BJD
British Journal of Dermatology

Novel findings of Langerhans cells and interleukin-17 expression in relation to the acrosyringium and pustule in palmoplantar pustulosis

E. Hagforsen, H. Hedstrand, F. Nyberg†‡ and G. Michaëlsson

Department of Medical Sciences, University of Uppsala, SE-751 85 Uppsala, Sweden
†Department of Dermatology, University of Uppsala, Uppsala, Sweden
‡Institution for Clinical Sciences, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden

Correspondence
Eva Hagforsen.
E-mail: eva.hagforsen@medsci.uu.se

Accepted for publication
9 April 2010

Key words
acrosyringium, immunohistochemistry, innate immune system, nicotine, smoking

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2010.09819.x

Summary

Background Palmoplantar pustulosis (PPP) is a chronic and intensely inflammatory skin disease with pustules, erythema and scaling localized to the palms and soles. To date, no specific treatment is known. Earlier findings indicate the acrosyringium as the target for the inflammation.

Objectives To identify specific features of the PPP inflammatory cell infiltrate and mediators of inflammation, which might provide insight into the pathogenesis and possible future treatment of the disease.

Methods Skin biopsies were taken from 23 patients with typical PPP (23 from involved skin and seven from noninvolved skin) and from 18 healthy controls (10 nonsmokers, eight smokers). Cell infiltrates and inflammation mediators were studied with immunohistochemistry.

Results A strong inflammation was observed in lesional skin of PPP. Our main findings of Langerhans cells and interleukin-17 close to or in the acrosyringium differs from findings in psoriasis vulgaris. Other inflammatory cells such as CD4+, CD8+, regulatory T cells and CD11a+ cells were also accumulated close to the sweat duct in epidermis and papillary dermis. More CD4+, CD8+, Langerhans cells, plasmacytoid dendritic cells and a higher proportion of regulatory T cells/CD3+ cells were seen in noninvolved palmar skin from patients with PPP compared with healthy controls.

Conclusions Our novel findings indicate that the inflammation in PPP is initiated by the 'stand-by' innate immune system at the acrosyringium.

Palmoplantar pustulosis (PPP) is a chronic and intensely inflammatory skin disease, with pustules, erythema and scaling localized to the palms and soles. Ninety per cent of the patients are women and 95% of all patients are smokers at the onset of the disease.[1] Treatments used for plaque psoriasis are often less effective and might even cause pustular flares as a side-effect.[2]

The pustules are sterile and contain large numbers of eosinophils and neutrophils.[1] Eosinophils, lymphocytes and mast cells infiltrate the papillary dermis, mainly below the pustules. Mast cells are also increased in the reticular dermis of PPP skin.[1] The reason for this infiltration of inflammatory cells is not known. Structure loss in the acrosyringium in the lowest part of stratum corneum[1] and the migration of granulocytes upwards within the acrosyringium[3] indicate that this is the target for the inflammation.

The sweat gland apparatus seems to be an immune-competent structure that probably contributes to the defence of the skin. Within it, some proinflammatory cytokines such as interleukin (IL)-1α, IL-1β and tumour necrosis factor (TNF)-α are normally produced.[4] IL-1α is thought to be a link between the innate and adaptive immune response as the increased expression of adhesion molecules, initiated by IL-1α, is followed by recruitment of T cells. Moreover, adhesion molecule interactions with cells such as Langerhans cells (LCs) and T cells are essential effectors in the development of skin inflammation.

Based on our previous studies, with findings of patients with PPP having other autoimmune diseases and with immunoreactions of PPP sera on skin sections,[4] our hypothesis is that PPP is an autoimmune disease. The massive invasion of mast

cells and neutrophil and eosinophil granulocytes in PPP might indicate that the innate immune system is more important than previously assumed.

Identification of specific features of the PPP cell infiltrate and mediators of inflammation may provide insight into the pathogenesis. Surprisingly little is known today about the expression of inflammatory markers in PPP, compared with the large amount of data for psoriasis vulgaris. The aim of this study was to investigate in detail the components of the inflammation in PPP. A particular interest was put on morphology, i.e. the relation of inflammatory markers to the sweat duct apparatus, especially the sweat duct in epidermis and papillary dermis.

