Epidermal Expression of Host Response Genes upon Skin Barrier Disruption in Normal Skin and Uninvolved Skin of Psoriasis and Atopic Dermatitis Patients

TO THE EDITOR

Recent studies have identified genetic risk factors for atopic dermatitis (AD) and psoriasis that may affect skin barrier function (Palmer et al., 2006; de Cid et al., 2009). Deficiencies in skin barrier formation or dysfunctional repair will expose epidermal cells to environmental stimuli, such as microbial components, which could evoke an inflammatory response, shaped by the genetic background of the host. These genetic studies, the demonstration of cell-autonomous differences between normal and patient keratinocytes (Zeeuwen et al., 2008), and the distinct antimicrobial protein (AMP) expression profiles in psoriasis and AD lesional skin (Ong et al., 2002; de Jongh et al., 2005) prompted us to investigate the responses of patients and controls to skin barrier disruption. We examined the effect of tape stripping and SDS-induced irritant contact dermatitis (Supplementary Materials and Methods online) on the expression of host defense genes in uninvolved epidermis of psoriasis and AD patients and healthy controls.

AMP gene expression levels were upregulated in all conditions of skin barrier disruption and subject groups, whereas expression profiles of pattern recognition receptors and inflammasome-related signaling genes were only marginally affected by barrier disruption, irrespective of the subject group (Table 1, Supplementary Tables 1 and 2 online, Supplementary Figure 1 online). As cytokines control the expression of AMPs, we analyzed the response of several psoriasis- and AD-associated cytokines on skin barrier disruption. However, no significant differences in expression of Th1, Th2, and Th17

cytokines were found (not shown). The sole significant difference between normal and patient skin was down-regulation of Toll-like receptor-3 in non-lesional skin of psoriasis and AD patients. Caspase-1 was the only gene that showed significant differential expression between the barrier disruption models (twofold higher expression in SDS-treated skin). At the protein level, AMP expression was strongly induced 48 hours after skin barrier disruption (Figure 1). Protein expression was rather similar in the different conditions of barrier disruption, even though there seemed to be a tendency toward stronger staining in the SDS-treated samples.

Interestingly, experimental barrier disruption induced similar high AMP levels in non-lesional skin of both psoriasis and AD patients, whereas in (untreated) lesional skin, these levels are much higher in psoriasis (Ong et al., 2002; de Jongh et al., 2005). Possibly, lesional cytokine levels account for the latter, as the AD-specific Th2 cytokines IL-4 and IL-13 were found to down-regulate AMP expression in keratinocytes (Nomura et al., 2003; Albanesi et al., 2007). This is a likely scenario *in vivo*, as IL-4 and IL-13 were only expressed in purified epidermal sheets of AD lesions and not in tape-stripped non-lesional skin of AD patients (not shown). We therefore examined the effect of Th2 cytokine addition on Th1 cytokine-induced AMP expression in cultured primary keratinocytes from healthy subjects and patients with psoriasis and AD. Cells were stimulated with IFN-γ, tumor necrosis factor-α and IL-1α (referred to as Th1 cytokines), Th2 cytokines (IL-4 and IL-13), or both. Indeed, Th2 cytokines caused

significant suppression of the Th1-mediated induction of DEFB4, DEFB103A, PI3, and SLPI in all groups (Supplementary Figure 2a online, Supplementary Table 3 online). Moreover, several AMPs were expressed at significantly lower levels in AD patient-derived keratinocytes compared with both healthy and psoriasis patient-derived keratinocytes on stimulation with both Th1 and Th2 cytokines (Supplementary Figure 2 online, Supplementary Table 4 online). At the protein level, Th1-mediated induction of hBD-2, but not elafin, was significantly suppressed by Th2 cytokines (Supplementary Figure 2b online, Supplementary Table 3 online). Stimulation by a combination of Th1 and Th2 cytokines resulted in upregulation of Toll-like receptor-3 in keratinocytes from psoriasis and AD patients, which was significant compared with intra-individual unstimulated samples, but not compared with healthy subjects (Supplementary Tables 3 and 4 online).

