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Deficiency of IL-22 Contributes to a Chronic Inflammatory Disease: Pathogenetic Mechanisms in Acne Inversa

Kerstin Wolk,*† Katarzyna Warszawska,* Conny Hoeflich,‡ Ellen Witte,* Sylke Schneider-Burrus,§ Katrin Witte,∗ Stefanie Kunz,* Annette Buss,* Hans Joachim Roewert,§ Markus Krause,§ Ansgar Lukowsky,§ Hans-Dieter Volk,‡ Wolfram Sterry,§ and Robert Sabat*†

Overexpression of the T cell cytokine IL-22 is linked to the development of some chronic diseases, but little is known about IL-22 deficiency in humans. As demonstrated in this study, acne inversa (AI; also designated as Hidradenitis suppurativa) lesions show a relative deficiency of IL-22 and IL-20, but not of IL-17A, IL-26, IFN-γ, IL-24, or IL-1β. Moreover, AI lesions had reduced expression of membranous IL-22 and IL-20 receptors and increased expression of the natural IL-22 inhibitor, IL-22 binding protein. AI is a chronic inflammatory skin disease with prevalence up to 4% of the population and in which cutaneous bacterial persistence represents an important pathogenetic factor. Accordingly, we also found a relative deficiency of antimicrobial proteins (AMPs) in AI lesions and a positive correlation between lesional IL-22 and IL-20 versus AMP levels. IL-22, like its tissue cell downstream mediator IL-20, upregulated AMPs in reconstituted human epidermis and was critical for increased AMP levels under inflammatory conditions. The relative IL-22 deficiency in AI was not linked to lesional T cell numbers or Th22/Th1/Th17 subset markers and -inducing cytokines. However, IL-10 was highly expressed in AI lesions and correlated negatively with IL-22 expression. Moreover, IL-10 inhibited IL-22 but not IL-17 production in vitro. The IL-10 overexpression, in turn, was not associated with an elevated presence of regulatory T cells but with the enhanced presence of an IL-10–inducing cytokine. We conclude that IL-22 deficiency may contribute to the pathogenesis of certain chronic disorders as postulated in this paper for AI. The Journal of Immunology, 2011, 186: 000–000.

Acne inversa (AI; also referred to as Hidradenitis suppurativa) is a chronic inflammatory skin disease with a prevalence rate of up to 4% (1–6). It affects the intertriginous skin of perianal, inguinal, and axillary sites (2, 5). Initially, the occlusion of apocrine or follicular ducts leads to stasis as well as to accumulation and propagation of bacteria in the apocrine hair follicle unit followed by dilatation and rupture of the unit, inflammation, and tissue destruction. The persistence of streptococci and staphylococci in obstructed and ruptured hair follicles maintains and boosts the inflammation and leads to a superfluous exudate of purulence. Left untreated, AI results in the development of painful, fistulating sinuses and wide scars.

The exact etiology of AI, however, is unknown. Smoking, obesity, a respective genetic background, and hormonal factors may play a role in its development and/or course (5). The assumption of cutaneous bacterial persistence in affected skin as a crucial pathogenetic factor is in line with the fact that an antibiotic treatment was successful in AI patients (7).

Antimicrobial proteins (AMPs) play an important role in the cutaneous defense against bacteria and are produced by keratinocytes (8, 9). Examples for AMPs in humans include β-defensin (BD) 1, BD2, BD3, and the S100 proteins S100A7 (also known as psoriasin), S100A8 (calgranulin A), and S100A9 (calgranulin B) (8, 10, 11). AMPs are constitutively expressed in human epithelia, although at low levels, and are upregulated by bacterial products and specific mediators (8, 12–17). The increase of AMP expression may be crucial for the prevention or limitation of cutaneous infections in situations in which the epidermal barrier is disrupted (e.g., in wounds and some skin diseases). In this regard, the difference in the expression of AMPs between psoriasis (Ps) and atopic dermatitis (AD) is interesting and has been intensively investigated: in lesional Ps, skin AMPs are strongly upregulated, and infections rarely occur (18, 19). In contrast, the AMP upregulation in lesional skin from AD patients is significantly lower, predisposing those patients to frequent skin infections with Gram-positive bacteria (streptococci and staphylococci) (20–23).

The aim of this study was to shed light on the molecular mechanisms behind the weakness of antibacterial defense in AI, which may form the basis of future therapy options. To this end, we analyzed the AMP expression in AI, compared it with that of Ps and AD, and searched for mechanisms underlying the observed deficiency in AMP expression in the AI patients.
Materials and Methods