Materials and methods

Patients

In total, 23 punch biopsy specimens (3 mm) were taken from involved skin from patients (22 women aged 20–72 years and one man aged 60 years) with typical PPP of the hands and/or feet and seven biopsies (six women aged 28–65 years and one man aged 60 years) from noninvolved skin. None of the patients was taking beta-blockers or lithium. Most were using only emollients at the time of examination. At the onset of PPP 22 patients were smokers. At examination, five had stopped smoking or had reduced the number of cigarettes they smoked in recent years. Moreover, three patients had autoimmune thyroid disease and two had diabetes.

Control specimens from palmar skin were taken from 18 healthy persons: 10 nonsmokers (nine women aged 24–64 years and one man aged 24 years) and eight smokers (six women aged 41–55 years and two men aged 37 and 68 years). Biopsies were either immediately frozen in isopentane/acetone at −70 °C and stored until sectioned, or fixed in 4% buffered formalin and embedded in paraffin.

The local Medical Ethics Committee approved the study. All participants gave their informed consent.

Immunohistochemistry

The numbers of specimens used for the different antibodies are presented in Table S1 and the antibodies used in this study together with their dilution and supplier are shown in Table S2 (see Supporting Information). Controls, replacement of the primary antibody with IgG of the same isotype and from the same species as the primary antibody and omitting the primary antibody, were all negative.

Evaluation of the histopathological findings

All evaluations were made on coded sections by the same observer in a semiquantitative fashion. In examination of some antibodies, primary observations were performed under the supervision of E.H.

The staining intensity with intercellular adhesion molecule (ICAM)-1, CD62E and IL-1α antibodies was classified as absent, weak, medium or strong in the epidermis (stratum granulosum, stratum spinosum and stratum basale), endothelium (in the papillary and reticular dermis), the sweat gland and duct in the reticular dermis, papillary dermis, living epidermis and stratum corneum. The total number as well as the number of stained coils and ducts in the reticular dermis, papillary dermis, viable epidermis and stratum corneum were counted. The proportion of the numbers with each staining intensity was calculated. The expression of myxovirus protein A (MxA) was studied in epidermis, endothelium and inflammatory cells.

The numbers of cells that were CD4+, CD8+, CD1a+, CD56+, CD3−, FoxP3+, CD3+, CD3+ or BDCA-2+ were counted in epidermis, papillary dermis, papillary dermis below pustules and in reticular dermis. The percentage of FoxP3+ cells of the total number of CD3+ cells was calculated in the respective area.

The numbers of CD11a+, CD68+ and IL-17+ cells in epidermis were counted and the area of epidermis measured with the help of Leica Qwin (Leica, Wetzlar, Germany). In papillary and reticular dermis the CD11a+ area was measured and divided by the total area of the respective region, while the number of CD68+ cells was counted and related to the area of papillary and reticular dermis, respectively. The number of IL-17+ cells was counted in papillary and reticular dermis. The numbers of CD11a+ and IL-17+ cells were also counted around the sweat glands.

The inflammatory infiltrate was estimated in each section as follows: *, no or very few inflammatory cells; **, some inflammatory cells; ***, large number of inflammatory cells.

Statistics

Statistical differences were evaluated by the Mann–Whitney U-test using the software StatView (SAS Institute Inc., Cary, NC, U.S.A.). $P < 0{\cdot}05$ was considered as statistically significant.