Previously, induction of psoriasin, hBD-2, hBD-3, and RNase 7 protein was reported after tape stripping of normal skin (Glaser et al., 2009; Harder et al., 2010), which is in line with our results. Our study shows that skin barrier disruption, either mechanically induced or through irritant contact dermatitis, elicits a striking increase in mRNA and protein expression levels of many AMPs, whereas the expression levels of pattern recognition receptors and some inflammasome-related signaling molecules were largely unaltered. Interestingly, almost all examined host defense gene expression levels were similarly influenced by barrier disruption in healthy skin and non-lesional skin of psoriasis or AD patients. This proves that non-lesional AD patient epidermis is equally

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Abbreviations: AD, atopic dermatitis; AMP, antimicrobial protein

Table 1. Relative epidermal mRNA expression levels of host defense genes after SDS application or tape stripping

HUGO gene symbol	Synonym	Fold change[1]				P-value compared with US[2]			
		SDS[3]	TS NS[4]	TS PS[5]	TS AD[6]	SDS	TS NS	TS PS	TS AD
Toll-like receptors									
TLR2		1.1	0.6	0.7	0.8	*0.921*	**0.019**	*0.077*	*0.147*
TLR3		0.6	0.4	0.1	0.2	**0.028**	**0.003**	**<0.001**	**<0.001**
TLR5		0.6	0.9	0.8	0.9	*0.203*	*0.309*	*0.139*	*0.262*
C-type lectins									
CLEC2B	CLECSF2	6.8	3.3	2.7	2.2	**0.001**	**0.013**	*0.152*	*0.348*
CLEC7A	Dectin-1	1.4	1.6	2.3	2.0	*0.679*	*0.204*	**0.003**	**0.028**
Nucleotide-binding oligomerization domain-like receptors									
NLRP1	NALP1	0.3	0.4	0.6	0.4	**0.007**	**0.006**	**0.020**	**0.005**
NLRP2	NALP2	3.3	1.3	0.4	0.3	*0.577*	*0.062*	*0.053*	**0.014**
NOD1	CARD4	0.6	0.9	2.3	1.3	**0.020**	*0.357*	*0.053*	*0.364*
NOD2	CARD15	1.1	5.7	6.8	4.9	*0.742*	*0.316*	**0.006**	**0.016**
RIG-like helicases									
DDX58	RIG-I	1.6	0.9	2.6	1.7	*0.305*	*0.373*	*0.311*	*0.131*
IFIH1	MDA5	1.6	1.9	4.1	3.1	**0.008**	*0.223*	*0.097*	**0.030**
Diverse									
RIPK2	RIP2	1.2	1.2	1.9	2.0	*0.463*	*0.166*	*0.180*	**0.045**
PYCARD	ASC	2.7	3.0	1.6	2.1	**0.006**	**0.012**	*0.310*	*0.054*
ICEBERG	Caspase-1 inhibitor	0.8	0.4	0.4	1.3	*0.286*	*0.058*	**0.005**	*0.989*
CASP1	ICE	1.6	0.8	0.7	1.2	*0.056*	*0.103*	**0.004**	*0.650*
IL1RN	IL-1RA	2.5	2.7	2.3	3.3	*0.748*	*0.465*	*0.196*	*0.061*
IL1A	IL-1α	4.9	1.1	2.3	9.6	*0.634*	*0.705*	*0.267*	*0.420*
IL1B	IL-1β	8.3	1.8	9.2	2.4	*0.191*	*0.932*	**0.047**	*0.119*
IL18	IL-18	1.2	2.0	0.8	0.7	**0.033**	*0.391*	*0.281*	**0.049**
Antimicrobial peptides									
DEFB4	hBD-2	1,901	21,760	9,394	9,359	**<0.001**	**0.001**	**0.001**	**0.002**
DEFB103A	hBD-3	12	43	43	41	**0.025**	**0.010**	**0.032**	**0.003**
PI3	Elafin	368	597	780	627	**<0.001**	**<0.001**	**<0.001**	**<0.001**
S100A7	Psoriasin	120	113	77	136	**<0.001**	**0.002**	**<0.001**	**<0.001**
S100A8	MRP8	941	333	139	655	**0.001**	**<0.001**	**<0.001**	**<0.001**
S100A9	MRP14	1,548	2,018	781	1,739	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SLPI	SLPI	6.3	5.8	3.2	4.8	**0.004**	**<0.001**	**0.004**	**<0.001**
LYZ	Lysozyme	7.9	3.1	2.6	7.4	*0.052*	**0.031**	**0.004**	*0.052*
CAMP	LL37	15	123	357	85	*0.091*	**<0.001**	**0.001**	**0.001**

Abbreviation: RIG, retinoid acid inducible gene.

