonoverlapping fields. Values represent the mean ± SEM of cells per mm^2 beyond the frontiers of the in vitro injury. The Student's t test was used for statistical analysis.

RHE model

For histological and immunohistochemical studies, RHE, grown for 12 days at the air-medium interface, were purchased from SkinEthic Laboratories. They consist of a multilayered epidermis that exhibits morphological and growth characteristics similar to human skin (25). As recommended, RHE were grown for 1 day in SkinEthic growth medium before stimulation in the absence or presence of 20 ng/ml IL-22 for 4 days. They were then fixed in a balanced 10% formalin solution and embedded in paraffin. Four-micrometer vertical sections were stained with H&E or with a specific Ab and peroxidase-conjugated Ab and counterstained with hematoxylin, according to standard protocols (25). Anti-K10 keratin, anti-filaggrin, and anti-Ki67 mAbs were obtained from Lab Vision Corporation. The number of Ki67-positive cells was counted in six nonoverlapping fields.

For gene expression profiling using cDNA macroarrays, 17-day-old RHE were grown for 1 day in SkinEthic maintenance medium before stimulation in the absence or presence of 20 ng/ml IL-22 for 24 h. Total RNA was isolated, and cDNA arrays were performed as described above.

Results

IL-22RA1 expression in normal human skin

The functional IL-22R complex consists of the tissue-specific receptor component IL-22RA1 and the relatively ubiquitous receptor component IL-10RB (5, 6). This has led to the hypothesis that IL-22 responsiveness is limited by the expression pattern of IL-22RA1. Thus, to further investigate the spectrum of tissues that might respond to IL-22, we surveyed IL-22RA1 expression in normal tissue samples from a wide variety of organs by microarray analysis. As shown in Fig. 1, the highest expression of IL-22RA1 is noted in the pancreas, and a clear expression is also noted in tissues of the gastrointestinal tract and in the skin.

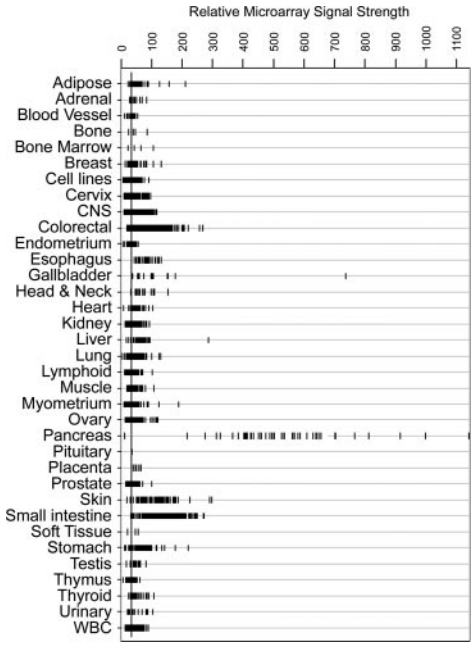

FIGURE 1. Relative expression of IL-22RA1 mRNA in human tissues. Shown are microarray expression data for IL-22RA1 mRNA in human tissue samples. Tissue samples were considered normal and without obvious pathology. The observed microarray signal strength for each sample is indicated by a bar in the appropriate tissue. Each tissue is assessed by multiple independent samples. Tissues are as indicated on the *left*. Lymphoid tissue refers to spleen, tonsil, and lymph node samples. The number of samples per tissue varies. The line drawn across the plotted expression data indicates the approximate limit of accurate signal detection for this probe set. WBC, white blood cell.

IL-22R expression and signaling in keratinocytes

Results from RT-PCR analysis showed that NHEK and the keratinocyte cell line SVK14 express mRNA coding for both the IL-22RA1 and IL-10RB subunits but not for the IL-10RA chain (Fig. 2*A*). Fresh skin biopsies have the same pattern of mRNA chain expression (data not shown). Moreover, IL-22RA2 mRNA is very low or undetectable (Fig. 2*A*). As expected, the colorectal cell line HT29 and the hepatoma cell line HepG2, both known to be sensitive to IL-22 (3, 9) and used as positive controls, express the IL-22RA1 chain mRNA, whereas resting human PBMC express transcripts for IL-10RA, and activated PBMC express transcripts for both IL-10RA and IL-22RA2 (7, 11). In accordance, the presence of the IL-22RA1 protein in NHEK and SVK14 cells was demonstrated by Western blot experiments (Fig. 2*B*). Furthermore, IL-22 induced STAT3 phosphorylation in NHEK, SVK14, and HepG2 cells whereas IL-10 did not. In contrast, IL-10, but not IL-22, induced STAT3 phosphorylation in PBMC (Fig. 3*A*). Time course study up to 24 h showed that the IL-22-induced STAT3 phosphorylation was transient, whereas IL-10 remained without any effect (Fig. 3*B*). These results are in accordance with the pattern of IL-10RA or IL-22RA1 mRNA chain expression detected by RT-PCR and demonstrate the functionality of the IL-22R expressed by human keratinocytes.

