Cytokine Milieu of Atopic Dermatitis, as Compared to Psoriasis, Skin Prevents Induction of Innate Immune Response Genes

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Atopic dermatitis (AD) and psoriasis are the two most common chronic skin diseases. However patients with AD, but not psoriasis, suffer from frequent skin infections. To understand the molecular basis for this phenomenon, skin biopsies from AD and psoriasis patients were analyzed using GeneChip microarrays. The expression of innate immune response genes, human β defensin (HBD)-2, IL-8, and inducible NO synthetase (iNOS) was found to be decreased in AD, as compared with psoriasis, skin (HBD-2, p = 0.00021; IL-8, p = 0.044; iNOS, p = 0.016). Decreased expression of the novel antimicrobial peptide, HBD-3, was demonstrated at the mRNA level by real-time PCR (p = 0.0002) and at the protein level by immunohistochemistry (p = 0.0005). By real-time PCR, our data confirmed that AD, as compared with psoriasis, is associated with elevated skin production of Th2 cytokines and low levels of proinflammatory cytokines such as TNF-α, IFN-γ, and IL-1β. Because HBD-2, IL-8, and iNOS are known to be inhibited by Th2 cytokines, we examined the effects of IL-4 and IL-13 on HBD-3 expression in keratinocyte culture in vitro. We found that IL-13 and IL-4 inhibited TNF-α- and IFN-γ-induced HBD-3 production. These studies indicate that decreased expression of a constellation of antimicrobial genes occurs as the result of local up-regulation of Th2 cytokines and the lack of elevated amounts of TNF-α and IFN-γ under inflammatory conditions in AD skin. These observations could explain the increased susceptibility of AD skin to microorganisms, and suggest a new fundamental rule that may explain the mechanism for frequent infection in other Th2 cytokine-mediated diseases. The Journal of Immunology, 2003, 171: 3262–3269.

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topic dermatitis (AD)³ and psoriasis are the two most common chronic inflammatory skin diseases found in the general population (1, 2). However, their mechanisms for skin inflammation and propensity for skin infection are quite different. The immune response in AD is Th2-mediated contributing to the high IgE levels and eosinophilia characteristic of this condition (3). In contrast, the immune response in psoriasis is Th1-mediated and associated with local neutrophil infiltration (4). Importantly, ~30% of AD patients suffer from frequent serious skin infections, and over 90% of AD patients are colonized with Staphylococcus aureus (5). In contrast, only 6.7% of psoriasis patients suffer from skin infections despite the fact that both skin diseases are characterized by defective skin barriers (6). This has recently been highlighted as a major public health problem because patients with AD are prone to eczema vaccinatum (7). Because nearly 15% of children have AD and close contacts are excluded from smallpox vaccination, it is estimated that nearly 50% of the general population cannot be vaccinated with the smallpox vaccine in any voluntary mass vaccination campaign to blunt the effects of a potential bioterrorist attack. Therefore, it is of great interest to better understand the mechanisms that make patients with AD prone to microbial skin infections.

Antimicrobial peptides have been shown to be key elements in the innate immune system providing the first line of defense in the skin against invading microbes (8). In normal skin, these peptides are present at only negligible levels. However, in response to injury or inflammation, keratinocytes produce high levels of these antimicrobial peptides, e.g., in the skin of patients with psoriasis. Animal models have shown that the induction of antimicrobial peptide expression is essential for skin to combat infection (9, 10). We have reported recently that both human β defensin (HBD)-2 and LL-37, a cathelicidin, are produced at low levels in the skin of patients with psoriasis. Antimicrobial peptides have been shown to be key elements in the innate immune system providing the first line of defense in the skin against invading microbes (8). In normal skin, these peptides are present at only negligible levels. However, in response to injury or inflammation, keratinocytes produce high levels of these antimicrobial peptides, e.g., in the skin of patients with psoriasis. Animal models have shown that the induction of antimicrobial peptide expression is essential for skin to combat infection (9, 10).