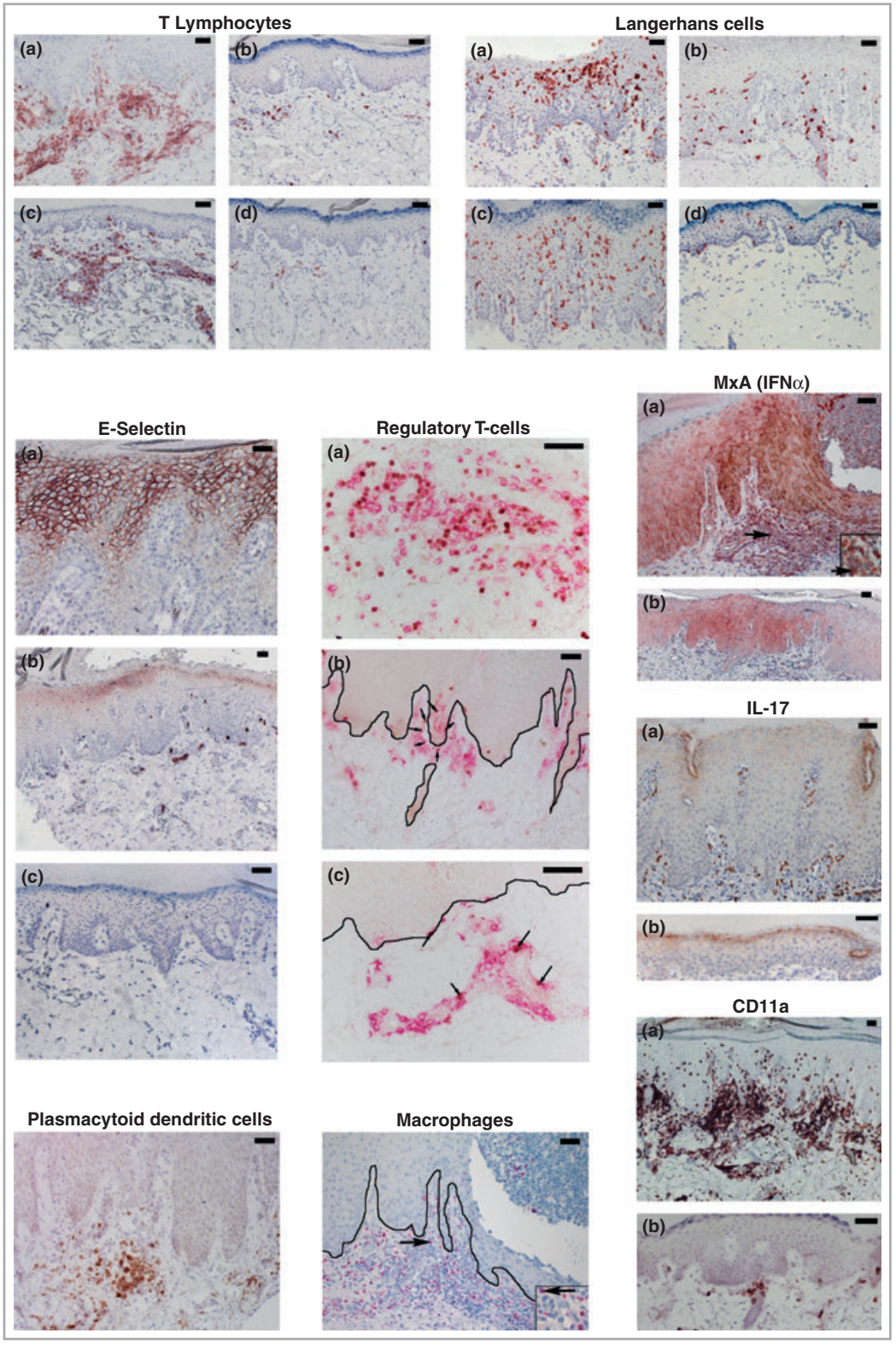
Results

In this study, we aimed at an in-depth investigation of markers of the innate and adaptive immune response in PPP, in relation to morphology of the lesion. Figures 1 and 2 show the results of the immunohistochemical analysis. Detailed data are published online in Table S3 (see Supporting Information). There was a very strong inflammation in lesional PPP skin, where both the innate and adaptive immune responses were represented, and the inflammatory infiltrates were significantly increased also in noninvolved palmar skin of patients with PPP as compared with healthy controls.