Patients
For mRNA expression analyses and immunohistochemistry (IHC), biopsies obtained from surgically excised skin areas (AI) or 4-6-mm punch biopsies (Ps, AD, control skin) were obtained from affected skin of adult patients with AI (18–61 y [mean ± SD: 36 ± 14 y], 58% male; disease severity: moderate to severe with involvement of at least two body areas; samples derived from inguinal [27%], axillary [45%], perianal/sacral [27%] skin; 30% of patients had isotretinoin therapy), chronic plaque Ps (23–82 y [mean ± SD: 49 ± 15 y], 67% male; disease severity: 83% moderate to severe; skin samples derived from leg [38%], trunk [35%], upper arm [4%], hand [12%], feet [4%], head [3%], inguinal [4%]; at least one patient had local steroid and vitamin D therapy), and and AD (19 to 54 y [mean ± SD: 27 ± 11 y], 33% male; severe skin involvement; skin samples derived from leg [67%], trunk [17%], arm [18%]; 50% of patients had local steroid therapy) from nonlesional skin of adult patients with Ps (paired to lesional skin samples of Ps patients) and from healthy skin of adult control participants (22 to 67 y [mean ± SD: 46 ± 14 y], 82% male; skin samples derived from leg [18%], trunk [64%], axilla [18%]). All skin biopsies were approved by the clinical institutional review board of the Charité University Hospital, Berlin, Germany.

Cell culture
Underdeveloped Epiderm-201 reconstituted human epidermis (RHE) tissues (MatTek, Ashland, MA) were cultured as described previously (24). In the first experimental setting, culture medium was supplemented or not (control) with recombinant human (rh)IL-22 (20 ng/ml), rhIL-20 (20 ng/ml), rhIL-17A (10 ng/ml), rhIFN-γ (10 ng/ml), or rhTNF-α (2 ng/ml), a cytokine mix containing rhIL-22 (1 ng/ml), rhIL-17A (1 ng/ml), rhIFN-γ (0.1 ng/ml), and rhTNF-α (0.1 ng/ml), with the same mix with the exception of one of the T cell cytokines lacking at each time, or with the same mix containing varying concentrations of IL-22 (0.1, 1, and 10 ng/ml) at each time. After 72 h, culture medium was harvested for ELISA and biopsies were taken and snap-frozen for either IHC or mRNA analysis. In the second experimental setting, RHE were stimulated or not with rhIL-22 for 72 h, and 500 μg/ml LPS from Escherichia coli 0111:B4 (Sigma-Aldrich, Taufkirchen, Germany) was added on the RHE surface for the following 24 h.
Human PBMCs were isolated from healthy donors and cultured as described previously (25, 26). Stimulation in the presence or absence of rhIL-10 (10 ng/ml) was done using either immobilized anti-CD3 Ab (1 μg/cm²; Orthoclone, Cilag, Sulzbach, Germany), anti-CD3 Ab (1 μg/cm²; Orthoclone) plus anti-CD28 Ab (1 μg/cm²; R&D Systems, Wiesbaden-Nordenstadt, Germany), a cytokine mix consisting of rhIL-1β and rhIL-2 (each at 10 ng/ml), or control medium. Nonstimulated cells served as the control. After 72 h, culture supernatants were harvested for ELISA.

Bacteria
Naïve CD4+ T cells were isolated by MACS based on negative selection using the naïve CD4+ T cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany). Obtained purified cells of CD4+ CD45RA+ cells were at least 85% viable and further directly analyzed or stimulated for 3 days in vitro in the presence of anti-CD3 and anti-CD28 mAb (anti-CD3 from Orthoclone OKT3, Cilag; and anti-CD28 from R&D Systems coated on the bottom of culture vessels [each at 1 μg/cm²] or Dynabeads Human T-Activator CD3/CD28 beads [Invitrogen, Karlsruhe, Germany; 1 bead/T cell]) in the presence of either 10 ng/ml rhIL-12 and 5 μg/ml anti-IL-4 mAb (Th1), 20 ng/ml rhIL-4, and 5 μg/ml anti-IFN-γ mAb (Th2), 10 ng/ml rhIL-1β, 10 ng/ml rhIL-6, 10 ng/ml rhIL-23, 5 ng/ml rhTGF-β1/2, 5 μg/ml anti-IL-4 mAb, and 5 μg/ml anti-IFN-γ mAb (Th17), 10 ng/ml rhIL-6, 10 ng/ml rhTNFα, 5 μg/ml anti-IL-4 mAb, 5 μg/ml anti-IFN-γ mAb, 5 μg/ml anti-TGF-β1/2 mAb, and 2 μM 6-formylindolo[3,2-b] carbazole (Enzo Life Sciences, Lörrach, Germany) (Th22), or 10 ng/ml rhIL-10, 10 ng/ml rhTGF-β1/2, 5 μg/ml anti-IL-4 mAb, 5 μg/ml anti-IFN-γ mAb, 5 μg/ml anti-TGF-β1/2 mAb, and 10 mM all-trans-retinoic acid (Sigma-Aldrich) (regulatory Th cells). Primary human keratinocytes (Lonza, Vervier, Belgium) were cultured as described previously (27). To study IL-20 induction, keratinocytes were stimulated or not (control) with rhIL-22 either at 10 ng/ml for 42 h, 10 ng/ml for 2, 6, 18, and 42 h (kinetic study), or at 1, 3, 2, 10, and 32 ng/ml for 42 h (study of dose dependency). All recombinant cytokines were purchased from R&D Systems.

