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IL-22 Is Required for Imiquimod-Induced Psoriasiform Skin Inflammation in Mice

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Psoriasis is a common chronic autoimmune skin disease of unknown cause that involves dysregulated interplay between immune cells and keratinocytes. IL-22 is a cytokine produced by the TH1, TH17, and TH22 subsets that are functionally implicated in the psoriatic pathology. We assessed the role of IL-22 in a mouse model where psoriasiform skin inflammation is triggered by topical application of the TLR7/8 agonist imiquimod. At the macroscopic level, scaly skin lesions induced by daily applications of imiquimod in wild-type mice were almost totally absent in IL-22−/− mice or in mice treated with a blocking anti–IL-22 Ab. At the microscopic level, IL-22−/− mice showed a dramatic decrease in the development of pustules and a partial decrease in acanthosis. At the molecular level, the absence or inhibition of IL-22 strongly decreased the expression of chemotactic factors such as CCL3 and CXCL3 and of biomarkers such as S100A8, S100A7, and keratin 14, which reflect the antimicrobial and hyperproliferative responses of keratinocytes. IL-22 also played a major role in neutrophil infiltration after imiquimod treatment. IL-23 was required for IL-22 production, and γδ TCR lymphocytes represented the major source of IL-22 in lymph nodes from imiquimod-treated mice. However, T cells were not absolutely required for IL-22 production because imiquimod-induced IL-22 expression in the skin is still preserved in Rag2−/− mice. Taken together, our data show that IL-22 is required for psoriasis-like lesions in the mouse imiquimod model and is produced by both T cells and innate immune cells. The Journal of Immunology, 2012, 188: 000–000.

IL-22 is a cytokine related to IL-10 for its structure and to IL-17 for its cellular pattern of production (1). This cytokine can hardly be classified as a pro- or anti-inflammatory cytokine. For instance, analysis of IL-22−/− mice indicated that it plays a pathogenic role in an arthritis model (2) while being protective in several colitis models (3). The main physiological role of IL-22 appears to be related to wound healing and innate antimicrobial responses, because its receptor is mainly expressed by nonhematopoietic cells at body barriers including epithelial cells of the lung and gastrointestinal tract and keratinocytes (1). In vitro studies showed that IL-22 activates STAT3 (4–6) and upregulates the expression of proinflammatory and antimicrobial molecules such as S100A7, S100A8, S100A9, and β-defensins in human keratinocytes (4–7), alone or in synergy with IL-17 or TNF (8, 9). IL-22 also regulates the cellular differentiation and proliferation of keratinocytes and induced hyperplasia of reconstituted epidermis (4).

These biological activities are expected to be beneficial during wound healing and infectious processes but might contribute to skin lesions of patients with psoriasis disease, one of the most common autoimmune-mediated chronic inflammatory skin disorders that affects ~2% of the general population (10). Increased levels of Th17 cells and their associated cytokines including IL-23, IL-17, and IL-22 have been found in human psoriatic skin (6, 11, 12). Elevated IL-22 concentrations were measured in plasma from patients and IL-22 production correlated positively with severity of the disease and negatively with response to therapy suggesting a significant role of this cytokine in psoriasis (5, 7, 13). T lymphocytes appeared to represent a major source of IL-22 in skin lesions (7) and treatment with cyclosporine A downregulated both IL-17 and IL-22 expression in those patients (14).

Psoriasis is not observed in animals other than humans, but a large number of mouse models have been set up to mimic at least some aspects of the human disease (15, 16). Psoriasis lesions are characterized by several histological features including 1) a thickened epidermis caused by keratinocyte proliferation that is called acanthosis, 2) retention of nuclei in the stratum corneum (parakeratosis) that arises from an aberrant differentiation of keratinocytes, and 3) inflammatory cell infiltrates in the epidermis and dermis (17, 18). IL-22–transgenic mice die soon after birth with acanthosis and hyperkeratosis that are reminiscent of such psoriatic lesions (19). Acanthosis can also be induced in wild-type mice by intradermal injection of rIL-23, and this effect is partially abolished in IL-22−/− mice (20). The role of IL-22 is also supported by a graft versus host disease (GVHD) mouse model that is based on the transfer of CD4+CD45RBhi T lymphocytes, depleted of regulatory T cells, into immunodeficient mice. The recipient mice develop acanthosis, elevated levels of antimicrobial

Abbreviations used in this article: GVHD, graft versus host disease; Ngg, neutrophilic granule protein; qPCR, quantitative PCR.