To further show the implication of the IL-22RA1/IL-10RB complex in IL-22 signaling, we studied the inhibition of the IL-22-induced STAT3 phosphorylation using IL-22RA2, anti-IL-10RB, and anti-IL-22RA1 Abs. As shown in Fig. 3*C*, NHEK preincubation with anti-IL-10RB or anti-IL-22RA1 Abs inhibited, respectively, 56 and 83% of the IL-22-induced STAT3 phosphorylation.

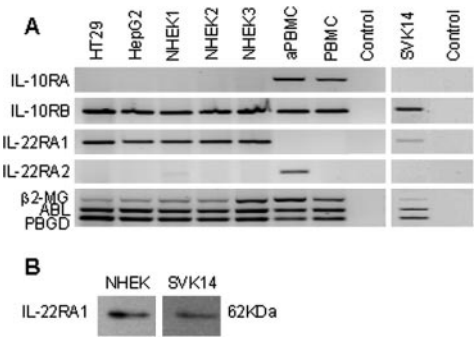

FIGURE 2. Expression of the IL-22RA1 membrane receptor by NHEK and keratinocyte cell line SVK14. *A*, Total RNA was extracted from NHEK of three independent donors and from the keratinocyte cell line SVK14. RT-PCR was performed with specific primers for IL-10RA, IL-10RB, IL-22RA1, IL-22RA2, β_2-microglobulin (β2-MG), PBGD, and abelson kinase (ABL) genes. PCR products were analyzed by agarose gel electrophoresis. The hepatoma cell line HepG2, the colorectal cell line HT29, and the activated human PBMC were used as positive controls. *B*, Twenty micrograms of cell lysate from NHEK and keratinocyte cell line SVK14 were separated by SDS-PAGE (10%) and transferred to nitrocellulose membrane. Detection of a 62-kDa IL-22RA1 band was assessed by Western blot analysis (representative of three independent experiments).

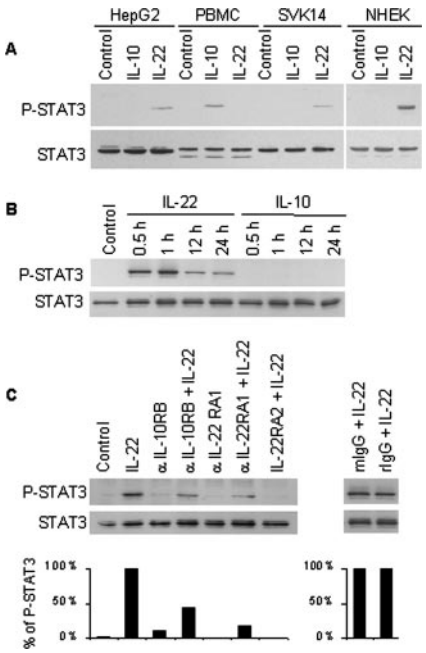

FIGURE 3. STAT3 phosphorylation induced by IL-22 in keratinocytes. *A*, PBMC, hepatoma cell line HepG2, keratinocyte cell line SVK14, and NHEK were stimulated or not for 30 min with IL-10 or IL-22. Twenty micrograms of cell lysate were separated by SDS-PAGE (10%) and transferred to nitrocellulose membrane. Phospho-STAT3 (P-STAT3) and STAT3 protein levels were assessed by Western blot analysis (representative of three independent experiments). *B*, NHEK were cultured with or without IL-10 or IL-22 for 0.5, 1, 12, and 24 h. P-STAT3 and STAT3 protein levels were assessed by Western blot analysis (representative of three independent experiments). *C*, NHEK were incubated for 1 h with either anti-IL-10RB mAb, anti-IL-22RA1 Ab, or with the appropriate isotype control and then stimulated or not with IL-22 for 30 min. IL-22RA2 was incubated for 1 h with or without of IL-22 prior to NHEK stimulation. P-STAT3 and STAT3 protein levels were assessed by Western blot analysis. The percentage of P-STAT3 was determined after quantification by densitometric analysis (representative of three independent experiments). mIgG, murine IgG; rIgG, rabbit IgG.