We have recently studied the role of human β defensins (HBD-2) and LL-37, a cathelicidin, in AD. HBD-2 was able to kill S. aureus in the presence of LL-37 but not by itself. Recently, a new antimicrobial peptide, HBD-3, has been identified which has potent killing activity against S. aureus by itself and can kill a broad spectrum of microbes (12, 13). Therefore, a deficiency of HBD-3 could contribute to the increased susceptibility of AD skin to infection. To capture a more complete picture of the innate immune response in atopic skin, we conducted the current study using a GeneChip microarray of multiple innate immune response genes using psoriasis skin samples as a control. The current study included a number of potentially important innate
immunity genes such as induced NO synthetase (iNOS) which can kill viruses, bacteria, and fungi through production of NO (14); IL-8 which attracts neutrophils into the skin for phagocytosis and killing of bacteria; as well as Toll-like receptors and their signal transduction system needed to recognize and respond against microbial organisms. Our data indicate that the cytokine milieu in AD, as compared with psoriasis, prevents the induction of multiple innate immune response genes due to lower levels of proinflammatory cytokines such as TNF-α or IFN-γ needed for induction of inducible antimicrobial peptides, and the increased Th2 cytokines (IL-4 and IL-13, known to be overexpressed in AD skin but suppress antimicrobial peptide expression).

Materials and Methods

Subjects

This study was approved by the Institutional Review Board at National Jewish Medical and Research Center (Denver, CO). Skin biopsies were obtained from the skin lesions of 15 different patients with moderate to severe AD (mean age = 33.0 years; skin involvement 20–60%; the samples of six different patients were used for GeneChip study, six different patients for immunohistochemistry, 10 different patients for real-time PCR; some patients’ skin biopsies were used in multiple assays), 15 psoriasis patients (mean age = 39.7 years; skin involvement 15–40%; the samples of seven patients were used for GeneChip study, 12 were used for real-time PCR), and 55 healthy controls (some samples were used in multiple experiments). A total of 125 skin samples were obtained from all subjects before the study. The skin samples were immediately submerged in 1 ml of RNA later (RNeasy protect mini kit; Qiagen, Valencia, CA) and frozen at −80°C for future RNA isolation or fixed for immunohistochemistry.

RNA extraction from skin biopsies

Skin biopsies, preserved in RNA later were put into 600 μl of buffer RLT (Qiagen RNeasy minikit) and homogenized using a Pyrex tissue grinder. U-95Av2 GeneChip probe arrays (Affymetrix, Santa Clara, CA) and hybridized at 45°C for 16 h. After washing and staining with streptavidin-PE, the probe arrays were scanned using a Gene-array scanner (Hewlett Packard, Palo Alto, CA). The data was analyzed with GeneChip Suite version 5.0 software (Affymetrix). The GeneChip array analysis yielded a hybridization intensity for each represented gene as the signal intensity. A difference between AD and psoriasis skin was defined as significant if 2-fold difference in average signal strength with a p value under 0.05 using a two-tailed unpaired t test was observed. Detailed protocols (16) and data analysis (17, 18) have been described in former reports.

Immunohistochemistry for HBD-3

For these experiments, skin biopsies were fixed in 4% paraformaldehyde in PBS for 4 h and transferred into 10% PBS-buffered formalin (Fisher Scientific, Fair Lawn, NJ). They were then transferred to PBS before being processed and embedded in paraffin. Three micron sections were cut onto poly-l-lysine-coated slides, dried and immunostained using polyclonal rabbit anti-human Ab directed against HBD-3 (OrbiGen, San Diego, CA). The Ab was used at 1/100 dilution (10 μg/ml) which gave optimal staining following a titration study. The Ag-Ab complex was detected with an avidin-biotin peroxidase complex method, as described before (19). The signal was visualized with diaminobenzidine. As a negative control, isotype Ab (purified nonimmune rabbit IgG; Southern Biotechnology Associates, Birmingham, AL) was used. The immunoreactivity was presented as the percentage of keratinocytes which were positively stained with a score of 0 to 8, where 0 was defined as no staining, 1 = up to 12.5%; 2 = up to 25% with stepwise increase by 12.5% to 8 = up to 100%.

