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Mechanism of HBD-3 deficiency in atopic dermatitis

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Abstract Extrinsic atopic dermatitis (EAD) and intrinsic atopic dermatitis (IAD) patients suffer from recurrent bacterial and viral infections. In this study, we demonstrate significantly decreased expression of human beta defensin (HBD)-3, a potent antimicrobial peptide (AMP), in lesional skin of both IAD ($p < 0.01$) and EAD patients ($p < 0.01$), as compared to psoriasis patients. Using primary keratinocytes from EAD and IAD patients, we determined that the deficiency in HBD-3 expression is an acquired rather than a constitutive defect. Furthermore, we demonstrate the down-regulatory effect of IL-4, IL-10, and IL-13 – which are over-expressed in the skin of AD patients – on HBD-3 expression in keratinocytes. Additionally, treatment of EAD skin explants with antibodies against IL-4, IL-10, and IL-13 augmented the expression of HBD-3. These studies suggest that neutralizing the Th2 cytokine milieu in AD skin may augment the innate immune response against bacterial and viral pathogens.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that affects approximately 17% of children and is associated with recurrent bacterial and viral skin infections [1–3]. AD can be broken down into two subsets: extrinsic AD (EAD) patients have elevated serum IgE and allergic sensitization and comprise approximately 70–80% of AD patients. Intrinsic AD (IAD) or non-atopic eczema patients make up 20–30% of AD patients and exhibit normal serum IgE levels and lack allergen-specific sensi-

Abbreviations: AD, atopic dermatitis; AMP, antimicrobial peptide; EAD, extrinsic atopic dermatitis; HBD, human beta-defensin; IAD, intrinsic atopic dermatitis; IL, interleukin; PBMC, peripheral blood mononuclear cells.

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tization [4]. It has been shown that patients with IAD, as compared to EAD, express significantly lower levels of IL-4 and IL-13 in their skin and peripheral blood [5–7]. This explains the normal levels of serum IgE found in IAD as compared to EAD. However, it does not explain why both groups of AD suffer from recurrent skin infections. Both IAD and EAD have been shown to over-express IL-10, a cytokine which inhibits or down-regulates the pro-inflammatory and Th1 immune responses which may promote host defense [8–12].

Human β -defensin (HBD)-3 is a 5-kDa peptide produced primarily in the epithelium [13]. In recent years, HBD-3 has been shown to exhibit anti-bacterial activity against a variety of bacteria including *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes* [13–15]. Recently we found that HBD-3 was decreased in the skin of AD patients [16]. Understanding the mechanism underlying the decrease in HBD-3 is important since approximately 90% of patients with AD are colonized with *S. aureus* [1,3].

The current studies were therefore conducted to investigate the expression of HBD-3 in eczematous skin lesions from IAD and EAD patients to determine whether HBD-3 was equally low in these two forms of AD and investigate the role of IL-4, IL-10, and IL-13, cytokines over-expressed in the skin of IAD and EAD patients [6], on the expression of HBD-3.

Materials and methods

Subjects

Study participants included six healthy controls (mean age: 44.2 years), seven IAD patients (mean age: 29.1 years; SCORAD: 22.2 ± 5.6 ; serum IgE: 34–146 kU/l), fourteen EAD patients (mean age: 38.7 years; SCORAD: 45.0 ± 11.5 ; serum IgE: 288–5000 kU/l), and eight psoriasis patients (mean age: 39.7 years; 15–40% skin involvement). Sera from AD patients were tested against house dust mite, *Aspergillus*, *Candida albicans*, birch pollen, timothy grass pollen, ragweed, apple, milk, egg, peanut, hazelnut, *Staphylococcus enterotoxin A*, and *Staphylococcus enterotoxin B* for specific IgE with the Immulite 2000 system (DPC Biemann, 61231 Bad Nauheim Germany). The skin prick test was conducted with the following allergens: house dust mite, grass, birch pollen, *Aspergillus*, *C. albicans*, milk, egg, soy, and peanut. Patients with IAD had no allergen-specific IgE and were skin prick test negative to inhalant and food allergens. In contrast, patients with EAD had allergen-specific IgE and were skin prick test positive to inhalant and food allergens.

None of the patients had received oral corticosteroids within 1 month of skin explant and topical corticosteroids were not allowed for a period of at least 1 week prior to enrollment. These studies were conducted according to the Declaration of the Helsinki Guidelines and approved by the institutional review board at National Jewish Medical and Research Center in Denver, University of Bonn Medical Center in Germany, and the Istituto Dermatologico dell'Immacolata in Italy. All subjects gave written informed consent prior to enrollment.