Mice
To study cutaneous AMP and cytokine expression, 14-wk-old female BALB/c mice were ip injected with either 1 μg recombinant mouse IL-22 (10 μg/ml; R&D Systems), 100 μg LPS from E. coli 0111:B4 (1 mg/ml; Sigma-Aldrich) or a respective volume (100 μl) of PBS (control). Before and 1, 3, 6, 24, and 48 h postinjection, mice were sacrificed, and skin samples were taken for quantitative real-time RT-PCR (qPCR) analysis. These studies were approved by the regional authorities for provisions on labor, health, and technical safety, Berlin, Germany.

qPCR
Isolation of total cellular RNA, reverse transcription of mRNA, and quantitative gene expression analysis by TaqMan real-time PCR either using the ABI Prism 7700 Sequence Detection System or the StepOne plus (both from Applied Biosystems, Weiterstadt, Germany) of human and mouse samples was done as described previously (14, 25). Oligonucleotide sequences used for expression analysis of the human genes BD1, BD2, BD3, IL-4, IL-10, IL-20, IL-22, IL-24, IL-26, IL-10R1, IL-22R1, IL-10R2, IL-20R1, IL-20R2, IL-22 binding protein (BP), and hypoxanthine phosphoribosyltransferase 1 (HPRT) are listed in Supplemental Table I. All other detection systems were purchased from Applied Biosystems.

ELISA
Frozen Epiderm-201 tissues were processed as described previously (24). Sections were stained with a mouse Ab to human S100A7 (clone 47C1068; Acris, Herford, Germany) or the corresponding isotype control mouse Ab to human IgG1 (clone DAK-G01; DakoCytomation, Hamburg, Germany). Detection of primary Ab binding was performed using the LSAB+ System-AP kit (DakoCytomation). Skin biopsies were fixed in 10% neutral buffered formalin, embedded in paraffin, routinely processed, sectioned at 5 μm, and stained with an Ab to CD3 (clone T3-8B5; DakoCytomation). Detection of primary Ab binding was performed using the LSAB2 System-IRP kit (DakoCytomation). All sections were finally counterstained and covered as described previously (24). Counting of stained T cells in skin sections was performed at 1:200 magnification using an ocular counting net.

Statistical analysis
Data are presented as the mean ± SEM. For further analyses, SPSS 14.0 software (SPSS) was used. Results from patients/control participants and mice were analyzed using the Mann–Whitney U test (two-tailed). Correlations were analyzed based on the Spearman’s correlation coefficient. Results on in vitro-treated RHE, PBMC, and conventional primary keratinocyte cultures were tested using the Wilcoxon matched-pairs signed-rank test (two-tailed).

Results
AI skin lesions show a relative deficiency of AMPs
Bacterial propagation in obstructed and ruptured hair follicles represents a crucial pathogenetic event in the development of AI skin lesions. We hypothesized that an attenuated increase of AMPs may be responsible for the survival of bacteria in these lesions. Therefore, we first analyzed the expression of BDs (BD1, BD2, and BD3) and S100 proteins (S100A7, S100A8, and S100A9) in AI lesions and, as a comparison, in lesional skin from Ps and AD patients as well as in healthy skin from control participants. In line with previous results from others and ourselves (8, 14, 15, 17, 20–23), we found constitutive AMP expression in healthy skin and a strong upregulation of all AMPs except of BD1 in lesions from Ps and AD patients (Fig. 1). The upregulation was very strong in Ps, whereas AD lesions showed a lower upregulation of some of these AMPs: BD2, S100A7, S100A8, and S100A9 (Fig. 1). Importantly, in AI lesions, AMPs were also present, but levels of actually all analyzed AMPs were clearly lower than those found in Ps lesions (Fig. 1). Furthermore, the BD1, BD3, and S100A7 levels were even lower in AI than in AD. Finally, the expression of BD1 was even downregulated compared with healthy skin. Our data show that AI lesions show a relative deficiency of AMP expression compared with Ps skin, and this deficiency is actually broader than that observed in AD patients.
AI lesions show reduced upregulation of IL-22 and IL-20 expression

Immune mediators regulate AMP expression in the skin (9). Having demonstrated a relative deficiency of all analyzed AMP in AI lesions, we then examined the levels of cytokines that might regulate AMP expression and that are produced preferentially by either T cells (IL-22, IFN-γ, IL-17A, IL-26), keratinocytes (IL-20, IL-24), or monocytes/macrophages (IL-24, IL-1β).

The lesions of all patient groups showed a significant upregulation of almost all analyzed cytokines compared with the healthy skin (Fig. 2). However, when comparing the expression levels between AI and Ps, those of IL-17A, IL-26, IFN-γ, IL-24, and IL-1β were similar (IL-17A, IL-26, IFN-γ, IL-24) or higher (IL-1β) in AI, whereas the expression of IL-22 and IL-20 was significantly lower in AI. Interestingly, the expression of IL-22 and IL-20 in AI lesions was even lower than in AD (Fig. 2).