Inflammatory cells in relation to the pustules

More CD8+ cells (dermis, $P = 0{\cdot}01$) (Fig. 2c), FoxP3+ cells (regulatory T cells, Tregs) (papillary dermis, $P = 0{\cdot}03$) and

macrophages (papillary dermis, P = 0·003; reticular dermis, P = 0·004) were observed in PPP skin with pustules than in PPP skin without pustules. These cells were also, together with LCs and CD11a+ cells, abundant below the pustules. There were also significantly more plasmacytoid dendritic cells (pDCs) in sections with pustules than in those without pustules (epidermis, P = 0·005; reticular dermis, P = 0·04). All pustules had large numbers of IL-17+ cells within them. Two



of these sections had IL-17+ cells in epidermis just below the pustules.

Natural killer (NK) cells were found in low numbers and not in relation to any specific structure. There were more NK cells in reticular dermis in lesional PPP skin as compared with skin from healthy nonsmokers ($P = 0{\cdot}004$).

Inflammatory cells in relation to the sweat duct and gland in palmoplantar pustulosis skin

The number of LCs was increased around sweat ducts in epidermis in PPP skin compared with skin from healthy controls, smokers ($P = 0{\cdot}01$) and nonsmokers ($P = 0{\cdot}02$) (Fig. 2a). Both Tregs and LCs were observed close to sweat ducts in papillary dermis in PPP skin, as well as CD4+ and CD8+ T cells, and CD11a+ cells [Fig. 1: Regulatory T cells (b), Langerhans cells (b) and CD11a (a)].

Inflammatory cells in noninvolved palmoplantar pustulosis skin

There were significantly more CD4+ T cells ($P = 0{\cdot}02$) and CD8+ T cells ($P = 0{\cdot}04$) in dermis in noninvolved PPP skin compared with healthy controls (Fig. 2c). Both CD4+ and CD8+ T cells were observed in papillary dermis below epidermal sweat ducts. Cells expressing CD11a were more abundant than in healthy controls in epidermis ($P = 0{\cdot}02$), papillary dermis ($P = 0{\cdot}009$) and around sweat glands ($P = 0{\cdot}01$). There were also more LCs mm^{-2} in epidermis in nonlesional PPP skin compared with palmar skin from healthy smokers ($P = 0{\cdot}04$) and healthy nonsmokers ($P = 0{\cdot}01$). Significantly more pDCs were observed in papillary dermis in noninvolved PPP skin compared with healthy controls ($P = 0{\cdot}02$). Nonlesional PPP skin also had a higher proportion of Tregs/CD3+ cells compared with healthy controls ($P = 0{\cdot}03$).

Inflammatory markers in palmar skin in healthy controls (smokers and nonsmokers)

In papillary dermis in healthy smokers there were more LCs per section than in healthy nonsmokers ($P = 0{\cdot}01$) (Fig. 2a). pDCs were seen in palmar skin from healthy smokers (epidermis and reticular dermis), while none was seen in nonsmoking controls. More IL-17+ cells were seen around the sweat glands ($P = 0{\cdot}05$) in smoking controls compared with nonsmokers ($P = 0{\cdot}05$), but the reverse distribution was found in papillary dermis ($P = 0{\cdot}01$).

Other findings

The adhesion molecule ICAM-1 was expressed mostly on the endothelium and, to some extent, on inflammatory cells. The strongest expression was seen in the endothelium in lesional PPP skin (data not shown).