Peripheral blood mononuclear cells (PBMC) culture

PBMC were isolated from heparinized blood using Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) centrifugation and suspended in RPMI 1640 (Bio-Whittaker, Walkersville, MD) [17]. PBMC were isolated from normal subjects and seeded at 1×10^6 cells/ml in 25 cm² flasks in the presence or absence of 100 ng/ml of anti-CD3 for 24 h at 37°C. Following the incubation, supernatant was collected from stimulated PBMC and added to skin explants from EAD patients or cultured HaCaT keratinocyte cells (2×10^5 cells/well) in 24-well plates. In some experiments, IL-10 (50 ng/ml; R&D Systems, Minneapolis, MN) was added to PBMC cultures to investigate the effects of IL-10 on anti-CD3 stimulated normal PBMC.

Cytokine protein analysis

Cytokine quantification was performed on supernatants from stimulated PBMC. The Bio-Plex human cytokine 17-plex panel was purchased from Bio-Rad Laboratories (Hercules, CA) and analyzed using the Bio-Plex array reader (Bio-Rad Laboratories) according to the manufacturer's guidelines. Data were calculated as mean picograms (pg) per ml $\pm$ standard error.

Keratinocyte cultures

Primary keratinocytes were isolated from six patients with moderate to severe chronic IAD or EAD (age range: 19–45 years), six patients with moderate to severe chronic plaque psoriasis (age range: 32–49 years) and six healthy control subjects (age range: 25–50 years). In these experiments, epidermal sheets were procured from the roof of suction blisters raised on normal-appearing skin of the forearms of patients with IAD or EAD, patients with psoriasis or healthy subjects, and primary keratinocyte cultures were obtained as described [18]. Keratinocytes were routinely grown in serum-free Keratinocyte Growth Medium® (Clonetics, San Diego, CA), prepared from the essential nutrient solution Keratinocyte Basal Medium® supplemented with 10 ng/ml epidermal growth factor (Clonetics), 0.4 μ g/ml hydrocortisone (Clonetics), bovine pituitary extract (Clonetics) and antibiotics. To study HBD-3 gene expression, keratinocyte cultures were differentiated in the presence of 1.3 mM CaCl₂ for 3 days and then stimulated for 24 h with a combination of 20 ng/ml TNF- α (R&D Systems, Abingdon, U.K.) and 200 U/ml IFN- γ (R&D Systems). The cells were then washed once and homogenized in buffer RLT (Qiagen). Total RNA was isolated according to the manufacturer's protocol in the presence of DNase I (Qiagen) for real-time RT-PCR.

The HaCaT human keratinocyte cell line was grown in Dulbecco's modified Eagle's medium (Cellgro, Herndon, VA), supplemented with 10% fetal calf serum (Gemini Bio Products, Calabasas, CA) and 1% of the following: penicillin/streptomycin, L-glutamine, minimal essential medium (MEM) with non-essential amino acids (GIBCO, Grand Island, NY), MEM vitamins solution (GIBCO) until confluent. Once confluent, cells were split and seeded in 24-well plates at 2×10^5 cells/well and cultured for 24 h to allow adherence.

HaCaT cells were additionally stimulated with supernatants from anti-CD3 stimulated PBMC experiments. In

some cases, IL-4 (50 ng/ml; R&D Systems) and IL-13 (50 ng/ml; R&D Systems) were added to the cultures in order to determine the combinatory effect of IL-4/IL-13 on the keratinocytes and IL-10 on the pro-inflammatory cytokines and HBD-3 expression. Cells were cultured for 24 h and RNA isolated from HaCaT cells according to manufacturer's guidelines (Qiagen) for real-time RT-PCR.

Skin explant cultures

Skin explants were cultured under various conditions in complete RPMI 1640 (Bio-Whittaker). To investigate whether the baseline levels of HBD-3 expression are similar between normal and AD patients; punch biopsies were collected from non-lesional skin of 4 AD patients as well as 4 normal subjects and added to 96-well tissue culture plates. Culture supernatants from either media or anti-CD3 stimulated PBMC were added to skin explants and allowed to culture for 24 h. Following exposure, RNA was isolated from skin explants for real-time RT-PCR.

In another experiment, lesional skin from 4 EAD patients was cultured *ex vivo* in complete RPMI 1640 (Bio-Whittaker). Anti-IL-4 (1 µg/ml; R&D Systems), anti-IL-10 (1 µg/ml; R&D Systems) and anti-IL-13 (1 µg/ml; R&D Systems) were added to skin explants for 24 h to investigate the effect of Th2 cytokine neutralization on HBD-3 expression. Following exposure, RNA was isolated from skin explants for real-time RT-PCR.