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peptides and inflammatory infiltrates that can be abolished by anti–IL-22 Abs (21). Interestingly, this same GVHD mouse model can also result in colitis that is exacerbated by IL-22 inhibition, in contrast to the effect in the skin, demonstrating the dual activity of this cytokine in inflammation (3).

More recently, topical treatment with imiquimod, a ligand for TLR7 and TLR8 was reported as a novel mouse model for psoriasis-like skin inflammation, inducing acanthosis, parakeratosis, and a mixed inflammatory infiltrate (22). Imiquimod is used in humans for topical treatment of genital and perianal warts caused by human papillomavirus (23), acinic keratosis, and superficial basal cell carcinoma (24, 25). Clinical application of imiquimod can also induce psoriasis or exacerbate the disease in patients with a well-controlled psoriasis (26–30).

Recently, a pivotal role of the IL-23/IL-17 axis was demonstrated in the mouse skin inflammation induced by imiquimod (22). In this study, we show that IL-22–deficient mice are almost totally protected from psoriasis lesions induced by imiquimod treatment. T lymphocytes played a key role in this inflammatory process and γδ T cells, in particular, were found to represent a major source of both IL-22 and IL-17 in draining lymph nodes. However, we show that non-T cells from the innate immune system also largely contribute to IL-22 but not IL-17 production.

Materials and Methods

Methods

All mice in this study were bred in the animal facility of Brussels branch of the Ludwig Institute for Cancer Research under specific pathogen-free conditions. Rag2−/− BALB/c mice were originally purchased from Harlan (Horst, The Netherlands). IL-22–deficient mice were provided by B. Ryffel (University of Lausanne, Lausanne, Switzerland). The Abs were added for 1 h at 4˚C at 2 µg/ml purified rat anti-mouse CD16-CD32 mAb (FcγR Block; BD Pharmingen) before incubation with specific Abs for CD45 (30-F11 conjugated with PerCP) and CD11B (M1/70 conjugated with PerCP) and for Ly6G (RB6-8C5 conjugated with AlexaFluor 700) (all from BioLegend). The Abs were added for 1 h at 4˚C at 2 µg/ml. Cells were gated, based on forward and side scatter and on CD45+ cells with a FACSFortessa (BD Biosciences). Postacquisition analysis was performed using FlowJo software (Tree Star).

For cytosin preparation, CD45+ cells were isolated from dermal single-cell suspension using magnetic beads (MACS; Miltenyi Biotec). A total of 30,000 CD45+ cells were spread on microscope slides by cytosin centrifugation (Cytospin 3; Shandon, Pittsburgh, PA) before staining with Diff-Quick solutions (Medion Diagnostics Ag, Duedingen, Switzerland). Percentages of neutrophils were counted on five different areas (with ~200 cells) for each cytosin.

Cells from lymph nodes were extracted by mechanical disruption and resuspended in Ivoce Dullbecco medium. For intracellular staining, cells were incubated at 37˚C in the presence of GolgiStop solution (BD Biosciences) and IL-23 (10 ng/ml) and IL-2 (400 U/ml). After 4 h, cells were washed with Abs specific for cell surface Ags at 4˚C. Cells were then fixed, permeabilized, and processed for intracellular staining using the Cytofix/ Cyperm Plus Kit (BD Biosciences). The Abs specific to murine Ags used in this study were: HH2B2 (anti–IL-22) and MM17F3 (anti–IL-17A), which were produced in our laboratory (32), whereas PC61 (anti-CD25), 17A2 (anti-CD3), GK1.5 (anti-CD4), H57-597 (anti-TCRβ), and GL3 (anti-TCRγδ) were purchased from BioLegend.