Quantitative real-time PCR

The primers and probes used in the real-time PCR of HBD-3 mRNA were selected with Primer Express software (PerkinElmer/Applied Biosystems, Norwalk, CT) as follows: forward, 5’-GCTGGCTTCTCAAAAGGGAGA-3’ and reverse 5’-CTTCCGACAGATTGTC-3’ and 6FAM-ACGGGTACGACCTTGCCGAAT-TAMRA. Primers and probes for HBD-2 were the same as in the former report (11). Primers and probes for human IL-4, IL-8, IL-13, TNF-α, IFN-γ, IL-1β, GAPDH and 18S RNA were purchased from PerkinElmer/Applied Biosystems (Norwalk, CT). Total RNA was isolated as described above. One microgram of RNA was reverse-transcribed in a 20-μl reaction containing Random Primers (Invitrogen, Carlsbad, CA) and Superscript II enzyme (Invitrogen). Each PCR system (50 μl) contained 1 μl of cDNA, 1× gold buffer II, 4 mM MgCl2, 500 μM dNTP, primers (200 nM), 100 nM probe (labeled with 6-carboxyfluorescein), and 1.25 U AmpliTaq Gold DNA polymerase (PerkinElmer/Applied Biosystems). Real-time PCR was performed and analyzed by the dual-labeled fluorogenic probe method using an ABI Prism 7700 sequence detector (PerkinElmer/Applied Biosystems). Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer’s technical bulletin. A standard curve was generated using the fluorescent data from the 10-fold serial dilutions of total RNA of the highest expression sample. This was then used to calculate the relative amounts of target mRNA in test samples. Quantities of all targets in test samples were normalized to the corresponding GAPDH RNA transcript in the skin samples and 18S RNA transcripts in cultured keratinocytes.

Statistical methods

Data were presented as the means ± SEM. Data were analyzed using unpaired two-tailed t test or one-way ANOVA followed by the Scheffe’s F test or Bonferroni-Dunn test for multiple comparisons. An associated probability (p value) of <0.05 was considered significant.

Results

GeneChip microarray studies

GeneChip microarray analyses were conducted on biopsies of skin lesions from six different AD and seven different psoriasis patients studying all known innate immunity genes available in the human U-95Av2 GeneChip Affymetrix probe array. The GeneChip array analysis yielded a hybridization intensity for each represented gene as the signal intensity. The difference between AD and psoriasis skin was defined as significant if 2-fold or greater difference in average hybridization signal intensity with a p value under 0.05 using a two-tailed unpaired t test was observed. Table I compares expression of various groups of those genes.
Table 1. Surveyed innate immunity genes in GeneChip microarray