RNA preparation and analysis

Total RNA was isolated from skin explants by chloroform:phenol extraction and isopropanol precipitation according to manufacturer's guidelines (Sigma Chemical Co., St. Louis, MO). RNeasy Mini Kits (Qiagen) were used according to the manufacturer's protocol to isolate RNA from cell cultures and to further purify RNA from skin explants. One microgram of RNA was reverse transcribed in a 20 µl reaction containing Random Primers (Invitrogen, Carlsbad, CA), RNase Inhibitor (Invitrogen), and Superscript II enzyme (Invitrogen) for 60 min at 42°C and then 70°C for 15 min. Real-time PCR was performed and analyzed by the dual-labeled fluorogenic probe method using an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA). Primers and probes for GAPDH were purchased from Applied Biosystems. HBD-3 primer and probes were prepared as previously described [16]. The TaqMan probes were purchased from Applied Biosystems and were 5' labeled with 6-carboxyfluorescein (FAM) and 3' labeled with 6-carboxy-tetramethylrhodamine (TAMRA). Amplification reactions were performed in Micro-Amp optical tubes (Applied Biosystems) in a 25 µl volume containing 2× TaqMan Master Mix (Applied Biosystems), 900 nM forward primer, 900 nM reverse primer, 200 nM probe, and the template RNA. Thermal cycling conditions were: 50°C for 2 min, 95°C for 10 min for one cycle. Subsequently 40 cycles of amplification were performed at 94°C for 15 s and 60°C for 1 min. 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 ten-fold serial dilutions of total RNA of the highest expression sample. To allow for comparisons between samples and groups, quanti-

ties of all targets in test samples were normalized to the corresponding GAPDH levels in the skin explants, cultured keratinocytes, and PBMC and expressed as HBD-3 normalized to GAPDH.

Statistical analyses

Statistical analysis was conducted using Graph Pad Prism, version 4.01 (San Diego, CA) and SAS, Inc. (Research Triangle Park, NC). Statistical differences in gene expression or protein staining between groups were determined using a nonparametric Mann–Whitney test with significant differences conferred when $p \leq 0.05$. In cases where multiple exposure groups were compared, data were analyzed by a one-way analysis of variance (ANOVA) and significant differences between exposure groups were determined by a Tukey–Kramer test. Regression analysis was performed using PROC GLM in SAS.

Results

HBD-3 gene expression levels in psoriasis and AD skin

Skin explants were collected from the lesional skin of psoriasis, IAD and EAD patients and were evaluated for HBD-3 gene expression using real-time RT-PCR. In order to make comparisons between samples and patient populations, HBD-3 gene expression was normalized to the endogenous control, GAPDH, for all samples. As shown in Fig. 1, HBD-3 expression was significantly higher in skin lesions from psoriasis (mean: 7092 ± 2165 ng HBD-3/ng GAPDH) as compared to lesions from IAD (mean: 460.4 ± 130.5 ; $p < 0.01$) or EAD (mean: 371.2 ± 104.5 ; $p < 0.01$). As expected, levels of HBD-3 in healthy normal skin (612.4 ± 103.3) were significantly lower ($p < 0.01$) than psoriasis lesions since non-inflamed skin does not express increased levels of HBD-3. No significant differences in HBD-3 expression were observed between IAD and EAD skin lesions.

In order to exclude disease severity as a reason for deficient HBD-3 expression, we investigated the relationship between SCORAD and HBD-3 gene expression using regression analysis. There was no correlation between the severity of

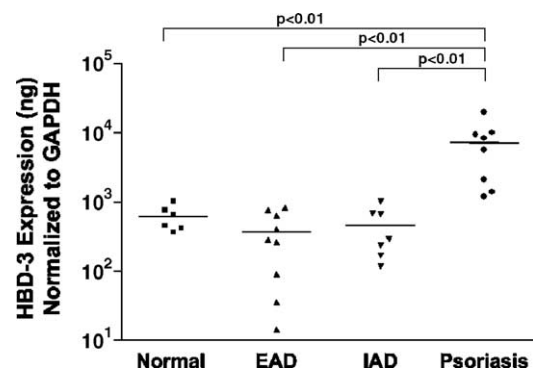


Figure 1 Decreased HBD-3 gene expression in lesional EAD and IAD skin. RNA was isolated from the skin of normal subjects ($n=6$), EAD ($n=9$), IAD ($n=7$), and psoriasis ($n=8$) patients and the levels of HBD-3 evaluated by real-time RT-PCR.

skin disease and HBD-3 expression ($p=0.36$) (data not shown).