T lymphocyte adoptive transfer

Spleen cell suspensions from BALB/c mice were prepared by mechanical dissection and ABC lysing solution (BD Biosciences). Postacquisition analysis was performed using FlowJo software (Tree Star). The IL-22–deficient mice were originally generated in the 129 background (University of Orle´ ans, Orle´ ans, France). Wild-type 129sv mice were originally purchased from Harlan (Horst, The Netherlands). IL-22–deficient mice were i.p. injected into Rag2−/− mice 3 d before the imiquimod treatment.

RT-PCR

Total RNA was isolated from two pieces of skin using TriPure isolation reagent (Roche, Mannheim, Germany). Reverse transcription was performed on 1 µg total RNA with oligo(dT) primer (Roche) and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA). Quantitative PCR (qPCR) amplifications were performed from cDNA corresponding to 25 ng total RNA using primer sets and TaqMan probes corresponding to murine β-actin, IL-17A, IL-22, S100A7, and S100A8 with qPCR Mastermix (Eurogentec, Seraing, Belgium). The sequences of primers and probes were as follows: β-actin, 5′-CTCTGCCCTCTCTGAGAAC-3′ and 5′-GCTGGAAGGTGGACAATGGAG-3′; IL-22, 5′-CTTACTTCTGAGGCTCTTCC-3′ and 5′-CTTACTTCTGAGGCTCTTCC-3′; IL-17A, 5′-GCTCCAGAAAGCCCTTCAG-3′ and 5′-CTTCTCCCACCGAATCTCC-3′; S100A7, 5′-CTTCTCCCACCGAATCTCC-3′ and 5′-GAGAGGTTCCTAACTACCAGC-3′; S100A8, 5′-GAGAGGTTCCTAACTACCAGC-3′ and 5′-GAGAGGTTCCTAACTACCAGC-3′, and probe, 5′-GTAAGTGGTCACCTGGTGCC-3′. 94˚C for 10 min, followed by 40 cycles of two-step PCR program at 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s. qPCR was done using MasterMix for SYBR Green (Eurogentec) and qPCR Mastermix TaqMan (Eurogentec, Seraing, Belgium). The sequences of primers and probes were as follows: CCL3, 5′-CTTACTTCTGAGGCTCTTCC-3′ and 5′-CTTACTTCTGAGGCTCTTCC-3′, and probe, 5′-GTAAGTGGTCACCTGGTGCC-3′.

Histopathological analysis

Paraffin tissue blocks of skin biopsies were prepared using routine methods and sectioned to obtain consecutive levels. The sections were stained with H&E. Microscope analysis of the staining was performed by two evaluators by reading three sections from each mouse. Proliferation of keratinocytes was measured by BrdU incorporation. A total of 1 µg BrdU (Roche) was injected 12 h before sacrifice. Back skin biopsies were fixed in formaldehyde and embedded in paraffin. Sections were deparaffinized, boiled in citrate buffer (5 mM [pH 5.5]), and incubated with mouse anti-BrdU, followed by HRP-labeled goat–anti-mouse IgG.

Statistics

Results are presented as the mean ± SEM for n = 4 mice/group. Statistical significance between groups was assessed by using one-tailed unpaired t test.
Student t test or one-way ANOVA with Tukey’s or Bonferroni’s multi-comparison test, using the InStat software.

Results
IL-22–deficient mice are protected from the development of psoriasis-like lesions induced by imiquimod