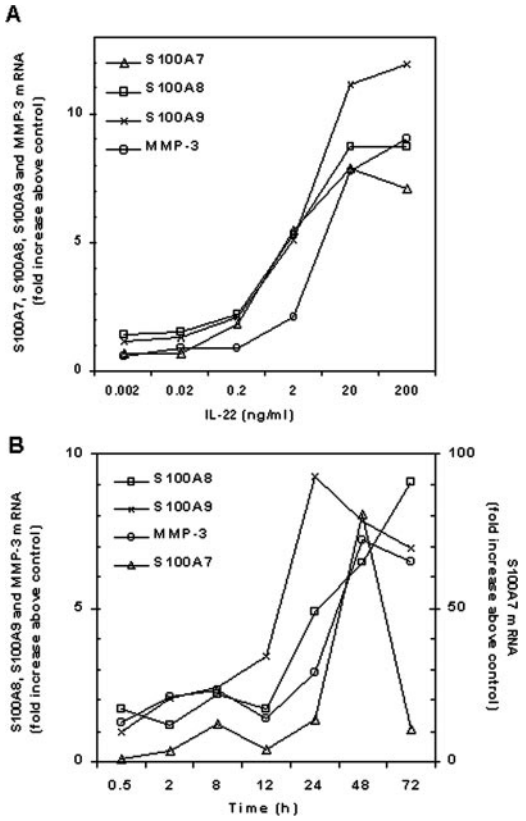

FIGURE 4. Effect of IL-22 on S100A7, S100A8, S100A9, and MMP-3 mRNA expression. NHEK were cultured with or without 0.002, 0.02, 0.2, 2, 20, and 200 ng/ml IL-22 for 24 h (*A*) or with or without 20 ng/ml IL-22 for 0.5, 2, 8, 12, 24, 48, and 72 h (*B*). Total RNA was extracted, reverse transcribed, and S100A7, S100A8, S100A9, MMP-3, and PBGD mRNA relative expressions were quantified by real-time PCR. PBGD was used as a housekeeping gene to normalize gene expression as detailed in *Materials and Methods*. Results, expressed as the relative expression of stimulated cells over control cells, are representative of two independent experiments.

In addition, the incubation of IL-22 with IL-22RA2 before NHEK stimulation completely abolished STAT3 phosphorylation.

Identification of IL-22-induced genes in human keratinocytes

Modification of the keratinocyte gene expression profile induced by IL-22 was analyzed using a cDNA array specially designed for the study of skin cells and keratinocytes and consisting of 475 different cDNAs, spotted in duplicate, that correspond to proteins involved in cell structure, metabolism, extracellular matrix, adhesion, differentiation, signaling, signal transduction, apoptosis, and stress (24). Analysis of Atlas cDNA array membranes revealed an IL-22-dependent increase in the expression of the calcium-binding protein S100A8 (GenBank accession no. X06234), S100A9 (GenBank accession no. X06233), and MMP-3 (GenBank accession no. X05232) (data not shown). Interestingly, the expression of these three proteins is known to be up-regulated in inflammatory tissues, indicating their involvement in the regulation of inflammation (26, 27). As for the other molecules, the hybridization signals for housekeeping genes did not change significantly following IL-22 stimulation.