Pro-inflammatory cytokines fail to induce HBD-3 in AD

HBD-3 is not highly expressed in un-stimulated skin, but rather needs stimulation by pro-inflammatory cytokines to induce the expression of HBD-3. Therefore, PBMC were isolated from normal patients and stimulated with anti-CD3 to trigger the release of pro-inflammatory cytokines into the supernatant, which would be used in subsequent experiments. Anti-CD3 stimulation of PBMC induced significantly higher levels of IL-1 β (3888 ± 612 pg/ml; $p<0.001$), IL-6 (6376 ± 948 pg/ml; $p<0.001$), TNF- α (20010 ± 2953 pg/ml; $p<0.001$), and IFN- γ (6393 ± 2229 pg/ml) as compared to media alone (IL-1 β : 236 ± 14 pg/ml; IL-6: 456 ± 42 pg/ml; TNF- α : 106 ± 3 pg/ml; IFN- γ : 105 ± 2 pg/ml) (Fig. 2A). These cytokines have been shown to induce HBD-2 and LL-37 expression [16,19–21] so purified TNF- α , IFN- γ , IL-1 β , and IL-6 were added to the HaCaT keratinocyte cell line to determine whether these cytokines also induce HBD-3. Fig. 2B illustrates that stimulation with the pro-inflammatory cytokines (20 ng/ml each) significantly induced HBD-3 expression (1.45 ± 0.13 ng HBD-3/ng GAPDH, $p<0.05$) as compared to media alone (0.84 ± 0.05). Furthermore, supernatant from anti-CD3 stimulated normal PBMC was added to HaCaT keratinocyte cells to evaluate whether it could induce the expression of HBD-3 in a similar manner. As shown in Fig. 2C, supernatant from anti-CD3 stimulated PBMC induced significantly greater expression of HBD-3 (3.00 ± 0.27 ng HBD-3/ng GAPDH; $p<0.01$) as compared to supernatant from media (1.35 ± 0.26).

Uninvolved skin was collected from 4 normal subjects and 4 EAD patients to determine whether HBD-3 could be induced in AD skin. Supernatants from either media or anti-CD3 stimulated PBMC were added to the skin biopsies and HBD-3 expression evaluated after 24 h. Supernatant from anti-CD3 stimulated PBMC induced significantly ($p<0.01$) greater expression of HBD-3 in skin from normal subjects (342 ± 102 μ g HBD-3/ng GAPDH) as compared to non-lesional skin from EAD patients (40 ± 17) (Fig. 3).

HBD-3 deficiency is acquired in AD

To investigate whether the reduced expression of HBD-3 in AD skin is constitutive or acquired, primary keratinocytes were isolated from the skin of IAD, EAD, psoriasis and normal subjects. Keratinocytes were cultured for three passages to eliminate the *in vivo* effects of inflammation or immune activation and then stimulated with TNF- α and IFN- γ to induce HBD-3. In comparison to non-treated cells (NT), HBD-3 was up-regulated following stimulation with TNF- α and IFN- γ (Fig. 4). However, there were no differences between the different keratinocytes types.

Down-regulatory role of Th2 cytokines on HBD-3 production

It has been shown that IL-4, IL-10, and IL-13 are over-expressed in AD patients [6,9,22]. While IL-4 and IL-13 can