IL-22 and IL-20, although produced by different cell types (IL-22 as a T and NK cell cytokine, and IL-20 being produced by activated keratinocytes and dendritic cells) are two structurally and functionally related cytokines (28, 29). The receptor complexes of both cytokines are known to be highly expressed in keratinocytes and are each composed of two different subunits: IL-22R1/IL-10R2 (receptor complex for IL-22), IL-20R1/IL-20R2 (receptor complex I for IL-20), and IL-22R1/IL-20R2 (receptor complex II for IL-20) (28, 29). For IL-22, there is also a soluble, single-chain receptor called IL-22BP that acts as a high-affinity inhibitor of IL-22 activity (30–34). In the next investigations, we further focused on the differences between AI and Ps and analyzed the cutaneous expression of these receptor subunits in these diseases. Interestingly, the expression of IL-22R1, IL-20R1, and IL-20R2 was decreased (Fig. 3A), whereas IL-22BP was expressed higher in AI than in Ps lesions (Fig. 3B). This therefore should further reduce the possibility of IL-22 and IL-20 to act in the AI lesions.

Our further studies focused on the reason why the upregulation of AMPs in AI was not as pronounced as in Ps, in which the antimicrobial competence of the disturbed skin barrier is increased to a level that almost perfectly prevents cutaneous infection. Interestingly, the correlation analysis of the data obtained from these patients revealed a positive association between the expression of IL-22 and IL-20 and the expression of all AMPs except BD1 (Table I). In contrast, for IL-17A, IL-26, IFN-γ, IL-24, or IL-1β versus AMP levels no significant correlations were found with the exception of those between IL-17A and BD2 and between IL-17A and BD3 (Table I).

IL-22 and IL-20 induce AMP production by reconstituted epidermis

The data described above led us to hypothesize that the relative deficiency of IL-22 and IL-20 is responsible for the relative AMP deficiency in AI lesions. We therefore analyzed the regulation of AMP expression by these cytokines in vitro and in vivo. As expected, treatment of RHE, an in vitro model mimicking human skin (35), with IL-22 or IL-20 resulted in a strong upregulation of BD1, BD2, BD3, S100A7, S100A8, and S100A9 mRNA (Fig. 4A), which was confirmed at the protein level for BD2 (Fig. 4B) and S100A7 (Fig. 4B, 4D).

As representatives of those cytokines upregulated similarly in AI and Ps lesions, we also included IFN-γ and IL-17A in these analyses. IFN-γ did not change BD2 or S100A7 production in RHE (Fig. 4B–D), although it induced the chemokines CXCL9, CXCL10, and CXCL11 (Fig. 4C and data not shown). IL-17A, which also has been described to increase AMP expression (16), induced BD2 and S100A7 production in RHE comparably to IL-22 (Fig. 4B, 4D). This suggested that in vivo during skin inflammation different cytokines may upregulate AMPs and that an attenuated increase of two mediators (IL-22 and IL-20) does not necessarily imply a limited AMP upregulation. Therefore, we next applied

![FIGURE 1. AI skin lesions show a relative deficiency in AMP expression. Biopsies of healthy skin from control participants (control; n = 8) and of skin lesions from patients with Ps (n = 14), AI (n = 7), and AD (n = 8) were analyzed for AMP mRNA expression by qPCR. Data are given as the mean ± SEM relative to the expression of the house-keeping gene HPRT (left panels). The p values that were calculated by the Mann–Whitney U test are indicated (right panels).](http://www.jimmunol.org/)

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<th></th>
<th>BD1</th>
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![Table I.](http://www.jimmunol.org/)
a mix of cytokines that included IL-22, IL-17A, IFN-γ, IL-20, and TNF-α at concentrations that should mimic the situation in Ps lesions to the RHE cultures and observed a high upregulation of BD1, BD2, BD3, S100A7, S100A8, and S100A9 as well as of the chemokine CXCL2 (Fig. 4E,4F). When leaving out individual T cell cytokines from the cytokine mix, IL-22 had the strongest overall effect on the AMP expression, whereas IL-17 was especially critical for BD2 expression (Fig. 4E,4F). In these experiments, a concentration of 1 ng/ml IL-22 was applied. We then tested the relevance of different IL-22 concentrations (10, 1, and 0.1 ng/ml) in the cytokine mix. As shown in Fig. 4G, decreasing IL-22 concentrations led to decreasing BD2 production by these cultures. A high versus a low concentration of IL-22 in that multicytokine stimulation system may be considered as a model reflecting the difference between the Ps lesion (high IL-22 levels) and the AI lesion (low IL-22 levels).

Next, we investigated the influence of bacterial products on AMP expression. Surprisingly, an E. coli LPS isolate that additionally contained protein and nucleic acid contaminations did not induce AMP expression in RHE but upregulated the expression of IL-1β (Fig. 4H).

Interestingly, even a single i.p. application of mIL-22 in BALB/c mice strongly upregulated the cutaneous expression of mBD3 (which corresponds to human BD2) and mS100A8 (mS100A7 has not been identified so far) (Supplemental Fig. 1A). LPS application that induces high systemic (14) as well as high cutaneous stimulation system may be considered as a model reflecting the difference between the Ps lesion (high IL-22 levels) and the AI lesion (low IL-22 levels).

FIGURE 2. The upregulation of cutaneous IL-22 and IL-20 expression in patients with AI is lower than that in Ps patients. Biopsies of healthy skin from control participants (control; n = 8) and of skin lesions from patients with Ps (n = 14), AI (n = 7), and AD (n = 9) were analyzed for cytokine mRNA expression by qPCR. Data are given as the mean ± SEM relative to the housekeeping gene HPRT (top and middle panels). The p values that were calculated by the Mann–Whitney U test are indicated (bottom panel).