IL-22 induces keratinocytes to produce S100A7, S100A8, S100A9, and MMP-3

Quantitative analysis of mRNA expression of the IL-22-responsive genes mentioned above was performed by real-time RT-PCR experiments. A strong induction of S100A8, S100A9, and MMP-3 gene expression was observed, with an increase of 26 ± 8-, 17 ± 6-, and 60 ± 20-fold, respectively (mean ± SEM of three independent experiments), after stimulation of NHEK by 20 ng/ml IL-22 for 24 h. In addition, we investigated the expression of a third member of the S100 family, i.e., the S100A7 or psoriasin, known to be involved in inflammatory responses (28). S100A7 mRNA levels were increased by 167 ± 14-fold after IL-22 stimulation. In a second set of experiments, we showed that S100A7, S100A8, S100A9, and MMP-3 mRNA were up-regulated markedly in a dose-dependent manner in response to IL-22 ranging from 0.2 to 20 ng/ml after 24 h of treatment, and the plateau was obtained between 20 and 200 ng/ml (Fig. 4*A*). Kinetics studies revealed a strong increase in S100A7, S100A8, S100A9, and MMP-3 mRNA expression starting at 8–12 h following stimulation with 20 ng/ml IL-22 (Fig. 4*B*).

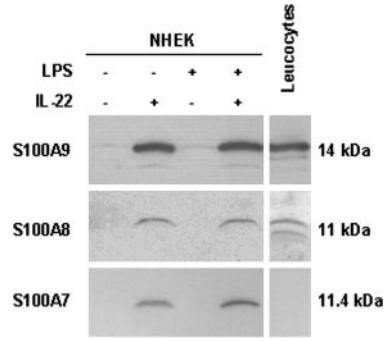

FIGURE 5. Effect of LPS and IL-22 on S100A7, S100A8, and S100A9 protein production. NHEK were cultured with or without 1 μg/ml LPS for 48 h and subsequently with or without 20 ng/ml IL-22 for 48 h. Twenty micrograms of cell lysate were separated by SDS-PAGE (16%) and transferred to nitrocellulose membrane. Ponceau red staining was used to control loading homogeneity. A peripheral blood leukocyte lysate was used as a positive control for S100A8 and S100A9 expression. Detection of an 11-kDa S100A8 and a 14-kDa S100A9 band was performed using goat polyclonal Abs, peroxidase-labeled rabbit anti-goat IgG, and ECL detection. An 11.4-kDa S100A7 band was detected using mouse mAb, peroxidase-labeled anti-mouse IgG, and ECL detection (representative of three independent experiments).

To determine whether the IL-22-induced increase in S100A7, S100A8, and S100A9 mRNA levels were observed at the protein level, we performed a Western blot analysis and showed that

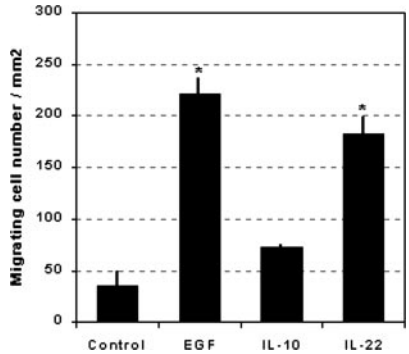

FIGURE 6. Effect of IL-22 on keratinocyte migration. In vitro wounds were introduced in mitomycin-treated confluent NHEK culture, and the keratinocytes were cultured for 48 h with or without 20 ng/ml IL-10, IL-22, or EGF. Cell migration to the cell-free area was assessed as described in *Materials and Methods*. Each bar represent the mean ± SEM of one experiment representative of three. ∗, a value of $p < 0.001$ compared with respective control without cytokine based on the Student's *t* test.