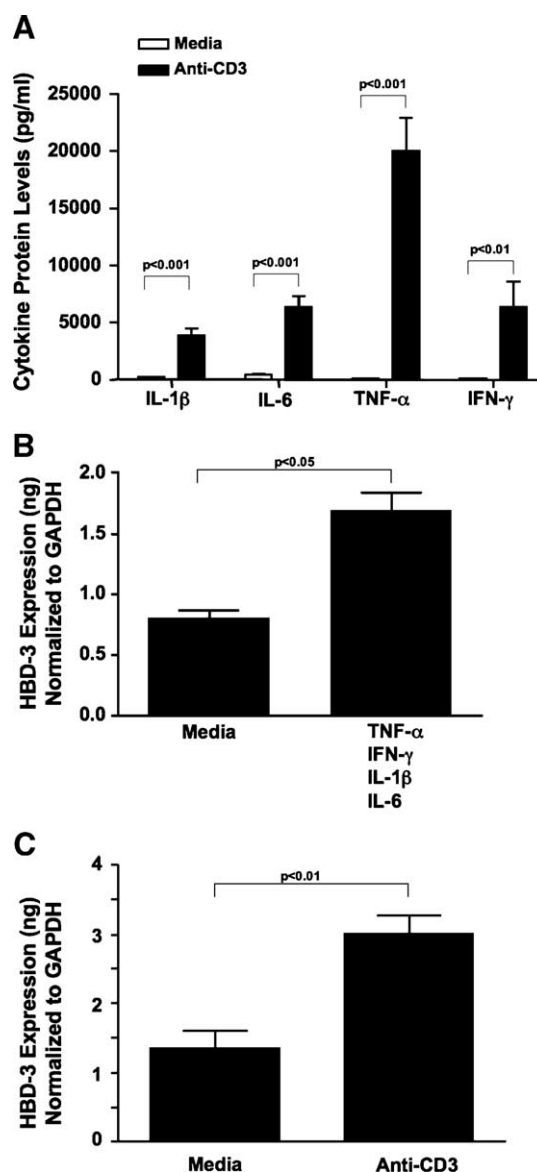


Figure 2 Anti-CD3 stimulation of PBMC induces pro-inflammatory cytokines which induce HBD-3 in human keratinocytes. PBMC were separated from the whole blood of normal subjects ($n=5$) and stimulated with 100 ng/ml of anti-CD3 for 24 h. (A) Levels of IL-1 β , IL-6, TNF- α , and IFN- γ were evaluated in the PBMC supernatants using the BioRad Multiplex assay. (B) HBD-3 expression was evaluated by real-time RT-PCR in HaCaT cells that were stimulated with purified IL-1 β (20 ng/ml), IL-6 (20 ng/ml), TNF- α (20 ng/ml), and IFN- γ (20 ng/ml). (C) HBD-3 expression was evaluated by real-time RT-PCR in HaCaT cells that were stimulated with supernatants from anti-CD3 stimulated PBMC.

directly down-regulate the expression of HBD-3 in keratinocytes [16], IL-10 does not act directly on keratinocytes [23] but can act indirectly by down-regulating the HBD-3 expression via inhibition of TNF- α and IFN- γ [9]. Therefore, we investigated the combinatorial effects of Th2 cytokines on HBD-3 expression. Fig. 5A illustrates that in the absence of IL-10, supernatants from anti-CD3 stimulated normal PBMC significantly induce the expression of HBD-3 (3.58 ± 0.40 ng HBD-3/ng GAPDH, $p<0.01$) as compared to media (1.06 ± 0.35). However, addition of IL-10 (1.81 ± 0.44)

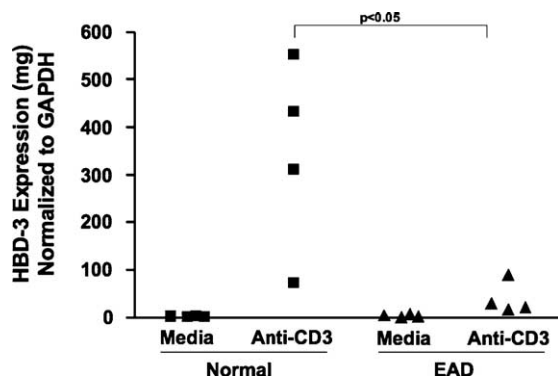


Figure 3 Pro-inflammatory cytokines induce HBD-3 in normal skin, but not AD skin. Supernatant from anti-CD3 stimulated PBMC was added to skin explants from normal subjects ($n=4$) or EAD patients ($n=4$) for 24 h. HBD-3 expression in the skin explants was evaluated by real-time RT-PCR.

or IL-4/IL-13 (1.86 ± 0.46) reduced the expression of HBD-3 by approximately 50%. The combination of the indirect effect of IL-10 on the pro-inflammatory cytokines and the direct effect of IL-4 and IL-13 on keratinocytes significantly reduced HBD-3 expression even further (1.13 ± 0.35 ; $p < 0.01$) in comparison with anti-CD3 alone. Direct stimulation of HaCaT cells with anti-CD3 did not induce HBD-3 (data not shown). To further define the down-regulatory effect of the Th2 cytokines, PBMC were collected from AD patients and stimulated with anti-CD3 in the same manner as above. In contrast to normal PBMC, anti-CD3 supernatants from AD patients failed to significantly induce HBD-3 expression (0.62 ± 0.15) in keratinocytes as compared to media alone (0.59 ± 0.10) (Fig. 5B).