| Classification                        | U-95 Av2 Accession No. | Mean AD ± SE | Mean Ps ± SE | t Test |
|----------------------------------------|------------------------|--------------|--------------|--------|
| Cytokines and chemokines               |                        |              |              |        |
| TNF-α                                  | 1852_at                | X02910       | 156 ± 32     | 162 ± 14 |
| IL-1β                                  | 39402_at               | M15330       | 195 ± 28     | 692 ± 229 |
| IL-6                                   | 38299_at               | X04430       | 38 ± 14      | 45 ± 11  |
| IL-12                                  | 563_at                 | M65290       | 18 ± 6       | 16 ± 3 * |
| IFN-γ                                  | 40702_at               | X13274       | 32 ± 11      | 54 ± 15  |
| IL-8                                   | 35372_r_at             | M17017       | 63 ± 12      | 600 ± 210 | p = 0.044 |
| IL-8/IRβ                               | 664_r_at               | L19593       | 62 ± 9       | 122 ± 12 | p = 0.0023 |
| IL-4                                   | 1574_s_at              | M13982       | 72 ± 17      | 68 ± 14  |
| IL-13                                  | 494_at                 | U31120       | 135 ± 35     | 84 ± 20  |
| Toll-like receptors                     |                        |              |              |        |
| TLR1                                   | 36243_at               | AL050262     | 97 ± 21      | 81 ± 17  |
| TLR2                                   | 40310_at               | AF051152     | 108 ± 10     | 161 ± 26 |
| TLR3                                   | 33488_at               | U88879       | 52 ± 16      | 25 ± 5   |
| TLR5                                   | 34473_at               | AF051151     | 62 ± 12      | 69 ± 15  |
| TLR6                                   | 34144_at               | AB020807     | 86 ± 18      | 79 ± 16  |
| Phagocytosis-promoting receptors       |                        |              |              |        |
| MARCO                                  | 40331_at               | AF035819     | 126 ± 41     | 94 ± 41  |
| β1 integrins                           | 32808_at               | X07979       | 2489 ± 132   | 2305 ± 228 |
| E-cadherin                             | 2082_s_at              | L08599       | 378 ± 69     | 379 ± 32 |
| CD36                                   | 36656_at               | M98399       | 49 ± 7       | 74 ± 11  |
| CD11b                                  | 38533_s_at             | J03925       | 52 ± 26      | 35 ± 11  |
| CD35                                   | 35892_at               | Y00816       | 38 ± 7       | 34 ± 10  |
| Mannose receptor                       | 36908_at               | M93221       | 312 ± 74     | 217 ± 38 |
| CD14                                   | 36661_s_at             | X06882       | 108 ± 10     | 161 ± 26 |
| Complement                              |                        |              |              |        |
| C1r                                    | 39409_at               | M14058       | 2869 ± 326   | 2467 ± 109 |
| C1q B chain                            | 38796_at               | X03084       | 620 ± 141    | 815 ± 249 |
| C1r                                    | 40496_at               | J04080       | 2485 ± 464   | 2310 ± 408 |
| C4                                     | 40766_at               | U24578       | 253 ± 44     | 200 ± 48 |
| C5                                     | 37816_at               | M57729       | 44 ± 9       | 32 ± 10  |
| Anti-microbial peptides, enzymes        | (from neutrophils)     |              |              |        |
| Defensin, α1                           | 31793_at               | AL036554     | 61 ± 12      | 73 ± 24  |
| Defensin, α4                           | 34546_at               | AI250799     | 66 ± 9       | 49 ± 14  |
| Defensin, α6                           | 34622_at               | U33317       | 19 ± 9       | 18 ± 3   |
| Defensin, α5                           | 34623_at               | M97295       | 49 ± 23      | 51 ± 14  |
| Azurocidin                             | 33963_at               | M96326       | 55 ± 9       | 34 ± 3   |
| Cathepsin C                            | 133_at                 | X87212       | 2875 ± 476   | 2063 ± 287 |
| Cathepsin D                            | 239_at                 | M93138       | 4496 ± 287   | 6488 ± 444 |
| Cathepsin H                            | 37021_at               | X16832       | 1630 ± 148   | 2604 ± 312 |
| iNOS                                   | 1948_f_at              | U31511       | 65 ± 9       | 299 ± 71  | p = 0.016 |
| HBD-1                                  | 39660_at               | AI309115     | 3255 ± 148   | 4279 ± 348  | p = 0.027 |
| HBD-2                                  | 32464_at               | AF071216     | 13312 ± 426  | 45863 ± 4177 | p = 0.00021 |
| Signal transduction                    |                        |              |              |        |
| MyD88                                  | 38536_at               | U70451       | 1063 ± 120   | 1235 ± 118 |
| TRAF6                                  | 1209_at                | U78798       | 72 ± 11      | 81 ± 12  |
| NF-κB1                                 | 1377_at                | M58603       | 743 ± 70     | 808 ± 61 |
| NF-κB2                                 | 40363_r_at             | U09609       | 78 ± 10      | 64 ± 8   |

*a Mean signal intensity ± SE of six AD skin biopsies and seven psoriasis (Ps) skin biopsies.
*b Two-tailed unpaired t-test comparing AD vs psoriasis.
*c Signal intensity under 100 is considered as under the detection limit by software used.
*d MARCO, macrophage scavenger receptor with collagenous structure; MyD88, myeloid differentiation factor 88; TRAF, TNFR-associated factor.

that belong to the innate immunity system in AD and psoriasis skin. Interestingly, most of them revealed similar levels of expression in atopic and psoriatic skin lesions. Using the GeneChip microarrays lower levels of IFN-γ, IL-1β, and TNF-α were observed in AD, as compared with psoriasis, skin. However these differences did not achieve statistically significant differences. In contrast, expression of HBD-2 (p = 0.00021), iNOS (p = 0.016), and IL-8 (p = 0.044) was found to be significantly lower in AD skin lesions as compared with psoriasis skin lesions. Expression of HBD-1, Toll-like receptor-2, CD14, cathepsin D and H were elevated in psoriatic skin samples but was not greater than a 2-fold difference.