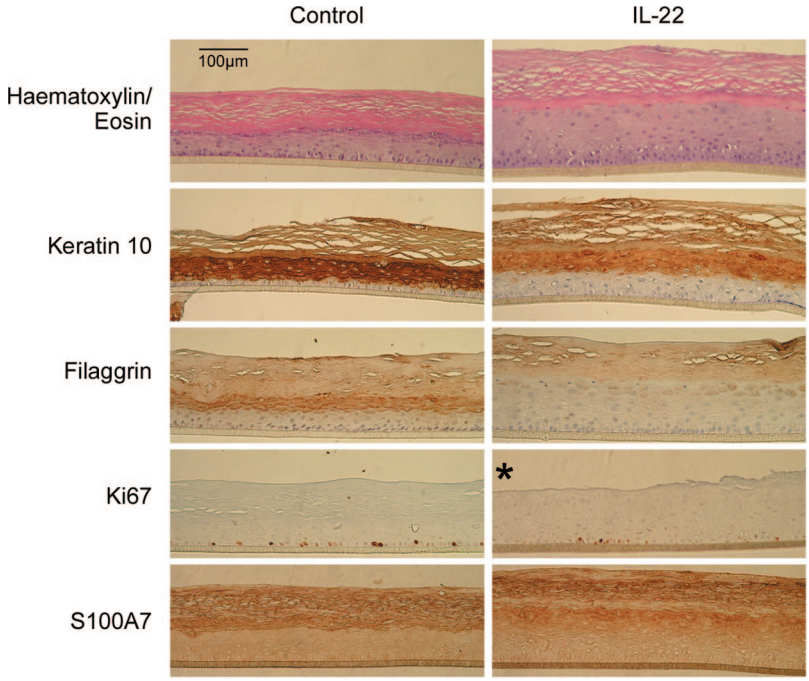

FIGURE 7. Histological and immunohistochemical analysis of RHE stimulated or not with 20 ng/ml IL-22 for 4 days. RHE were fixed and embedded in paraffin. Four-micrometer vertical sections were stained with H&E or reacted with anti-K10 keratin mAb, anti-filaggrin mAb, anti-Ki67 mAb, or anti-S100A7 mAb and then photographed under a microscope (magnification, ×200). *, The cornified layer is missing in this sample (representative of three independent experiments).

NHEK exposure to 20 ng/ml IL-22 for 48 h resulted in a clear expression increase of these three S100 proteins (Fig. 5). We next tested whether preincubation of the cells with LPS was able to modulate this induction by IL-22. As shown in Fig. 5, the endotoxin did not modulate the IL-22-induced expression of the S100 proteins.

IL-22 induces keratinocyte migration in vitro

Once established that IL-22 induces proinflammatory gene expression in human keratinocytes, we investigated the potential effect of the cytokine on wound healing using an in vitro keratinocyte migration assay. NHEK were stimulated either with EGF, which is known to induce keratinocyte migration (29), IL-22, or IL-10. IL-22 induced, at a magnitude comparable to those induced by EGF, a significant migration of human keratinocytes to the in vitro injury, whereas IL-10, as expected, had no effect (Fig. 6).

IL-22 triggers hyperplasia of RHE and reduces the expression of differentiation-related Ags

To further approach the dynamic of epidermal differentiation, we tested the biological effect of IL-22 on in vitro RHE to assess the basal cell layer proliferation and the graduated epidermal differentiation processes. Histological analysis of control RHE showed a keratinized, multistratified epithelium resembling epidermis in vivo, containing intact basal, spinous, granulous, and cornified cell layers and numerous keratohyalin granules in the upper granular layer (Fig. 7). IL-22 triggered the hyperplasia of the keratinocytes layers, leading to an increase in the overall thickness of the RHE. However, this effect did not result from an increased basal keratinocyte proliferation because the number of Ki67-positive cells counted in six nonoverlapping fields was not different significantly between control and IL-22-treated RHE (17 ± 4 and 15 ± 4 Ki67-positive cells/500 μm, respectively) (Fig. 7). In addition, we observed a loss of keratohyalin granules in the granular layer and the presence of picnotic nuclei (Fig. 7). cDNA array profile analysis of RHE demonstrated that IL-22 treatment up-regulates keratin 6, CXCL5 (epithelial-derived neutrophil-activating peptide 78) chemokine, platelet-derived growth factor (PDGF)-A, and S100A7, S100A8, and S100A9 gene transcription (Table I). Using immunohistochemistry, we confirmed the S100A7 protein up-regulation in IL-22-treated RHE (Fig. 7). In contrast, IL-22 down-regulates at least seven genes associated with keratinocyte differentiation, such as *involucrin*, *loricrin*, *filaggrin*, *27-kDa heat shock protein* (*Hsp*), *calmodulin-related proteins*, and *heme oxygenase 1* (Table I; Refs.