Consistent with a previous report (22), topical application of imiquimod on the shaved back skin of wild-type mice for 7 d induced psoriasis-like lesions including redness, scales resulting from desquamation, and crust formation. Because it was previously shown that mouse IL-22 expression is transiently induced by imiquimod (22), we took advantage of IL-22–deficient mice to assess the functional role of this cytokine in this model of skin inflammation. As shown macroscopically in Fig. 1A, IL–22–deficient mice were almost totally protected from imiquimod-induced skin inflammation, showing less desquamation and no crusts in comparison with the wild-type animals. Microscopic evaluation of skin sections from wild-type mice treated for 7 d showed characteristic changes of psoriasis lesions, such as acanthosis (thickening of the epidermis), parakeratosis (presence of nuclei in the stratum corneum), desquamation, and dermal infiltrate. By contrast, imiquimod application had qualitatively and quantitatively fewer effects on IL-22–deficient mice, including almost no pustules, a small, yet statistically significant, decrease in acanthosis, as well as less desquamation and parakeratosis (Fig. 1B, 1C). Hyperproliferation of keratinocytes was also monitored by BrdU incorporation. In imiquimod-treated wild-type mice, as shown in Fig. 1D, BrdU was incorporated into DNA within keratinocytes that are located throughout the different layers of the epidermis, reflecting deregulated proliferation. In contrast, positive BrdU cells are restricted to the basal layer of the epidermis in IL-22–deficient mice. At the molecular level, the imiquimod treatment induces expression of keratinocyte-specific genes and of inflammatory markers, including cytokines, granulocyte markers, and antimicrobial peptides (Fig. 2). In the skin from IL-22–deficient mice, we observed a significant reduction in the imiquimod induction of several inflammatory chemokines such as CCL3 (MIP-1α) and CXCL3 (MIP-2β), of a granulocyte marker (Ngp), of antimicrobial peptides (S100A7 and S100A8), and of keratin 14, which is associated with hyperproliferative keratinocytes. Expression of loricrin, the major protein component from the corneified cell envelope of terminally differentiated keratinocytes, remained higher in imiquimod-treated IL–22–deficient mice compared with wild-type, thereby demonstrating, at the molecular level, that the absence of IL-22 prevents the de-differentiation of the epidermis (Fig. 2). Interestingly, IL-17 and CCL20, which are, respectively, the hallmark cytokine produced by, and chemokine for the trafficking of Th17 cells are similarly induced by imiquimod in IL–22–deficient and wild-type mice. This observation indicates that IL–22 is not required for the development of a Th17 immune response by imiquimod. Rather, IL–22 acts to induce the expression of proteins that point to a function of IL–22 for granulocyte recruitment in this model of skin inflammation.

![FIGURE 1](http://www.jimmunol.org/) IL–22–deficient mice are protected against psoriasis-like lesions induced by imiquimod treatment. Imiquimod was applied daily to 129sv wild-type and IL–22–deficient mice. After seven days, pictures of mice were taken and mice were sacrificed to analyze skin lesions. A, Macroscopic phenotypical representation of psoriasiform lesions in 129sv wild-type or IL–22–deficient mice treated with imiquimod for 7 d (four representative mice are presented) or untreated (one representative mouse). B, H&E staining of back skin sections from wild-type and IL–22–deficient mice, treated or not with imiquimod (original magnification ×20). One representative picture is shown for each treatment regimen. Acanthosis (a), parakeratosis (p), pustules (pu), and desquamation (d) were observed in wild-type mice treated with imiquimod. C, Acanthosis was evaluated by counting the number of epidermal cell layers throughout the skin section of four mice per group. The number of pustules divided by the length of the section is also represented. Bar graphs represent mean for four mice ± SD. D, BrdU was injected into mice 12 h before sacrifice. BrdU incorporation in the back skin was detected by immunohistochemistry. BrdU-positive cells were distributed across the epidermis in the wild-type mice, whereas, in IL–22–deficient mice, the staining was restricted to the basal layer of the epidermis (original magnification ×20). Data are representative of three experiments with four mice per group. *p < 0.05 wild-type treated mice versus IL–22–deficient mice.
The absence of IL-22 activity prevented the dermal infiltration of neutrophils induced by imiquimod

To further analyze granulocyte infiltration after imiquimod treatment, we isolated cells from the dermis and analyzed them by flow cytometry, using the pan-leukocyte marker CD45 in combination with the CD11B and Ly6G granulocyte markers. After 5 d of imiquimod treatment, we observed an increase in the CD11B+ Ly6G+ cell population in wild-type mice but not in IL-22–deficient mice (Fig. 3A). To confirm this observation, cytospins were prepared with MACS-purified CD45+ cells from imiquimod-treated mice. Eosin–thiazin staining showed increased neutrophils in wild-type mice compared with IL-22–deficient mice as illustrated in Fig. 3B. Percentages of neutrophils counted on these slides (Fig. 3C) correlated with those measured by flow cytometry, confirming that IL-22 plays a key role on neutrophil infiltration in this model.