**Immunohistochemistry of HBD-3 in AD, psoriasis, and normal skin**

The U-95Av2 GeneChip Affymetrix probe array that was used in this study did not include a probe for HBD-3 which is a newly identified antimicrobial peptide that has been found to be active against a broad spectrum of microbes (12, 13). In particular, HBD-3 has strong killing activity at physiologic concentrations.
against *S. aureus* by itself while HBD-2 requires presence of LL-37 to kill *S. aureus* (11). Therefore, a defect in HBD-3 could be an important deficit in the pathogenesis of AD skin infections.

To determine whether HBD-3 expression in AD was lower than psoriasis skin at the protein level, immunohistochemistry studies were conducted (Fig. 1). Strong HBD-3 staining in keratinocytes from psoriasis skin lesions (Fig. 1a) was observed while AD skin samples revealed absent staining within keratinocytes (Fig. 1b). The specificity of staining was confirmed with the absence of immunostaining using an isotype control in psoriasis skin sample (Fig. 1c) and the lack of staining in normal skin (Fig. 1d). The staining for HBD-3 of six different psoriasis skin biopsies was significantly stronger (*p* = 0.0005) than six different AD skin biopsies (Fig. 1e).

*Gene regulation of HBD-3 by cytokine treatment of cultured keratinocytes*

To examine the mechanism of suppression of HBD-3 in AD skin, mRNA expression of HBD-3 was studied in differentiated primary human keratinocyte cell cultures. Differentiated cells were stimulated with TNF-α, IFN-γ, or their combination for 24 h. Stimulated cells revealed increased HBD-2 and HBD-3 mRNA expression as compared with the medium control (Fig. 2), confirming that both antimicrobial peptides can be induced by proinflammatory cytokines. The combination of TNF-α/IFN-γ was the most potent stimuli for induction of HBD-3 transcripts in keratinocyte cultures. The proinflammatory cytokine IL-1β was also tested based on differences observed between skin from AD and psoriasis patients (Table I). However, stimulation of keratinocyte cultures with IL-1β did not augment the induction of HBD-2 or HBD-3 observed following stimulation with the TNF-α/IFN-γ combination (data not shown).

Because induction of HBD-2, iNOS and IL-8 are known to be inhibited by Th2 cytokines, Th2 cytokines might play an active role also in the inhibition of HBD-3 gene expression. To explore this possibility, we studied the effects of IL-4 and IL-13 on the gene expression of HBD-3 in primary human keratinocytes. Our current data was consistent with previous observations that Th2 cytokines suppress HBD-2 induction in transformed HaCAT cell lines (11). Fig. 2 shows that IL-4 in combination with IL-13 significantly inhibited the up-regulation of HBD-2 and HBD-3 mRNA by TNF-α and IFN-γ combination. Because AD lesions are characterized by a predominance of IL-4 and IL-13 expression (20, 21), the suppression of HBD-3 mRNA by these cytokines may represent an important mechanism for the observed low expression of HBD-3 in AD lesions.

*Real-time PCR of HBD-3 and cytokines in AD and psoriasis skin samples*

To confirm the finding in immunohistochemistry and to determine the clinical relevancy of cultured keratinocyte experiments in Fig. 2, real-time PCR to evaluate HBD-3 and expression of various cytokines (TNF-α, IFN-γ, IL-4, IL-8, and IL-13) in skin lesions from psoriasis and AD patients was performed. Although the gene array is a good method to determine initial differences between populations, evaluation of individual gene targets using real-time PCR is a much more sensitive and quantitative method. Because TNF-α and IFN-γ were the main inducers for HBDs as shown in Fig. 2, we initially evaluated levels of TNF-α and IFN-γ mRNA in skin samples of all study groups by real-time PCR. We found that TNF-α and IFN-γ gene expression were significantly increased (TNF-α, *p* = 0.0365; IFN-γ, *p* = 0.0192) in psoriasis as compared with AD skin samples (Fig. 3).