Table I. *Effect of IL-22 on the gene expression profile from reconstituted human epidermis*

Gene Name	GenBank	Fold Increase or Decrease after IL-22 Treatment[a] Expt. 1	Expt. 2
Up-regulated genes			
Keratin 6A	L42583	2.0	3.4
Keratin 6B	L42592	2.1	4.2
Keratin 6D	L42607	2.1	3.3
CXCL5/ENA–78	X78686	13.9	26.2
PDGF-A subunit	X06374	12.7	18.7
S100A7	M86757	200.5	404.9
S100A8	X06234	13.5	11.9
S100A9	X06233	9.0	20.9
Down-regulated genes			
27-kDa Hsp	X54079	2.2	2.2
CLSP	AF172852	9.1	13.1
Calmodulin-related protein NB-1	M58026	5.9	4.1
Epidermal filaggrin	AF043380	5.0	8.9
Heme oxygenase 1	X06985	3.1	3.8
Involucrin	M13903	2.2	2.2
Loricrin	M61120	4.0	6.1

[a] RHE were cultured with or without 20 ng/ml IL-22 for 24 h. Total RNA was isolated, treated with DNase 1, and used to make ^{33}P-labeled cDNA probes, which were hybridized to cDNA arrays. The computer images were obtained after 4 days of exposure to a Molecular Dynamics Storm storage screen and additional scanning. After local background substraction, an average signal intensity from duplicate spots was normalized for differences in probe labeling using the values obtained for housekeeping genes. For each gene, the IL-22-induced modulation was expressed as the relative expression value for the stimulated vs the control sample. Shown are the results of two experiments performed with two independent RHE.

30–33). Immunohistochemical analysis performed on RHE sections also confirmed the inhibition of keratinocyte differentiation, as indicated by the decrease of filaggrin and keratin 10 expression in IL-22-treated RHE (Fig. 7).

Discussion

In the present study, we show that in addition to the pancreas (4) and the gastrointestinal tract, normal human skin is a major site for the expression of a functional IL-22R that is composed of the IL-22RA1 chain, associated with the IL-10RB chain, a signaling component of the IL-10R complex. Expression of the IL-22RA1 transcript was detected in fresh skin biopsies and in cultured human keratinocytes from normal epidermis, in agreement with a recent report (18), as well as in the keratinocyte cell line SVK14, and the presence of the protein was observed in NHEK and SVK14 cells. Furthermore, IL-22 was found to induce STAT3 phosphorylation in human keratinocytes, demonstrating the functionality of the receptor. Blocking experiments demonstrated that both IL-10RB and IL-22RA1 are required to generate optimal STAT3 phosphorylation. However, the absence of complete inhibition observed with the anti-IL-22RA1 or anti-IL-10RB Abs cannot totally exclude the involvement of other partners. Interestingly, a complete inhibition of IL-22-induced STAT3 phosphorylation was obtained with IL-22RA2, the natural soluble receptor antagonist of IL-22. The expression of IL-22RA2 was detected weakly or undetected in keratinocytes, although it is strongly expressed in skin samples (11), suggesting an expression by skin cells other than keratinocytes. In contrast, we failed to detect neither IL-10RA1 mRNA expression, nor IL-10-induced STAT3 phosphorylation in human keratinocytes, confirming the results previously reported in the literature (14). It appears that cells, such as keratinocytes, hepatocytes, and renal carcinoma cells, expressing a functional IL-22R, are unresponsive to IL-10 (2, 3, 5, 8). In contrast, hemopoietic cells such as B lymphocytes, MOLT-4 T cells, macrophages, and peritoneal cells, which all express a functional IL-10R, are unresponsive to IL-22 (2, 5, 34). This discrepancy could be of biological relevance: although IL-10 and IL-22 have very different biological functions, they both activate JAK1 and Tyk2 tyrosine kinases and STAT3 phosphorylation, in addition to specific signaling pathways (7, 8).

