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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SUPPLEMENTARY MATERIAL

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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

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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

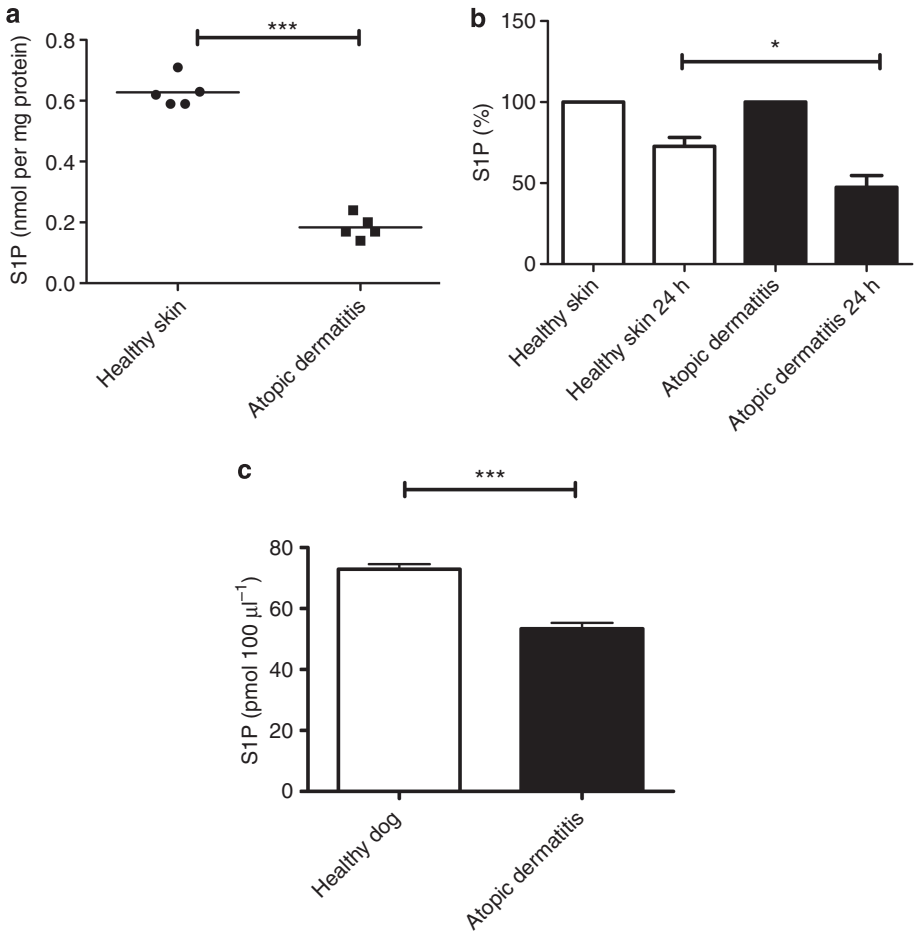

Figure 1. Concentration of sphingosin-1-phosphate (nmol of S1P per mg protein) in the epidermis of healthy skin and lesional skin of dogs suffering from atopic dermatitis. (**a**) When epidermal skin homogenate was spiked with S1P, there was a significantly higher degradation of S1P in the lesional epidermis of atopic dogs (**b**). There was also a significantly lower concentration of S1P in the plasma of dogs with atopic dermatitis (**c**). $n = 5$ (4 for plasma values) in each group. $^{*}P<0.05$, $^{***}P<0.001$.

In a recent study, we demonstrated the action of topically administered S1P in a murine model of allergic contact dermatitis. Interestingly, S1P inhibited the inflammatory reaction in the elicitation phase of allergic contact dermatitis. In the sensitization phase, S1P reduced the weight and cell count of the draining auricular lymph node, as well as of immigrated dendritic cells provoked by repetitive topical administration of the hapten. Correspondingly, the density of Langerhans cells in the epidermis was higher in S1P-treated mice than in mice treated with vehicle (Reines *et al.*, 2009).

In the light of previous reports suggesting that S1P-lyase activity is altered in atopic lesions in humans (Seo *et al.*, 2006) and dogs (Wood *et al.*, 2009), the present study was performed to determine the concentration of S1P in plasma and healthy skin compared with plasma and lesional skin of dogs with atopic dermatitis in order to evaluate a possible imbalance in the S1P–S1P–lyase axis in atopic dermatitis. With the consent of the owners, eight-millimeter biopsy punches were taken from the inguinal region of five atopic dogs. (Supplementary Table S1 online lists the sex, breed, and age of each dog.) Atopic dermatitis was diagnosed by excluding flea allergy and adverse food reactions as well as by skin biopsy and blood testing. Immediately after euthanasia, healthy skin was taken from the inguinal area of dogs, which had to be killed for reasons not related to this study. Alternatively, skin was taken, which had to be removed within a surgery procedure. Epidermal pieces taken from the biopsied samples were homogenized in culture medium. A portion of the homogenate was spiked with S1P. One nonspiked sample and one S1P-spiked sample were taken after thorough mixing and frozen at −20°C until measurement of S1P. The remaining two samples were cultivated at 37°C, in 5% CO_2 for 24 hours, and then frozen at −20°C. S1P was measured as previously described (Ruwisch *et al.*, 2001; Reines *et al.*, 2009).