As shown in Fig. 4a, we found that HBD-3 mRNA expression was significantly lower (*p* = 0.0002) in AD skin lesions than in psoriasis skin lesions. Although IL-4 mRNA expression showed increased levels in AD (3.07 ± 1.38), as compared with psoriasis skin samples (0.70 ± 0.18), the gene expression level in AD was quite variable and did not reach statistically significant difference between these two skin conditions (*p* = 0.06). Expression of IL-8 showed a statistically significant up-regulation in psoriasis skin compared with AD, confirming the GeneChip results (data not shown). The most interesting relationship was observed for IL-13 (Fig. 4b). IL-13 mRNA expression was significantly higher in AD,

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1.** Immunohistochemistry demonstrates increased HBD-3 staining in the skin of psoriasis, as compared with AD, patients. a. Psoriasis skin lesions show strong staining for HBD-3 in their keratinocytes; b. AD skin lesions show weak staining within keratinocytes. c. Absence of immunostaining in isotype control in psoriasis skin sample. d. Minimal HBD-3 staining is observed in normal skin. e. Summary of HBD-3 staining intensity in six different AD patients, six different psoriasis patients, and four different normal control skin biopsies. The intensity of the immunostaining was graded as the percentage of keratinocytes which were positively stained with a score of 0 to 8, where 0 is no staining, 1 = up to 12.5%, 2 = up to 25% with stepwise increase in scoring by 12.5% until 8 = up to 100%.
as compared with psoriasis skin. Importantly, as shown in Fig. 4c, a statistically significant inverse relationship was found between in vitro mRNA expression of IL-13 and HBD-3 (\( p = 0.032, r = 0.456 \)). This data supports our observation in cultured keratinocytes that Th2 cytokines suppress HBD-3 expression.

**Discussion**

Our current study demonstrates the novel observation that a recently identified antimicrobial peptide, HBD-3, which independently has antimicrobial activity against a broad spectrum of microbes including *S. aureus* (12) is extremely low at both the mRNA and protein level in AD skin and may therefore contribute to the increased susceptibility of such patients to skin infections especially to *S. aureus*. We have also confirmed and extended previous reports of separately examined expression of individual antimicrobial molecules in AD. Using GeneChip microarray technology we found the orchestrated suppression of a constellation of innate immune response genes in AD skin lesions as compared with psoriasis skin lesions. In addition to HBD-3, we found that AD skin lesions contain significantly lower levels of iNOS, IL-8, and HBD-2 transcripts. Combined with a previous report that the levels of LL-37 and HBD-2 in psoriasis skin, but not AD skin, can kill *S. aureus* (11), and our current observation that the majority of AD patients have undetectable or negligible levels of other antimicrobial molecules, deficient production of iNOS and IL-8, the lack of an adequate inducible innate immune response likely accounts for the failure of patients with AD to mount an adequate host response against a variety of microbes. Due to the limited amounts of RNA that were isolated from normal skin samples we were unable to compare levels of innate immune response genes in normal skin vs AD skin using the GeneChip Microarray technology. However, a sufficient amount of RNA was isolated to evaluate individual target genes by real-time PCR.

We propose that the reason antimicrobial molecules such as HBD-3 are suppressed in AD skin may be due to the overexpression of Th2 cytokines (20, 21) and low levels of TNF-\( \alpha \), and IFN-\( \gamma \)
production in AD skin as compared with psoriasis. This concept is supported by the following findings in our current study. First, using real-time PCR we have found that inflammation in AD skin is associated with negligible levels of TNF-α and IFN-γ which are considered to be the main inducers of antimicrobial peptides. Second, we have demonstrated a significant inverse correlation between in vivo expression of IL-13 mRNA transcripts and levels of HBD-3 in AD and psoriasis skin lesions. High IL-13 expression in skin lesions was always associated with decreased expression of HBD-3 in AD skin. In contrast, psoriatic lesions showed high levels of HBD-3 transcripts and low IL-13 expression. IL-4 mRNA expression showed increased levels in AD, as compared with psoriasis skin samples, but the difference observed was not statistically significant. This may be accounted for by the transient nature of IL-4 mRNA expression in the skin because our skin biopsies were not timed for peak expression of IL-4 in AD and other studies have found significantly increased levels of IL-4 in AD, as compared with psoriasis (20). Third, IL-4 and IL-13 were found to suppress the generation of TNF-α- and IFN-γ-induced HBD-2 and HBD-3 in primary human keratinocytes. This is consistent with our recent report that IL-4 and IL-13 suppress the generation of TNF-α-induced HBD-2 in the HaCaT keratinocyte tumor cell line (11).