The use of a cDNA array approach, specially designed for the analysis of gene expression in human skin, enabled us to identify IL-22 target genes in human keratinocytes and to demonstrate the involvement of IL-22 in a variety of processes, including migration and differentiation. In particular, the strong, dose-dependent, IL-22-mediated induction of the expression of S100A7, S100A8, and S100A9 proteins in NHEK and RHE demonstrates the possible proinflammatory and chemotactic effects of the cytokine. The opposing effects of IL-10 and IL-22 in cutaneous inflammation are underscored by the IL-10-induced down-regulation of S100A8 and S100A9 release by monocytes (35). S100A7, S100A8, and S100A9 belong to the pleiotropic S100 family of calcium-binding proteins (26). Although their main functions are as yet unclear, they appear to play prominent inflammatory functions (26, 36, 37) and to be involved in the fine regulation of a large number of intra- and extracellular activities such as the dynamic of motility of cytoskeletal components or chemotaxis (38). Interestingly, whereas all three S100A7, S100A8, and S100A9 proteins have been reported to be expressed at low or undetectable levels in normal skin epidermis and nondifferentiated cultured keratinocytes, they are expressed highly in abnormally differentiated psoriatic keratinocytes (39) during wound repair (40) and in epithelial skin tumors (37, 41, 42). Because of the chemotactic effects of S100A7 on inflammatory cells, in particular, neutrophils and $CD4^+$ T lymphocytes, it has been suggested that this protein may be involved in the genesis of psoriatic lesions (37). Because S100A7 acts upstream of these mechanisms, we speculate that IL-22 could be a key molecule for the induction of S100A7 under pathological conditions and, indirectly, could be involved in the pathological state. The modulation of two other genes is also in favor of the proinflammatory and chemotactic roles of IL-22. Indeed, the induction of neutrophil attractant chemokine CXCL5/epithelial-derived neutrophil-activating peptide 78, together with the down-regulation of heme oxygenase 1, which antagonizes inflammation by attenuating adhesive interaction and cellular infiltration in the skin, could contribute to the neutrophil influx in the skin (33, 43).

IL-22-induced MMP-3 expression is also of interest in the context of inflammatory cutaneous diseases and wound repair. Whereas MMP-3 cannot be detected in normal skin, it is expressed by proliferative keratinocytes of the basal layer after injury (44). During progression of many diseases, MMP-3 is involved in epidermis remodeling by removal of the extracellular matrix during tissue resorption (27, 44), and mice that lack the *MMP-3* gene are deficient in wound repair of the epidermis (45). Using an in vitro wound assay, we demonstrate that keratinocyte migration is increased by IL-22 stimulation. These data are in agreement with the demonstration that STAT3 deficiency in keratinocytes leads to impaired migration (46). Under inflammatory conditions, IL-22 could be one of the mediators enhancing keratinocyte migration and wound healing, via MMP-3- or S100A8-S100A9-dependent mechanisms. Additional evidence to speculate the involvement of IL-22 in wound healing is the strong induction of PDGF in RHE, a major proliferative and migratory stimulus for connective tissue during the initiation of skin repair processes (47).

Finally, we show that IL-22 increases the overall thickness of the keratinocyte layer of RHE. This process appears not to be related to basal cell hyperproliferation because Ki67 expression is not induced in response to IL-22, but more likely results from an inhibition of terminal keratinocyte differentiation, as shown by the decreased production of filaggrin, loricrin, or involucrin. In agreement with this notion, we show that IL-22 down-regulates the expression of the calmodulin-like skin protein (CLSP) and calmodulin-related protein NB-1, two members of the calmodulin family directly related to keratinocyte differentiation (31, 48). CLSP binds transglutaminase-3, a key enzyme implicated in the formation and assembly of proteins, such as loricrin or involucrin, to form the cornified cell envelop of the epidermis (30). In addition, the expression of Hsp27, a small Hsp, that functions as a chaperone in cornification and that colocalizes with both loricrin and involucrin (32) is also down-regulated by IL-22. The modulatory effects of IL-22 on the keratin expression profile, i.e., keratin 6 overexpression and keratin 10 inhibition, also supports the notion of an inhibition of epidermal differentiation. Keratin 6 is known to be induced under hyperproliferative and/or inflammatory situations, including wound healing, psoriasis, carcinogenesis, or by agents such as retinoic acid that provoke epidermal hyperplasia (49, 50). In contrast, keratin 10, normally expressed in terminally differentiating epidermal keratinocytes, is reduced during wound healing (51).

Additional investigations are necessary to characterize the potential skin sources of IL-22 and IL-22RA2 in physiological and pathological conditions. Because psoriasis is a Th1 cell-mediated disease and blocking T cells led to inhibition of psoriasis development (52), particular attention should be given to psoriatic, epidermis-infiltrating T lymphocytes. Likewise, we will focus on mast cells because an increased number of epidermal mast cells are detected in psoriatic lesions and that mast cells are known to produce IL-22 (2, 53).

Taken together, these data underscore the importance of IL-22 in the expression of several molecules involved in human keratinocyte proliferation/differentiation program. Additional studies will have to address the possibility that certain characteristics of inflammatory skin in diseases such as psoriasis could be mediated directly by IL-22.

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Disclosures

The authors have no financial conflict of interest.

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