Ab neutralization of IL-22 decreased psoriasis-like lesions induced by imiquimod treatment

To confirm the effect of IL-22 in this model, we treated wild-type mice with an IL-22–neutralizing or isotype control Ab, starting 1 d before the first application of imiquimod. As shown in Fig. 4A, mice that received an IL-22–blocking Ab presented with reduced skin lesions as compared with control mice. Similar results were obtained with a second IL-22 mAb (data not shown). As observed for IL-22–deficient mice, IL-22 Ab-treated mice had less desquamation and almost no crusts. Histopathological analysis, as presented in Fig. 4B, showed that mice receiving a control Ab had acanthosis, parakeratosis, desquamation, and inflammatory infiltrates, whereas mice that were treated with an IL-22–neutralizing Ab were partially protected, showing a decrease in acanthosis with almost no desquamation and parakeratosis. The protective effect of IL-22 neutralization was also observed at the molecular level. Quantitative RT-PCR analysis of RNA from the skin demonstrated that IL-22 blockade led to a significant decrease in several inflammatory markers such as CCL3 and CXCL3 and the neutrophilic marker Ngp (Fig. 4C). In addition, transcript levels for S100A7 antimicrobial peptide was also reduced with IL-22 Ab-treated mice. As observed for IL-22–deficient mice, administra---
tion of IL-22 Ab to imiquimod-treated mice did not suppress the expression of IL-17. In addition, the expression of the loricrin differentiation marker was higher than with the administration of the control Ab, indicating that blockade of IL-22 activity prevented dedifferentiation of the keratinocytes in the presence of imiquimod (Fig. 4C).

**FIGURE 4.** IL-22 Ab neutralization prevented the development of imiquimod-induced skin lesion. Imiquimod was applied daily to 129sv wild-type mice that were treated biweekly with 500 μg of a neutralizing IL-22 Ab (AM22.1) or isotype control Ab. After 7 d, pictures of mice were taken and mice were sacrificed to analyze skin lesions. **A**, Macroscopic phenotypical representation of psoriasiform lesions in two 129sv mice that received control Abs and in three mice that were treated with anti–IL-22 Abs. **B**, H&E staining of back skin sections from imiquimod-treated mice (original magnification ×20). One representative picture is shown. **C**, Quantitative RT-PCR analysis was performed for each indicated gene. RNA was isolated from two different pieces of back skin. Data correspond to the mean ± SEM from four mice. Data are representative of at least two independent experiments. *p < 0.05 and ***p < 0.001 (one-way ANOVA and Bonferroni multiple comparisons).

**IL-22 production upon imiquimod treatment is IL-23 dependent**

To better characterize the regulation of the Th17-associated cytokines IL-22, IL-23, and CCL20 in this model, we investigated the kinetics of expression for the corresponding RNA in mice that were treated everyday with imiquimod. As shown in Fig. 5A, CCL20 is induced early with a peak at day 1 or 2. IL-23p19 induction was increased at day 1 and peaked between days 2 and 3. Finally, IL-22 expression became detectable at day 2 and peaked between days 3 and 5. Because IL-23 is a major inducer of IL-22 expression, these data suggested that IL-23 was responsible for IL-22 production in this model of skin inflammation. Indeed, the role of IL-23 was supported by the observation that p19−/− mice did not express IL-22 upon imiquimod treatment (Fig. 5B). In contrast, the absence of IL-23 did not affect CCL20 expression, consistent with the early production of this chemokine in this process (Fig. 5C).

**FIGURE 5.** Induction of IL-22 expression in imiquimod-induced lesions is IL-23 dependent. **A**, Imiquimod was applied daily to 129sv wild-type mice. After different periods of time, RNA was extracted from two different pieces of skin, and quantitative RT-PCR was performed for IL-22, IL-23, CCL20, and β-actin. Data correspond to the mean ± SEM from four mice per group. Data are representative of two independent experiments. **B** and **C**, Wild-type and IL-23 p19−/− mice are treated for the indicated period of time with imiquimod and RNA was extracted. Quantitative RT-PCR was performed for IL-22, CCL20, and β-actin. Data correspond to the mean ± SEM from four mice. Data are representative of at least three independent experiments.