The mean concentration of S1P in homogenized epidermis from healthy skin was 0.63 nmol per mg protein. The mean concentration of S1P in lesional skin was significantly lower, 0.18 nmol per mg protein (Figure 1a), whereas the concentration of sphingosine is comparable in the two groups (1.49 ± 0.92 nmol mg^{-1} in healthy skin vs. 1.48 ± 0.99 nmol mg^{-1} in atopic skin). Directly after spiking with S1P, there was a comparable concentration of S1P in

samples of healthy and lesional skin (2.95 ± 0.40 nmol mg^{-1} and 2.45 ± 0.21 nmol mg^{-1}, respectively). However, 24 hours after incubation at 37°C there was a nonsignificant decrease in samples of nonlesional skin (2.07 ± 0.19 nmol mg^{-1}), whereas S1P decreased significantly in lesional skin (1.16 ± 0.22 nmol mg^{-1}). The percentage of decrease was significantly higher in atopic lesions (28% decrease of S1P in healthy skin vs. 53% decrease in lesional skin (Figure 1b)).

In the plasma of dogs with healthy skin, the mean concentration of S1P was 72.9 pmol 100 µl^{-1}. The concentration was significantly lower—53.4 pmol 100 µl^{-1}—in dogs suffering from atopic dermatitis (Figure 1c). This was confirmed in a separate experiment using the serum of nine other dogs (21.5 pmol 100 µl^{-1} in dogs with healthy skin vs. 14.5 pmol 100 µl^{-1} in dogs with atopic dermatitis; $P<0.05$).

The results of this study suggest an imbalance in the S1P–S1P-lyase axis in atopic dermatitis. In a recent review (Kumar and Saba, 2009), S1P lyase was also characterized as a potential therapeutic target for immunological disorders, which is in accordance with the imbalance that we observed. It is noteworthy that the variation in S1P concentration in the skin of healthy dogs was so small given the wide variation in breeds and age (1.8–12 years) of the dogs studied. Similarly, the concentration range of S1P in lesional skin was very narrow in spite of the variety of breeds and ages (1–5 years). This is indicative of a very robust and significant alteration in atopic dogs, which is reflected in the altered plasma concentration.

The findings are consistent with the enhanced S1P-lyase activity found in mRNA of lesional skin from human atopic dermatitis patients (Seo *et al.*, 2006). In a gene expression study in dogs with atopic dermatitis, a significant difference in mRNA expression between atopic and healthy skin was found for S1P lyase (Wood *et al.*, 2009). However, unlike in human skin, the mRNA expression was downregulated rather than upregulated.

To rule out the possibility that S1P was dephosphorylated by the S1P phosphatase, sphingosine was measured in the homogenate of spiked samples. There was only a marginal increase of sphingosine within 24 hours of incubation (11.2 ± 1.6 nmol mg^{-1} to 13.5 ± 1.3 nmol mg^{-1} in healthy skin vs. 12.1 ± 1.4 nmol mg^{-1} to 13.3 ± 1.5 nmol mg^{-1} in atopic dermatitis), which indicates that S1P was not extensively dephosphorylated but, instead, metabolized by S1P lyase, resulting in a vastly reduced S1P concentration in dog skin with acutely affected atopic lesions (Figure 1a). Thus, it can be speculated that topical S1P has local immunosuppressive activity in the epidermis and that reduced S1P levels by enhanced S1P-lyase activity in skin might be involved in activation of Langerhans cells.

With regard to allergic diseases such as atopic dermatitis, it can be postulated that topical administration of S1P may be beneficial. Another promising aspect of topical administration is the effect of S1P on keratinocytes. S1P reduces proliferation and induces differentiation of keratinocytes. The hyperproliferative epidermis in chronic skin lesions resembling lichenification (Olivry and Hill, 2001) might be another therapeutic target, given S1P's potential to restore keratinocyte homeostasis (Herzinger *et al.*, 2007; Schuppel *et al.*, 2008).

It is interesting that the plasma concentration of S1P is also reduced; this indicates a systemic dysregulation of the S1P pathway, possibly stemming from genetic predisposition.

With regard to atopic dermatitis, it is noteworthy that there exist several similarities (as well as some differences) between the canine and human forms of the condition, as described in detail in a recently published review (Marsella and Girolomoni, 2009). Considering the enhanced S1P-lyase mRNA in atopic skin lesions in humans (Seo *et al.*, 2006), it would be worthwhile to determine the exact concentrations of S1P and lyase products in the skin and plasma of humans with atopic dermatitis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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