[1] Relative epidermal mRNA expression levels calculated by taking the mean of the treated samples each divided by the relative mRNA expression level of its intra-individual control sample as calculated by Livak's delta–delta quantitative PCR cycle times method.

[2] P-value of dCt of treated samples compared with intra-individual control samples of untreated skin (US). Shaded: upregulated more than 10 times. Bold: P-value of analysis of variance and Bonferroni *post hoc* test below 0.05.

[3] Mean ratio in SDS-treated skin.

[4] Tape-stripped healthy normal skin (TS NS).

[5] Tape-stripped non-lesional skin of psoriasis patients (TS PS).

[6] Tape-stripped non-lesional skin of atopic dermatitis patients (TS AD).
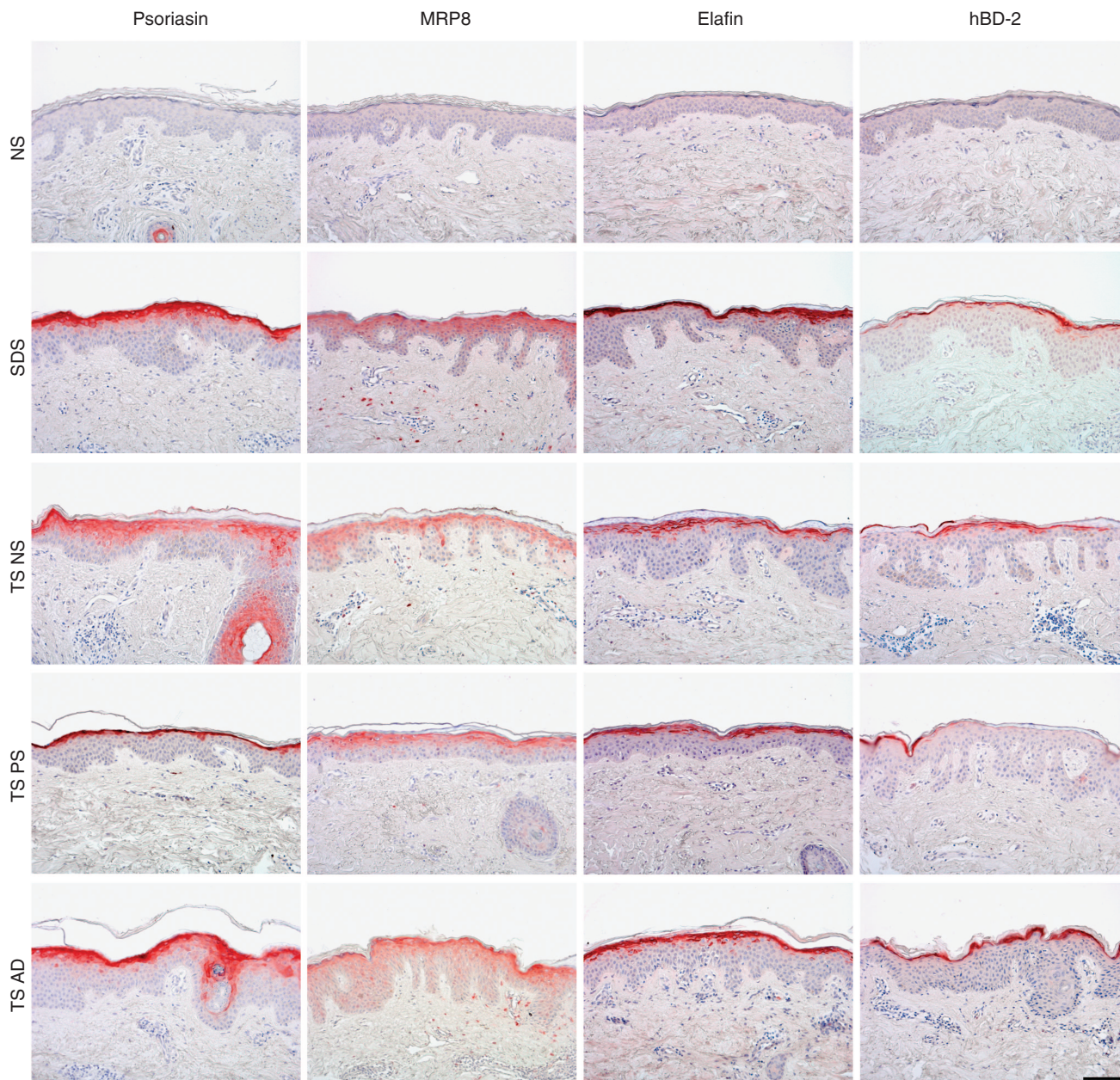
Figure 1. **Protein expression of psoriasin, MRP8, elafin, and hBD-2 after SDS application or tape stripping of human skin.** Immunohistochemical staining of normal skin, and skin 48 hours after barrier disruption by means of either SDS application of normal skin, or tape stripping of normal skin (TS NS) or uninvolved skin of psoriasis (TS PS) and atopic dermatitis (TS AD) patients. Treated skin was compared with intra-individual healthy control skin. Each picture is representative of data from six different individuals; the healthy controls, from 24 different individuals. Bar = 100 μm.