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FIGURE 3. AI patients show reduced cutaneous expression of IL-22 and IL-20 receptor subunits, but increased expression of mIL-22BP compared with Ps patients. Biopsies of skin lesions from patients with Ps (n = 14) and AI (n = 7) were analyzed for cell-associated (A) and soluble (B) cytokine receptor mRNA expression by qPCR. Data are given as the mean ± SEM relative to the house-keeping gene HPRT. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001, Mann–Whitney U test.
Relative deficiency in IL-22 expression is not caused by reduced T cell infiltration

Our data suggested a causal relationship between the relative IL-22/IL-20 deficiency and the relative AMP deficiency in AI lesions compared with Ps lesions. We then asked for the mechanism(s) behind the IL-22 and IL-20 deficiency. Because IL-22 is produced by T cells in Ps lesions (36–38), we first analyzed the extent of T cell infiltration. Fig. 5 shows macroscopic (Fig. 5A) and microscopic (Fig. 5B) pictures of AI and Ps lesions, with indication of T cells in the microscopic picture by IHC staining of CD3. Compared to uninvolved skin from Ps patients, the T cell numbers were significantly increased in both AI and Ps lesions (Fig. 5B, 5C). However, AI and Ps lesions did not differ in this respect (Fig. 5B, 5C). Among T cells, IL-22 was described to be produced by the Th1 (25), Th17 (16, 39), and very recently by the Th22 (40, 41) cell subsets. To our knowledge, there is no data available showing direct comparison of these Th cell subsets’ capacity to produce IL-22 in humans so far. Our comparison of the IL-22 production capacity of human T cell subsets generated starting from blood-derived naïve CD4-positive T cells (see Materials and Methods) demonstrated high production by Th22 cells, moderate production by Th1 cells, but only minor production by Th17 cells (although these cells highly secreted IL-17A) (Fig. 5D). Among others, the IL-22–producing T cell subsets differ by their expression of chemokine receptor and transcription factor patterns: Th22 cells express CCR4 (the receptor for CCL17 and CCL22), CCR6 (the receptor for CCL20), and CCR10 (the receptor for CCL27) and contain high levels of the aryl hydrocarbon receptor; Th1 cells are characterized by their expression of CXCR3 (the receptor for CXCL9, CXCL10, CXCL11) and T-bet, but often also express CCR6; Th17 cells, apart from CCR4 and CCR6, express retinoic acid–related orphan receptor γt (29, 40–45). We investigated the cutaneous expression of selected molecules distinguishing these T cell subsets. None of the molecules was decreased in its expression in AI lesions compared with Ps lesions: whereas CXCR3, CCR4, CCR10, retinoic acid–related orphan receptor γt, and aryl hydrocarbon receptor expression did not differ between these diseases, the CCR6 and the T-bet expression in AI lesions was even enhanced compared with Ps lesions (Fig. 5E). These data point to mechanisms underlying the relative IL-22 deficiency other than the degree of lesional infiltration of Th22, Th1, or Th17 cells. We also measured the expression of chemokines attracting these T cell subsets in comparison with other chemokines. As demonstrated in Fig. 5F, the expression of the chemokines CCL17, CCL27, CXCL9, and CXCL8 was not significantly different between AI and Ps lesions. However, the expression of CCL20 (attracting not only CCR6–positive T cell subsets but also dendritic cells and macrophages) and CCL22 was decreased and that of CXCL2 (attracting neutrophilic granulocytes) was clearly increased in AI, matching very well with the clinical picture of AI as purulent chronic inflammation.

IL-10 inhibits IL-22 production and is overexpressed in AI lesions

Because another potential cause of the relative IL-22 deficiency in AI lesions could be a deficiency of cytokines essential for the generation/activation of IL-22–producing cells, we investigated the expression of IL-12p35, IL-12/IL-23p40, IL-23p19, IL-6, and TNF-α. As demonstrated in Fig. 6A, however, there was no deficiency in the expression of Th22 cell–inducing cytokines in AI. In fact, cutaneous TNF-α expression was virtually identical between AI and Ps patients, and IL-6 was even higher in AI. Interestingly, IL-12p35 was strongly upregulated in AI compared with Ps lesions, matching the high expression of T-bet (Fig. 5E) in AI.