Our current study also confirms a previous report that iNOS is suppressed in AD skin compared with psoriasis skin (14). iNOS is known to be produced by keratinocytes, and can kill HSV (22–24), bacteria (25–27), and fungi through the generation of NO. It is induced by bacterial endotoxins, staphylococcal lipoteichoic acid, production in AD skin as compared with psoriasis. This concept is supported by the following findings in our current study. First, using real-time PCR we have found that inflammation in AD skin is associated with negligible levels of TNF-α and IFN-γ which are considered to be the main inducers of antimicrobial peptides. Second, we have demonstrated a significant inverse correlation between in vivo expression of IL-13 mRNA transcripts and levels of HBD-3 in AD and psoriasis skin lesions. High IL-13 expression in skin lesions was always associated with decreased expression of HBD-3 in AD skin. In contrast, psoriatic lesions showed high levels of HBD-3 transcripts and low IL-13 expression. IL-4 mRNA expression showed increased levels in AD, as compared with psoriasis skin samples, but the difference observed was not statistically significant. This may be accounted for by the transient nature of IL-4 mRNA expression in the skin because our skin biopsies were not timed for peak expression of IL-4 in AD and other studies have found significantly increased levels of IL-4 in AD, as compared with psoriasis (20). Third, IL-4 and IL-13 were found to suppress the generation of TNF-α- and IFN-γ-induced HBD-2 and HBD-3 in primary human keratinocytes. This is consistent with our recent report that IL-4 and IL-13 suppress the generation of TNF-α-induced HBD-2 in the HaCaT keratinocyte tumor cell line (11).

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TNF-α, IL-1β, and IL-8. Because AD skin is densely colonized with *S. aureus*, iNOS should be induced by staphylococcal lipoteichoic acid. Consistent with the inhibitory effects of Th2 cytokines on HBDs, induction of iNOS is known to be inhibited by IL-4 (28).

Interestingly, IL-8, a chemokine now referred to as CXC chemokine ligand 8, is one of the most potent neutrophil chemoattractants (29), and was found in our current study to be decreased at the mRNA level in the skin of AD compared with psoriasis. Neutrophils play an important role in bacterial killing cooperating with antimicrobial-specific Abs and complement. In contrast to psoriasis, absence of neutrophils is one of the most prominent histologic features of AD. Suppression of IL-8 expression may explain this phenomenon and could significantly impair host defense against various infectious agents in AD skin. It is well-established that IL-8 is induced by TNF-α and IL-1 through activation of the transcription factor, NF-κB. Several reports have indicated that Th2 cytokines can suppress IL-8 production in airway smooth muscle cells (30), bronchial epithelial cells (31), and monocytes (32). Therefore, increased expression of Th2 cytokines could cause IL-8 suppression as well in AD skin.

Taken together, the induction of various innate immune response genes has been found to be suppressed in AD skin, i.e., HBD-2, HBD-3, IL-8, and iNOS. These genes are known to be up-regulated by proinflammatory cytokines like TNF-α and IFN-γ. Interestingly, our data indicate that TNF-α and IFN-γ are decreased in AD skin, as compared with psoriasis, this by itself may result in a relative inability to induce production of the above mentioned innate immunity genes. Although psoriasis skin samples showed higher levels of IL-1β mRNA in psoriasis, as compared with AD skin, this does not account for the difference in antimicrobial peptide production in these two skin conditions because IL-1β does not induce HBDs in keratinocyte cell cultures (data not shown). This observation is consistent with a recent observation by Sorensen et al. (33) which also failed to demonstrate significant elevations in either HBD-2 or HBD-3 gene expression in primary keratinocytes following IL-1β stimulation.