capable of producing massive amounts of AMPs on skin barrier perturbation as psoriasis patient epidermis. This is remarkable in view of the lower AMP levels in cultured keratinocytes from AD patients, either or not stimulated with Th1 or Th2 cytokines (Zeeuwen *et al.*, 2008) or both cytokine mixtures (this study). Previous studies showed that, depending on the specific AMP,

these molecules are expressed at equal or higher levels in lesional AD skin than in normal skin, but these expression levels are exceeded considerably by those in lesional psoriasis skin (de Jongh *et al.*, 2005; Gambichler *et al.*, 2008; Ballardini *et al.*, 2009; Harder *et al.*, 2010). Apart from cell-autonomous (genetically programmed) low AMP expression levels in lesional AD

keratinocytes, the particular local cyto-kine environment (Th1/Th17 versus Th2) may also be involved, as demonstrated by the Th2-mediated partial inhibition of Th1-induced AMP expression in kerati-nocytes from healthy subjects and pa-tients with AD and psoriasis.

Altogether, our data show that enhan-ced expression levels of epidermal AMPs *in vivo* can be induced by barrier

disruption, irrespective of the genetic predisposition of the keratinocytes (normal, psoriasis, or AD background). This phenomenon may possibly depend on the massive damage-induced release of preformed cytosolic stimuli, such as IL-1α. Modifying factors such as genetic programming (for example, filaggrin or LCE3B/C deficiency, differential sensitivity to cytokines) and cytokine environment could have a role in the repair process, which may be qualitatively different in psoriasis and AD. Continued barrier deficiency will stimulate the production of factors that induce inflammation and recruitment of immune cells, eventually including Th1/Th17 cells in psoriasis and mainly Th2 cells in AD. This process will also determine epidermal host defense gene expression levels: a full-blown antimicrobial defense in psoriasis or a dampened antimicrobial response that promotes skin colonization and superinfection as observed in AD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Decreased Concentration and Enhanced Metabolism of Sphingosine-1-Phosphate in Lesional Skin of Dogs with Atopic Dermatitis: Disturbed Sphingosine-1-Phosphate Homeostasis in Atopic Dermatitis

TO THE EDITOR

Sphingosine-1-phosphate (S1P) is a unique lipid in that on the one hand it is part of the lipid fraction securing the epidermal permeability barrier and on the other hand, it has been shown to act as a critical signaling molecule and to elicit a variety of partially contrasting cellular effects. The significance of S1P in immune cell regulation became obvious when it was discovered that the novel immunosuppressive drug FTY720 (fingolimod) causes lymphopenia via S1P signaling (Mandala et al., 2002). In skin, sphingosine can be cleaved from ceramides, which account for 30–40% of stratum corneum lipids (Herzinger *et al.*, 2007). Sphingosine can then be phosphorylated by sphingosine kinases to S1P, which binds to a family of G-protein-coupled receptors, termed $S1P_1$–$S1P_5$. S1P signaling is irreversibly inactivated by an S1P lyase.

Abbreviation: S1P, sphingosine-1-phosphate