Table I. Correlation analysis of cytokine and AMP expression

<table>
<thead>
<tr>
<th></th>
<th>BD1</th>
<th>BD2</th>
<th>BD3</th>
<th>S100A7</th>
<th>S100A8</th>
<th>S100A9</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-22</td>
<td>0.41</td>
<td>0.064</td>
<td>0.69</td>
<td>0.001</td>
<td>0.75</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.14</td>
<td>0.533</td>
<td>0.57</td>
<td>0.007</td>
<td>0.50</td>
<td>0.021</td>
</tr>
<tr>
<td>IL-26</td>
<td>0.16</td>
<td>0.567</td>
<td>0.28</td>
<td>0.232</td>
<td>0.27</td>
<td>0.236</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>−0.13</td>
<td>0.659</td>
<td>0.10</td>
<td>0.674</td>
<td>0.25</td>
<td>0.298</td>
</tr>
<tr>
<td>IL-20</td>
<td>0.61</td>
<td>0.003</td>
<td>0.79</td>
<td>0.000</td>
<td>0.74</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-24</td>
<td>−0.33</td>
<td>0.148</td>
<td>0.13</td>
<td>0.563</td>
<td>0.23</td>
<td>0.313</td>
</tr>
<tr>
<td>IL-1β</td>
<td>−0.40</td>
<td>0.071</td>
<td>0.03</td>
<td>0.905</td>
<td>0.12</td>
<td>0.610</td>
</tr>
</tbody>
</table>

Cytokine and AMP mRNA expression data from skin lesions of AI and Ps patients (n = 21) were analyzed by qPCR and correlated by means of Spearman’s rank correlation analysis. For each field, the Spearman’s rank correlation coefficient and, in parenthesis, the p values are given. Significant correlations (p ≤ 0.05) are in boldface.

( Supplementary Fig. 1B) levels of various proinflammatory mediators, including mIL-1β and mTNF-α, led to a comparable (mS100A8) or even much lower (mBD3) cutaneous AMP upregulation when compared with IL-22 injection (Supplemental Fig. 1A).

The overexpression of IL-10 in AI lesions, however, may not be responsible for the relative IL-20 deficiency in AI lesions. In contrast to IL-22, IL-20 is mainly produced by keratinocytes (25, 47, 48), and IL-10 does not regulate the keratinocyte biology due to the lack of one of the IL-10 receptor subunits, IL-10R1 (Fig. 6E). Accordingly, IL-10 levels did not correlate with IL-20 levels in the inflamed skin (data not shown).

We very recently generated several results that describe in detail how IL-22 induced the IL-20 production in keratinocytes and the potential relevance of this cascade for the Ps pathogenesis (27). We
FIGURE 4. IL-22 and IL-20 are important regulators of keratinocyte AMP expression. A–D, RHE cultures were stimulated with 20 ng/ml rhIL-22, 20 ng/ml rhIL-20, 10 ng/ml rhIL-17A, 10 ng/ml rhIFN-γ, and 2 ng/ml TNF-α as indicated or were left untreated (controls) for 72 h. A, mRNA expression was analyzed by qPCR. Data from five experiments are given as the mean ± SEM relative to HPRT expression. B, Secreted BD1 and S100A7 was analyzed by ELISA. Data from six experiments (data on control, IL-20, IL-22; mean ± SEM) or one experiment (data on control, IL-17, IL-22 and on control, IFN-γ, IL-22) are given. C, mRNA expression was analyzed by qPCR. Data from three experiments are given as the mean ± SEM relative to HPRT expression. D, Cell-associated S100A7 expression was analyzed by IHC. Representative pictures from three experiments are given. Original magnification ×100. E, RHE cultures were stimulated or not (control) for 72 h with a mix of 1 ng/ml rhIL-22, 5 ng/ml rhIL-20, 1 ng/ml rhIL-17A, 0.1 ng/ml rhIFN-γ, and 0.1 ng/ml rhTNF-α or with the same mix with the exception of one of the T cell cytokines lacking at each time. mRNA expression was analyzed relative to HPRT expression by qPCR, and data from three experiments are given as the mean ± SEM as the percent of the mix-stimulated group. F, RHE cultures were treated as in E. BD2 levels in culture supernatant were measured by ELISA. Data from three experiments are shown. G, RHE cultures were stimulated or...
demonstrate in this study that this IL-22 effect in vitro became visible after already 2 h and at even low IL-22 concentrations (Fig. 7A), and its statistical significance could be demonstrated for the stimulation with 10 ng/ml IL-22 during 42 h (Fig. 7B). We therefore hypothesized that the relative IL-20 deficiency in AI lesions may be caused by the relative IL-22 deficiency. This assumption was substantiated by a strong positive correlation of IL-20 expression with IL-22 (Fig. 7C), but not with IL-17A or IL-26 expression (data not shown) in AI and Ps lesions.

Reasons for IL-10 overexpression in AI lesions

Potential mechanisms of enhanced IL-10 expression in AI include the elevated presence of IL-10–secreting T cells (e.g., regulatory T cells) and the elevated expression of mediators known to induce monocyctic IL-10 production. No difference in lesional Foxp3 expression, a marker of regulatory T cells, was found between AI and Ps patients (Fig. 7D). However, the higher cutaneous expression of IL-1β in AI lesions (Fig. 2) in combination with the positive correlation between IL-1β and IL-10 mRNA levels (Fig. 7E) (which was the only statistically significant, positive correlation found for IL-10 when analyzing cutaneous cytokine expressions) may point to an important role of IL-1β in inducing IL-10 expression in AI.

Discussion

In contrast to other chronic inflammatory skin diseases such as Ps, AI patients greatly suffer from bacterial persistence in the affected skin (2, 5, 7). In this study, we show for the first time, to our knowledge, that the AMP expression was significantly lower in AI lesions than in Ps lesions and even than in lesions from AD patients. Because cutaneous AMPs represent a major line of defense against skin infections, we speculate that this relative AMP deficiency may be responsible for bacterial propagation in AI lesions.