Neutralization of Th2 cytokines augments HBD-3 expression in AD skin

Since the deficiency is acquired, we then investigated whether low HBD-3 in AD skin could be reversed. Lesional skin from EAD patients was cultured with neutralizing

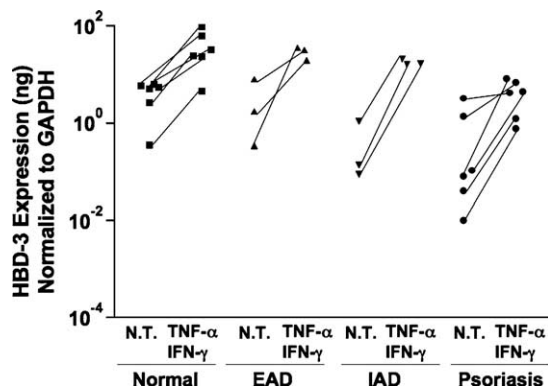


Figure 4 Reduced HBD-3 expression is an acquired defect in primary keratinocytes from AD patients. The levels of HBD-3 were evaluated by real-time RT-PCR in differentiated keratinocytes obtained from normal subjects ($n=6$), EAD patients ($n=3$), IAD patients ($n=3$), and psoriatic patients ($n=6$). HBD-3 was induced by 24-h stimulation with TNF- α plus IFN- γ . Samples treated with media alone are indicated by NT.

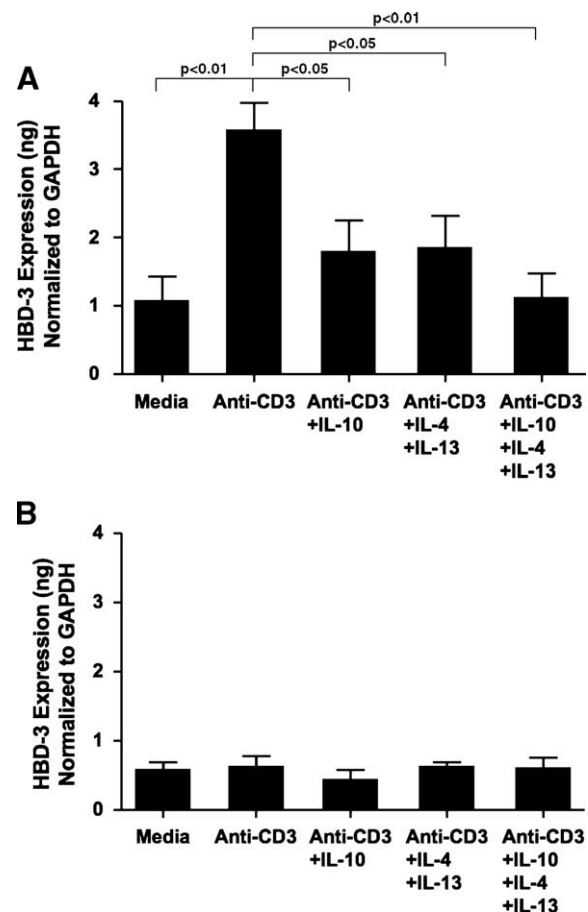


Figure 5 HBD-3 expression in HaCaT keratinocytes is down-regulated by Th2 cytokines. (A) RNA was isolated from keratinocytes stimulated with the supernatants from normal PBMC ($n=6$) cultured with media or anti-CD3 (100 ng/ml) in the presence or absence of Th2 cytokines. (B) RNA was isolated from keratinocytes stimulated with the supernatants from AD PBMC ($n=3$) cultured with media or anti-CD3 (100 ng/ml) in the presence or absence of Th2 cytokines. Levels of HBD-3 evaluated by real-time RT-PCR.

antibodies to IL-4, IL-10 and IL-13 and assayed for HBD-3 expression. Addition of neutralizing antibodies to skin explants resulted in a significant increase in HBD-3 expression (8644 ± 2036 ng HBD-3/ng GAPDH; $p < 0.05$) as compared to media alone (2662 ± 589) (Fig. 6). Treatment of AD skin with an isotype control antibody gave results similar to those observed with media alone (2328 ± 1383). To demonstrate that this effect was specific to AD skin, additional experiments were conducted using normal skin and lesional skin from psoriasis patients. Treatment with anti-IL-4, anti-IL-10, and anti-IL-13 had no significant effect on HBD-3 expression in either normal skin or lesional psoriasis skin (data not shown).