IL-22 is produced both by γδ T lymphocytes and by non T cells upon imiquimod application to the skin

To determine the cellular source of IL-22 in this model, we isolated cells from the dermis, the hypodermis, and the axillary and inguinal draining lymph nodes of wild-type 129sv mice treated with imiquimod, and cells were subsequently stimulated in vitro with IL-23 and IL-2. Surface and intracellular stains were then evaluated by FACS, using cells from IL-22-deficient mice to confirm the specificity of the IL-22 Ab for detecting intracellular cytokine. Significant numbers of IL-22–producing cells were observed in lymph nodes from imiquimod-treated wild-type mice in contrast to those from untreated mice (Fig. 6A). All the IL-22+ cells were CD3γδ and CD25+ (Fig. 6B), corresponding to activated T cells. Approximately two-thirds of the IL-22+ cells were γδTCR+ cells, whereas one-third were αβTCR+ cells (Fig. 6C). All of the IL-22+ γδTCR+ cells also produced IL-17 and are negative for CD4 and CD8 (Fig. 6C, 6D). By contrast, the IL-22γδTCR− population
contains CD4+ and CD8+ cells (Fig. 6E), and only one-third of them coexpressed IL-17 (Fig. 6C), whereas all IL-17–expressing cells coexpressed IL-22 (data not shown).

To further address the role of T lymphocytes, we treated Rag2-deficient mice with imiquimod. As shown in Fig. 7, comparable IL-22 expression was detected in the skin of Rag2−/− and wild-type mice, indicating that imiquimod induced IL-22 expression from innate cells and that T cells were not essential for this IL-22 production. The adoptive transfer of wild-type CD5+ T cells into IL-22–competent Rag2−/− recipient did not significantly increase IL-22 expression induced by imiquimod. However, when wild-type CD5+ cells were transferred into Rag2−/− IL-22–deficient mice, cells from the donor representing the only potential source of IL-22, IL-22 expression levels were fully restored to levels detected in the skin of Rag2−/− mice with imiquimod treatment. By contrast, IL-17 induction was completely absent in Rag2−/− mice unless they received CD5+ T cells, indicating that IL-17 production is strictly T cell dependent.

Taken together, these observations indicate that IL-22 is redundantly produced by cells of the adaptive and innate immune systems and plays an important role in the inflammatory response as well as in the dysregulation of keratinocyte proliferation and differentiation induced by imiquimod.

Discussion

The IL-23/Th17 pathway is considered to play a major role in psoriasis because polymorphisms of genes encoding components of IL-23 and its receptor have been associated with an increased risk of psoriasis (33–36), and because of the clinical effectiveness of Abs targeting IL-23/IL-12 p40 as well as IL-17 (37, 38). It has previously been shown that inhibition of IL-23 or IL-17 partially protected the skin from imiquimod-induced inflammation (22, 39). In this article, we show that blocking IL-22 can also protect mice from the skin inflammation induced by imiquimod.

A major difference between IL-17 and IL-22 production in this model is that IL-17 is exclusively produced by T lymphocytes, whereas wild-type and Rag2−/− mice, which have neither αβ nor γδ T cells in the draining lymph nodes.
γδ T cells, showed a similar skin IL-22 induction upon imiquimod application, highlighting the role of non-T cells. We were unable to define the cell types responsible for this innate IL-22 production in the skin. Potential innate IL-22 sources can be hypothesized, based on the numerous studies addressing this issue in the intestinal mucosae. Dendritic cells from the dermis or Langerhans cells from the epidermis might be involved, but there is little evidence so far that this cell type represents a significant source of IL-22 (40). Innate lymphoid cells, which include but are not limited to lymphoid inducer cells, represent the major source of IL-22 in the intestine (41–44) or in the spleen upon administration of TLR4 or TLR5 agonists (45, 46), but such cells were not described in the skin so far. In the K5.Stat3C transgenic model, intradermal administration of IL-23 induces IL-22 expression by CD56" cells from draining lymph nodes, pointing to NK cells as a potential source of IL-22 (39), although such NK markers are also shared by a subset of the above mentioned innate lymphoid cells (41, 42, 44). In our model, we did not detect any IL-22 production by NK cells from draining lymph nodes, suggesting that another innate cell type, possibly residing in the skin might be involved.