This raised the question of what the potential mechanisms underlying the relative AMP deficiency in AI lesions are. We found several hints suggesting that it is due to a relative deficiency of IL-22 and IL-20: first, the levels of IL-22 and IL-20, but not of IL-17A, IL-26, IFN-γ, IL-24, or IL-1β, although increased compared with healthy skin, were diminished in AI lesions compared with Ps lesions. Second, AI compared with Ps lesions showed a reduced expression of the membrane-associated receptors for IL-22 and IL-20 and a higher expression of the IL-22–neutralizing soluble receptor IL-22BP. Third, of all investigated mediators, only the lesional levels of IL-22 and IL-20 correlated with the levels of all analyzed AMPs. Forth, IL-22 and IL-20 strongly induced cutaneous AMP expression both in vitro (RHE) and in vivo (mice; only IL-22 was tested). Fifth, the absence or reduced concentration of IL-22 during RHE stimulation with a combination of several cytokines had the broadest negative effect on the induction of AMPs.

It seems that in general, upon inflammation, skin upregulates AMP expression compared with healthy skin to compensate the higher risk of infection arising due to impaired skin integrity. It is very likely that the cooperation of several cytokines (including IL-22, IL-17A, IL-20, IL-1β, TNF-α) is responsible for this AMP upregulation. However, our in vitro demonstration that the absence or reduction of only one special cytokine may cause a strong attenuation of AMP induction. This led us to suggest that the limited IL-22 and IL-20 upregulation is responsible for limited AMP levels in AI compared with Ps lesions.

Apart from AI, AD also represents an inflammatory skin disease with frequent bacterial persistence in the affected skin (20–23). We demonstrated in this study that, surprisingly, the extent of the relative AMP deficiency in AD was much lower than in AI. In contrast to AI, AD patients show IL-22 levels comparable to Ps patients (Fig. 2) (14). In these patients, however, the relative IL-17 deficiency, probably dependent on low numbers of skin-infiltrated Th17 cells, and the IL-4 overproduction, may be the key factors for the reduced AMP expression (Fig. 2) (21, 49). These known facts about AD further support the idea that, despite redundancy of the different mediators in inducing AMPs, the failure of only one or two of them may cause cutaneous antimicrobial incompetence.

The induction of AMPs by IL-22 and IL-20 is in line with previous observations from others and ourselves (12–14, 16, 17, 50, 51) regarding these mediators. We expanded upon these observations by illuminating the high significance of IL-22 as an inducer of AMPs in comparison with other inflammatory mediators and bacterial components. Recently, results from animal models demonstrated a very important role of IL-22 in the mucosal defense against extracellular bacteria in the gut and lungs (52, 53) and even against systemic infection with Salmonella enterica (54). Interestingly, the significance of IL-22 in the defense against pathogens seems to be dependent on the kind of affected tissue. Furthermore, the Mizoguchi group (55) showed very nicely the local positive role of IL-22 in a mouse colitis model and postulated the involvement of diminished IL-22 production in the pathogenesis of ulcerative colitis.

As mentioned above, IL-22 is mainly produced by various Th cell subsets (25). Regarding the human system, our data presented in this paper suggests that Th22 and Th1 cells are more important IL-22 producers than Th17 cells. Although we excluded reduced skin infiltration by Th22, Th1, and Th17 cells and reduced cutaneous expression of mediators essential for T cell subset generation/activation as being responsible for the relative IL-22 deficiency in AI lesions, we found an increased expression of IL-10 in these patients. The IL-10 levels negatively correlated with IL-22 expression in the inflamed skin. Furthermore, IL-10 partly inhibited IL-22 production in stimulated lymphocytes in vitro. Because, in turn, IL-22 induces IL-20 in keratinocytes, we speculate that in AI skin lesions, diminished IL-20 production is a result of reduced IL-22 production.

A further very interesting question is that regarding the cause of the overexpression of IL-10 in AI lesions. Different factors may play a role, including: nucleotide polymorphisms of the IL-10 gene (56, 57); accumulation of regulatory T cells (Foxp3-positive cells), of Tr1 cells (IL-10–secreting Foxp3-negative cells), or of highly IL-10–producing APCs (58–64); and the enhanced presence of IL-10–inducing cytokines. Having investigated the latter possibility, the positive correlation between lesional IL-10 and IL-1β levels found in the current study may indeed point to a role of IL-1β in upregulating IL-10. Nicotine, which is likely to be present in most AI patients because of the high prevalence of smokers, may be another reason for the increased IL-10 production. In fact, trans-
FIGURE 5. The cutaneous IL-22 deficiency in AI cannot be attributed to a differential lesional T cell infiltration. 

A, Macroscopic appearance of lesions from a Ps (top panel) and an AI (bottom panel) patient. 

B, Biopsies from skin lesions of Ps (left top panel) and AI (left bottom panel) patients and from healthy skin from control participants (right panels) were analyzed for CD3-positive cells by IHC. Scale bars, 200 μm. 