Discussion

The skin is the first line of host defense against invading microbial pathogens. In the event that this physical barrier is compromised, the innate immune response prevents

- O. Kreyden, R. Disch, B. Wuthrich, K. Blaser, H. Simon, T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis, *J. Invest. Dermatol.* 113 (1999) 628–634.
- [6] C.W. Jeong, K.S. Ahn, N.K. Rho, Y.D. Park, D.Y. Lee, J.H. Lee, E.S. Lee, J.M. Yang, Differential *in vivo* cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR, *Clin. Exp. Allergy* 33 (2003) 1717–1724.
- [7] M.K. Kagi, B. Wuthrich, E. Montano, J. Barandun, K. Blaser, C. Walker, Differential cytokine profiles in peripheral blood lymphocyte supernatant and skin biopsies from patients with different forms of atopic dermatitis, psoriasis, and normal individuals, *Int. Arch. Allergy Immunol.* 103 (1994) 332–340.
- [8] K. Asadullah, W. Sterry, K. Stephanek, D. Jasulaitis, M. Leupold, H. Audring, H.-D. Volk, W.-D. Docke, IL-10 is a key cytokine in psoriasis, *J. Clin. Invest.* 101 (1998) 783–794.
- [9] M.D. Howell, N. Novak, T. Bieber, S. Pastore, G. Girolomoni, M. Boguniewicz, J.E. Streib, C. Wong, R.L. Gallo, D.Y.M. Leung, IL-10 down-regulates anti-microbial peptide expression in atopic dermatitis, *J. Invest. Dermatol.* 125 (2005) 738–745.
- [10] D. Laouini, H. Alenius, P. Bryce, H. Oettgen, E. Tsitsikov, R.S. Geha, IL-10 is critical for Th2 responses in a murine model of allergic dermatitis, *J. Clin. Invest.* 112 (2003) 1058–1066.
- [11] J.D. Ohmen, J.M. Hanifin, B.J. Nickoloff, T.H. Rea, R. Wyzkowski, J. Kim, D. Jullien, T. McHugh, A.S. Nassif, S.C. Chan, R.L. Modlin, Overexpression of IL-10 in atopic dermatitis: contrasting cytokine patterns with delayed-type hypersensitivity reactions, *J. Immunol.* 154 (1995) 1956–1963.
- [12] M.R. Lester, M.F. Hofer, M. Gately, A. Trumble, D.Y.M. Leung, Down-regulating effects of IL-4 and IL-10 on the IFN- γ response in atopic dermatitis, *J. Immunol.* 154 (1995) 6174–6181.
- [13] J. Harder, J. Bartels, E. Christophers, J.M. Schroder, Isolation and characterization of human β -defensin-3, a novel human inducible peptide antibiotic, *J. Biol. Chem.* 276 (2001) 5707–5713.
- [14] K. Midorikawa, K. Ouhara, H. Komatsuzawa, T. Kawai, S. Yamada, T. Fujiwara, K. Yamazaki, K. Sayama, M.A. Taubman, H. Kurihara, K. Hashimoto, M. Sugai, *Staphylococcus aureus* susceptibility to innate antimicrobial peptides, B-defensins and CAP18, expressed by human keratinocytes, *Infect. Immun.* 71 (2003) 3730–3739.
- [15] G. Maisetta, G. Batoni, S. Esin, G. Raco, D. Bottai, F. Favilli, W. Florio, M. Campa, Susceptibility of *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* to bactericidal activity of human β -defensin 3 in biological fluids, *Antimicrob. Agents Chemother.* 49 (2005) 1245–1248.
- [16] I. Nomura, E. Goleva, M.D. Howell, Q.A. Hamid, P.Y. Ong, C.F. Hall, M.A. Darst, B. Gao, M. Boguniewicz, J.B. Travers, D.Y.M. Leung, Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes, *J. Immunol.* 171 (2003) 3262–3269.
- [17] P.J. Hauk, Q.A. Hamid, G.P. Chrousos, D.Y.M. Leung, Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens, *J. Allergy Clin. Immunol.* 105 (2000) 782–787.
- [18] S. Pastore, E. Fanales-Belasio, C. Albanesi, L.M. Chinni, A. Giannetti, G. Girolomoni, Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis: implications for sustained dendritic cell activation in the skin, *J. Clin. Invest.* 99 (1997) 3009–3017.
- [19] G. Erdag, J.R. Morgan, Interleukin-1 α and interleukin-6 enhance the antibacterial properties of cultured composite keratinocyte grafts, *Ann. Surg.* 235 (2002) 113–124.
- [20] A.Y. Liu, D. Destoumieux, A.V. Wong, C.H. Park, E.V. Valore, L. Liu, T. Ganz, Human β -defensin-2 production in keratinocytes is regulated by interleukin-1, bacteria, and the state of differentiation, *J. Invest. Dermatol.* 118 (2002) 275–281.