Fig 1. T lymphocytes. Palmoplantar pustulosis (PPP) skin with pustules showed larger numbers of CD4+ T cells and CD8+ T cells ($P = 0{\cdot}03$) as compared with PPP skin without pustules. (a) and (b) show CD4+ T cells in a patient with PPP and a healthy control, respectively. (c) and (d) show CD8+ T cells in a patient with PPP and a healthy control, respectively. **Langerhans cells (LCs).** (a) LCs were abundant in epidermis below pustules. (b) illustrates LCs gathering around the sweat duct in the papillary dermis in PPP skin. Around sweat ducts in viable epidermis the number of LCs was increased in lesional PPP skin compared with healthy controls, both in smokers ($P = 0{\cdot}014$) and in nonsmokers ($P = 0{\cdot}025$). (c) Specimen from involved PPP skin without pustules with a high number of LCs. In PPP skin some LCs were also observed in dermis. (d) Specimen from a healthy control with only a few LCs in epidermis. **E-selectin.** (a) Besides strong expression in the endothelium in lesional PPP skin, almost all (91%) of the PPP lesions showed expression of E-selectin in stratum spinosum (57% medium–strong). Moreover, 21% of the nonlesional PPP skin samples showed weak expression in epidermis. (b) Furthermore, we observed strong expression in the endothelium in papillary dermis. Note the expression on keratinocytes adjacent to the pustules. (c) No biopsies from healthy controls showed E-selectin expression. **Regulatory T cells (Tregs).** Double staining with CD3 and FoxP3 was used to visualize Tregs. Tregs are seen with brown nuclei. The percentage Tregs of the number of CD3+ cells in PPP skin was higher in all compartments, e.g. epidermis, papillary dermis and reticular dermis ($P = 0{\cdot}0009$, $P = 0{\cdot}0015$ and $P = 0{\cdot}0009$, respectively) compared with the percentage in healthy controls. (a) Lesional PPP skin with pustule that, compared with that without pustules, showed more Tregs and CD3+ T cells in the papillary dermis. (b) An interesting finding of Tregs around a sweat duct in papillary dermis (arrows) in PPP skin without pustules. (c) Only a few Tregs (arrows) were present in noninvolved skin from patients with PPP. Dermal-epidermal junction is marked in (b) and (c). **Myxovirus protein A (MxA)** is specifically induced by type I interferons (IFNs) and can thus be used as a substitute marker for IFN-α. Expression of MxA was seen in lesional PPP skin. The strongest expression was observed in sections with pustules, where the epidermis adjacent to the pustule had a pronounced expression (a). MxA was also seen in inflammatory cells especially in papillary dermis (arrow) (same cell in higher magnification in inset). (b) Moreover, we saw a strong expression near sweat ducts in epidermis (even if without pustules) in lesional PPP skin. **Plasmacytoid dendritic cells (pDCs).** Most pDCs were seen in papillary dermis in sections with large numbers of inflammatory cells. **Macrophages** were seen in all subjects, with the largest number in the papillary dermis in lesional PPP skin with pustules. In PPP skin with pustules there was significantly larger number mm^{-2} than in PPP skin without pustules (papillary dermis, $P = 0{\cdot}003$; reticular dermis, $P = 0{\cdot}004$). Image shows macrophages in a PPP specimen with pustule. Arrow indicates the macrophage in higher magnification in the inset. Dermal-epidermal junction is marked. **Interleukin-17 (IL-17)**-positive cells were found in palmar skin with the largest number in lesional PPP skin in dermis (papillary and reticular). (a) PPP lesional skin with expression in the acrosyringia. (b) Palmar skin from a healthy control with IL-17-like immunoreactivity in stratum granulosum and the upper part of the acrosyringium in living epidermis. **CD11a**-positive cells were abundant in lesional PPP skin seen in (a) with an accumulation close to the sweat ducts in papillary dermis. (b) illustrates CD11a-positive cells adjacent to the sweat duct in papillary dermis in a specimen from nonlesional PPP skin. All bars = 50 μm.