TNF-α is now believed to mainly act through the activation of NF-κB transcription factor. To date, three NF-κB binding sites have been reported within the HBD-2 promoter region (34). Mutation analysis of HBD-2 promoter construct revealed that two of them were essential for full LPS responsiveness of HBD-2 gene (34).

An increasing number of reports on defensins and other antimicrobial peptides provide strong evidence that they possess important roles in antimicrobial host defense. Molecular mechanisms of their transcriptional regulation are, however, only starting to be delineated (35). The role of several inflammatory stimuli in up-regulation of HBD-3 expression has been studied in epithelial cells (12, 36). TNF-α was found to be able to induce HBD-3 gene expression in primary keratinocytes as well as in primary tracheal epithelial cells at physiologically relevant concentrations (10 ng/ml) within 6 h. In addition, contact of epithelial cells with heat inactivated Gram-negative and Gram-positive bacteria like *Pseudomonas aeruginosa* and *S. aureus*, respectively, induced HBD-3 mRNA (12). Interestingly, HBD-3 mRNA expression in fetal lung explants and gingival keratinocytes was shown to be markedly induced by IL-1β, despite the absence of NF-κB consensus sequences in the immediate 5’ flanking sequence or intron of the gene. It was suggested that HBD-3 expression can be regulated from NF-κB sites at the 5’ end of the HBD-2 gene (36). In our studies, TNF-α stimulation induced 4-fold increased production of HBD-3. IFN-γ stimulation as well appeared to be able to induce HBD-3 in our experiments. Previously genetic analysis reported the presence of IFN-γ response elements within the 5’ flanking region of HBD-3 gene (36). Based on our observations, the most potent transcriptional activation of HBD-3 was achieved by a combination of TNF-α and IFN-γ. Our data as well indicated that Th2 cytokines suppress HBD-3 induction.

Recently, it has been reported that Th2 cytokines can inhibit the combination of TNF-α/NF-κB system through activation of STAT6 which can act as a transcriptional inhibitor of NF-κB target genes (37–39). Because Th2 cytokines are at high levels in AD skin, they may act directly by inhibiting the TNF-α/NF-κB system and thus inhibit the expression of innate immunity genes in the AD skin. We suggest that STAT6 induced by IL-4 and IL-13 can interfere with NF-κB binding to the consensus target sequences for NF-κB. Similar observations have been reported in the experimental model of NF-κB induced osteoclastogenesis (37). An excess of unlabelled consensus sequence STAT6, but not its mutated form, inhibited NF-κB binding. The presence of exogenously added STAT6 protein as well inhibited NF-κB/DNA interaction. STAT6 acts as an NF-κB antagonist, perhaps allosterically or by binding to DNA sequences overlapping with that of NF-κB (37). An analogous mechanism was described by Bennett et al. (38) in this study IL-4 was found to inhibit TNF-induced NF-κB DNA binding due to STAT6 antagonism in the E-selectin gene promoter of endothelial cells. It has been established that STAT6 as well can compete with IFN-γ-induced STAT1 for IFN-γ activation sequences in the promoters of sensitive genes. Interestingly, STAT1 binding to the IFN-γ activation sequence motif induces transactivation, while STAT6 binding to the same motive has no transactivation potential (39).

In conclusion, we have demonstrated that AD, as compared with psoriasis skin lesions are associated with the suppression of a constellation of innate immunity genes. We speculate that this orchestrated suppression of innate immune response genes appears to be a combination of two events, due to the low levels of TNF-α/IFN-γ production in AD skin and inhibition of TNF-α/IFN-γ-induced expression of these genes by Th2 cytokines, likely as the result of competition between STAT6 and NF-κB or IFN-γ response elements (STAT1) for the same consensus sequence elements in the promoter regions of these innate immune response genes (Fig. 5). Importantly, our current report suggests a new concept to potentially explain the mechanism for frequent infections that accompany other Th2-mediated disease conditions, e.g., hyper-IgE syndrome (40), Netherton syndrome, and superinfection of bacteria in the parasite-infected organ (41). Although further studies are needed to confirm this speculation, our current study suggests that the blockage of Th2 cytokine action may augment innate immune responses in such disease conditions.

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