- [21] P.Y. Ong, T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R.L. Gallo, D.Y. Leung, Endogenous antimicrobial peptides and skin infections in atopic dermatitis, *N. Engl. J. Med.* 347 (2002) 1151–1160.
- [22] Q.A. Hamid, M. Boguniewicz, D.Y.M. Leung, Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis, *J. Clin. Invest.* 94 (1994) 870–876.
- [23] M. Seifert, B.H. Gruenberg, R. Sabat, P. Donner, G. Gruetz, H.-D. Volk, K. Wolk, K. Asadullah, Keratinocyte unresponsiveness towards interleukin-10: lack of specific binding due to deficient IL-10 receptor 1 expression, *Exp. Dermatol.* 12 (2003) 137–144.
- [24] J. Harder, J. Bartels, E. Christophers, J.M. Schroder, A peptide antibiotic from human skin, *Nature* 387 (1997) 861.
- [25] R.L. Gallo, M. Murakami, T. Ohtake, M. Zaiou, Biology and clinical relevance of naturally occurring antimicrobial peptides, *J. Allergy Clin. Immunol.* 110 (2002) 823–831.
- [26] M.D. Howell, R.L. Gallo, M. Boguniewicz, J.F. Jones, C. Wong, J.E. Streib, D.Y.M. Leung, Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus, *Immunity* 24 (2006) 341–348.
- [27] M.D. Howell, A. Wollenberg, R.L. Gallo, M. Flaig, J.E. Streib, C. Wong, T. Pavicic, M. Boguniewicz, D.Y.M. Leung, Cathelicidin deficiency predisposes to eczema herpeticum, *J. Allergy Clin. Immunol.* 117 (2006) 836–841.
- [28] V. Nizet, T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R.A. Dorschner, V. Pestonjamasp, J. Piraino, K.M. Huttner, R.L. Gallo, Innate antimicrobial peptide protects the skin from invasive bacterial infection, *Nature* 414 (2001) 454–457.
- [29] K. Takeda, T. Tanaka, W. Shi, M. Matsumoto, M. Minami, S. Kashiwamura, K. Nakanishi, N. Yoshida, T. Kishimoto, S. Akira, Essential role of STAT6 in IL-4 signalling, *Nature* 380 (1996) 627–630.
- [30] K. Takeda, M. Kamanaka, T. Tanaka, T. Kishimoto, S. Akira, Impaired IL-13 mediated functions of macrophages in STAT6-deficient mice, *J. Immunol.* 157 (1996) 3220–3222.
- [31] Y. Ohmori, T.A. Hamilton, Interleukin-4/STAT6 represses STAT1 and NF- κ B-dependent transcription through distinct mechanisms, *J. Biol. Chem.* 275 (2000) 38095–38103.
- [32] A.E. Chernoff, E.V. Granowitz, L. Shapiro, E. Vannier, G. Lonnemann, J.B. Angel, J.S. Kennedy, A.R. Rabson, S.M. Wolff, C.A. Dinarello, A randomized, controlled trial of IL-10 in humans: inhibition of inflammatory cytokine production and immune responses, *J. Immunol.* 154 (1995) 5492–5499.
- [33] A. D'Andrea, M. Aste-Amezaga, N.M. Valiante, X. Ma, M. Kubin, G. Trinchieri, Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ -production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells, *J. Exp. Med.* 178 (1993) 1041–1048.
- [34] R. de Waal Malefyt, J.S. Abrams, S.M. Zurawski, J.C. Lecron, S. Mohan-Peterson, B. Sanjanwala, B. Bennett, J. Silver, J.E. de Vries, H. Yssel, Differential regulation of IL-13 and IL-4 production by human CD8 $^{+}$ and CD4 $^{+}$ Th0, Th1 and Th2 T cell clones and EBV-transformed B cells, *Int. Immunol.* 7 (1995) 1405–1416.
- [35] D.F. Fiorentino, A. Zlotnik, T.R. Mosmann, M. Howard, A. O'Garra, IL-10 inhibits cytokine production by activated macrophages, *J. Immunol.* 147 (1991) 3815–3822.
- [36] S. Ito, P. Ansari, M. Sakatusume, H. Deickensheets, N. Vazquez, R.P. Donnelly, A.C. Lerner, D.S. Finbloom, Interleukin-10 inhibits expression of both interferon α - and interferon γ -induced genes by suppressing tyrosine phosphorylation, *Blood* 93 (1999) 1456–1463.
- [37] M.M. Song, K. Shuai, The Suppressor of Cytokine Signaling (SOCS) 1 and SOCS3 but Not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities, *J. Biol. Chem.* 273 (1998) 35056–35062.
- [38] R.M. Grossman, J. Krueger, D. Yourish, A. Granelli-Piperno, D.P. Murphy, L.T. May, T.S. Kupper, P.B. Sehgal, A.B. Gottlieb, Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured keratinocytes, *Proc. Natl. Acad. Sci.* 86 (1989) 6367–6371.