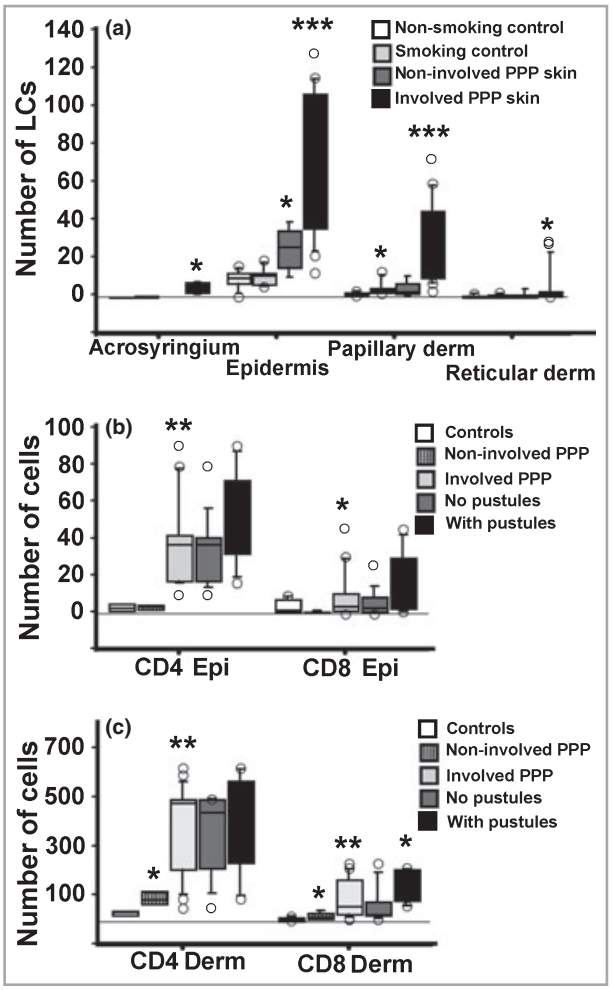


Fig 2. Boxplots showing that the number of (a) Langerhans cells (LCs) around the acrosyringium in epidermis was greatest in lesional palmoplantar pustulosis (PPP) skin. In epidermis there was a greater number mm^{-2} both in involved and in noninvolved PPP skin compared with healthy controls. In papillary and reticular dermis there was a greater number per section in PPP skin compared with healthy controls, and in papillary dermis the LCs per section were more numerous in skin from smokers than in nonsmokers. (b) CD4+ and CD8+ cells per section in epidermis were in greatest numbers in the involved PPP skin. (c) CD4+ and CD8+ cells per section in dermis were in greater numbers in both involved and noninvolved PPP skin compared with healthy controls. Furthermore, CD8+ cells per section in dermis were more abundant in PPP skin with pustules than in PPP skin without pustules. *P < 0·05; **P < 0·01; ***P < 0·001.

The strongest endothelial expression of E-selectin was observed in lesional PPP skin. E-selectin was also expressed in lesional PPP skin in keratinocytes especially in the stratum spinosum, particularly adjacent to the pustules [Fig. 1: E-selectin (a, b)]. The endothelial expression in nonlesional skin from patients with PPP was similar to that in healthy smoking controls, while nonsmokers had a very weak expression [Fig. 1: E-selectin (c)].

The proinflammatory cytokine IL-1α had a strong expression in the sweat gland and duct also in healthy controls. The coils had the strongest expression both in patients and controls. IL-1α expression was also seen in epidermis (data not shown).

MxA, a type I interferon (IFN)-induced protein, was expressed in epidermis (close to pustules and acrosyringium) and inflammatory cells in lesional PPP skin, with the strongest staining in sections with pustules [Fig. 1: MxA (a)]. Furthermore, there was a strong expression near sweat ducts in epidermis in lesional PPP skin without pustules [Fig. 1: MxA (b)]. Some inflammatory cells also showed expression of MxA [Fig. 1: MxA (a)].

IL-17 was not only expressed in inflammatory cells, but also in the sweat duct in viable epidermis both in palmar and in gluteal skin from patients with PPP and psoriasis, respectively, and in healthy controls (smokers and nonsmokers) [Fig. 1: IL-17 (a, b)]. Furthermore, in healthy controls, keratinocytes in stratum granulosum showed a IL-17-like immunoreactivity [Fig. 1: IL-17 (b)].

Discussion

The aim of this study was to characterize further the components of the inflammation in PPP. We have previously found the eccrine sweat gland apparatus to be the target for the inflammation in PPP[1,3] and our new results further underline the sweat gland as an immune-competent unit with an important role in the pathogenesis of PPP.