Among T cells, our data indicate that conventional Th17 T cells probably represent only a minority of the T lymphocytes involved in IL-22 production. The majority of IL-22+ T cells found in draining lymph nodes expressed a γδ TCR and coexpressed IL-17. By contrast, most of γδ-negative IL-22–producing cells did not coexpress IL-17 and included both CD4" and CD8" cells. The role of T lymphocytes in human psoriasis is well established (22, 47), but the Ags that are recognized remain ill-defined. The association of this disease with streptococcal throat infections supports the hypothesis that pathogenic T cells are specific determinants common to streptococcal M protein and keratin (48), but this observation might only be relevant for a subset of patients. The role of γδ T lymphocytes, which are activated by unconventional Ags, has been poorly studied in psoriasis, although there is evidence for local recruitment of these cells in psoriatic lesions (49). Interestingly, the Ags recognized by γδ T cells include nonpeptide compounds associated with MHC-like molecules such as CD1 (50, 51). In humans, it was recently shown that a particular population of CD4+ T cells homed to skin, where they produced IL-22 in response to CD1a on Langerhans cells (52). It is tempting to speculate that imiquimod activation of Langerhans cells similarly allows for presentation of nonpeptide Ags via MHC-like molecules to both γδ T cells or unconventional CD4+ or CD8+ T cells.

A striking common feature between all the different cell subtypes that are involved in IL-22 production is the expression of the CCR6 receptor that binds the CCL20 chemokine. Our data showed that this chemokine is rapidly induced by imiquimod and that this induction is independent of IL-22 and IL-23. Thus, recruitment of CCR6+ cells appears to be an early step in this inflammatory process, and its importance is illustrated by the observation that IL-23 injection failed to induce acanthosis as well as IL-17 and IL-22 expression in CCR6/" mice (53). In contrast, our results show that IL-22 did not, or only marginally, affect the Th17 response as IL-22 expression is maintained in IL-22–deficient mice. However, in the CD45RB/"GVHD model of skin inflammation, IL-22 blockade prevented IL-17 production (21), indicating that IL-22 might have different effects in these two models of skin inflammation. Keratinocytes likely represent the major IL-22 target in the skin, because these cells respond to IL-22 in vitro. As it was shown previously that blocking IL-17 prevents the development of skin lesions in this imiquimod model, our data suggest that IL-17 and IL-22, which are both induced by IL-23 in vivo, exert synergistic activities in skin inflammation, in line with their in vitro activity on keratinocyte cultures (8).

Among the different pathophysiological features of this model, the absence of IL-22 had a particularly stringent effect on neutrophil recruitment into the skin, most likely via the regulation of CXCL3 and CCL3 because induction of these chemokines was partially prevented in the absence of IL-22. Neutrophil recruitment is a typical activity attributed to IL-17, and this observation further highlights the cooperation between both cytokines. However, this might also result from the regulation of specific chemotactic factors. For instance, CXCL1 and CXCL5, which are often dependent on IL-17 for production, were poorly affected in IL-22–deficient mice (data not shown), whereas CXCL3 and CCL3 are not generally considered as typical mediators of IL-17 activity. In vitro studies focusing on the regulation of these chemokines would be required to determine whether the latter represent specific IL-22–responsive genes or depend on IL-22 and IL-17 cooperation for their expression. Nevertheless, the pathogenic role of neutrophils in psoriasis is supported by clinical observations describing disease remission during drug-induced granulocytosis (54). Thus, our data showing that blocking IL-22 can both affect markers of keratinocyte dysregulation and of neutrophil infiltration further support the potential clinical effectiveness of IL-22 inhibitors in psoriatic patients.

Disclosures
K.D.J. and L.A.F. are currently employees of Pfizer. The other authors have no financial conflicts of interest.

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