C, Epidermal and dermal CD3-positive cell numbers in biopsies from skin lesions of AI (n = 10) and Ps (n = 13) patients and from uninvolved skin from Ps patients (control skin; n = 5) were analyzed by IHC. Data are given as the mean ± SEM. 

D, Activated Th cell subsets were generated from naive human T cells and analyzed for IL-22 and IL-17A production by ELISA of cell culture supernatant. Data from five experiments are shown (mean ± SEM; *p ≤ 0.05 compared with all other T cell subsets, Wilcoxon matched-pairs signed-rank test). Biopsies of skin lesions from patients with Ps (n = 12) and AI (n = 7) were analyzed for the expression of chemokine receptors and transcription factors marking T cell subsets (E) and of chemokines (F) by qPCR. Data are given as the mean ± SEM relative to HPRT expression. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001, Mann–Whitney U test.
cutaneous nicotine administration in humans increased IL-10 levels after LPS application (65). Very probably, monocytic cells (66), the α4 and β2 subunits of nicotinic acetylcholine receptor (67), and the increase of intracellular cyclic 3’,5’-adenosin-monophosphate levels (68) are involved in this nicotine effect. Importantly, for a significant IL-10 secretion, the activation of protein kinase (PK) C and NF-κB as well as of the PKA pathway is necessary (69). Whereas in AI lesions, the elevated IL-1β levels may cause strong activation of the PKC/NF-κB pathway, nicotine may increase cyclic 3’,5’-adenosin-monophosphate levels that induce activation of PKA. The simultaneous presence of IL-1β/TNF-α and nicotine may therefore mediate strong IL-10 production in AI lesions.

In summary, we hypothesize an immunologically based pathogenetic cascade underlying the high frequency of bacterial infections in AI, which includes the following steps: 1) increased
IL-10 expression results in limited IL-22 production; 2) limited IL-22 production, in turn, leads to limited keratinocyte IL-20 production; and 3) limited IL-22 and IL-20 production are responsible for the relative deficiency of AMP that allows the bacterial persistence in AI lesions. In Fig. 8, we sketched a hypothesis regarding the pathogenesis of this disease. According to this hypothesis, several treatment options may be added to surgical excision, which is currently the treatment of choice (5): smoking

**FIGURE 8.** Hypothesized immunologically based pathogenic cascade underlying bacterial infections in AI.

**FIGURE 7.** The regulation of keratinocyte IL-20 expression by IL-22 may represent a pathogenetic cascade in AI. A, Primary human keratinocytes were stimulated with rIL-22 or left unstimulated (control) for the indicated times (left panel; 10 ng/ml) or at the indicated concentrations (42 h, right panel) and were analyzed afterward for IL-20 mRNA expression by qPCR. Data relative to HPRT expression from three experiments in each case are given as percent of respective control (mean ± SEM). B, Primary human keratinocytes were stimulated with 10 ng/ml rIL-22 or left unstimulated (control) for 42 h and were analyzed afterward for IL-20 mRNA expression by qPCR. Data relative to HPRT expression from six experiments are given as percent of the control (mean ± SEM). C, IL-20 mRNA expression data (obtained by qPCR relative to HPRT expression) were correlated with IL-22 mRNA expression data (obtained by qPCR relative to HPRT expression) from Ps and AI lesions (n = 21) by means of Spearman’s rank correlation analysis. The Spearman’s rank correlation coefficient (rₛ) is indicated. D, Biopsies of skin lesions from Ps (n = 12) and AI (n = 7) patients were analyzed for Foxp3 expression by qPCR. Data are given as the mean ± SEM relative to HPRT expression (no significance, Mann–Whitney U test). E, IL-10 mRNA expression data were correlated with IL-1β mRNA expression data (both obtained by qPCR relative to HPRT expression) from Ps and AI lesions (n = 21) by means of Spearman’s rank correlation analysis. The Spearman’s rank correlation coefficient (rₛ) is indicated. *p ≤ 0.05; Wilcoxon matched-pairs signed-rank test; **p ≤ 0.01; ***p ≤ 0.001.
cession, anti-inflammation without induction of IL-10, inhibition of IL-10 production or activity, IL-22 application, and AMP application. Interestingly, anti-TNF-α therapy [TNF-α is another major inducer of IL-10 production (69)] has proven to be successful in an initial number of cases with AI (70, 71), supporting our hypothesis.

Data from the human system demonstrated that elevated amounts of IL-22 play a fundamental role in the pathogenesis of some chronic inflammatory diseases such as Ps (12–14, 17, 24). However, little was known so far about deficient IL-22 expression in humans and its relevance. By shedding light on the molecular mechanisms underlying the weakness of antimicrobial defense in AI, we demonstrate an example of how a deficiency of IL-22 can lead to a chronic inflammatory disease in humans. Chronic sinustis and Crohn’s disease, like AI, are further examples of such disorders with significant T cell inflammation. However, the two major effects of IL-22, inducing AMPs and protecting against damage via increased regeneration, are minimally present in this study. Future studies must show whether these disorders also belong to the IL-22 deficiency diseases.

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Disclosures

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