PPP seems to have a different genotype,[5] as well as phenotype, than psoriasis vulgaris. PPP is a chronic inflammatory skin disease and is very resistant to treatment, in contrast to psoriasis vulgaris.[6] In psoriasis vulgaris, activated T cells play the crucial role in the inflammation, while the first line of immune defence seems to be more important in PPP.[1] A central finding in this study was the accumulation of several of the inflammatory markers such as IL-17 in the acrosyringium and LCs close to the sweat duct in epidermis and papillary dermis. This further suggests initial immunological events taking place in the sweat duct in PPP. Furthermore, the increased number of LCs in PPP skin (lesional and nonlesional) (see Fig. 2) suggests an antigen-driven inflammation.

Another striking finding is that even in seemingly noninvolved palmar skin from patients with PPP there are significantly more inflammatory cells such as CD4+ and CD8+ T cells, LCs, CD11a+ cells and pDCs and a higher proportion of Tregs/CD3+ cells compared with healthy controls, indicating an ongoing inflammation.

The proinflammatory cytokine IL-1α was expressed in the sweat gland apparatus in both lesional and healthy skin. Keratinocytes release IL-1α when injured and inflammation is thereby initiated, for example by the induction of expression of adhesion molecules, chemokines, nitric oxide synthase and cyclooxygenase. Moreover, IL-1α stimulates secretion of IL-8,[7] an important neutrophil attractant. IL-1α expression in the sweat gland apparatus may have a pathophysiological role in PPP because Sato and Sato[8] reported that sweat from palms contained about six times higher concentration of IL-1α as

compared with axillae and 18 times higher concentration than sweat from the back.

Our finding of expression of IL-17 in the sweat gland apparatus (in both lesional and healthy skin) has, to our knowledge, not been reported before. However, it has been shown before that not only inflammatory cells are able to produce IL-17. Sakai *et al.*[9] detected IL-17 in human salivary glands in the cytoplasm of ductal epithelial cells from patients with Sjögren's syndrome, sicca syndrome and from healthy persons.

It has been suggested that IL-17 is important for host defence at the epithelial surface by inducing different classes of antimicrobial molecules.[10] Our findings of acrosyringeal expression of IL-17 indicate that the sweat duct is an active part of the skin barrier and physiologically important for the protection against microbes and other antigens from the surroundings.

In PPP large numbers of granulocytes gather in the pustules. The majority of these cells showed expression of IL-17. The IL-17 family consists of six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17A and IL-17F are primarily produced by activated T cells. In plaque psoriasis the T-cell population includes a separate type called Th17 cells, i.e. IL-17-producing T cells. In contrast, IL-17B, IL-17C, IL-17D and IL-17E are expressed in a variety of tissues. The IL-17 antibody used in this study can show, according to the company, cross-reactivity with IL-17B–F and the staining of the differentiated keratinocytes in the granular layer in epidermis in healthy control skin may reflect the expression of these other forms of IL-17. Their functions partially overlap those of IL-17A although they have not been as thoroughly investigated.[11]

There are several publications that support a role for IL-17 as a promoter of neutrophil accumulation and activation. Ferretti *et al.*[12] showed that IL-17 is produced mainly by CD4+ cells, as well as by neutrophils, and plays a role in the mobilization of lung neutrophils after bacterial challenge. Exposure to smoke enhances neutrophilic airway inflammation characterized by high pulmonary IL-17 levels.[13]

Several other factors can be involved in the accumulation of neutrophils in the acrosyringium. IL-8 is a well-known neutrophilic chemoattractant. PPP skin has a high expression of IL-8[14] and in palmar skin from healthy persons there was a high expression of IL-8 in the sweat gland and duct.[14] An interesting early discovery was made by Shelley *et al.*[15] who injected streptococcal pyogenes antigen into 'normal' skin in a patient with pustular psoriasis and saw neutrophils 'swarming' into the sweat duct in dermis; these cells moved inside further up to the terminal sweat duct – the acrosyringium – where they formed a pustule. The authors postulated that 'an unidentified invisible antigen was secreted by the sweat gland'.

Furthermore, nicotine has been found to induce human neutrophils to produce IL-8 *in vitro*.[16] This could be important for the pathogenesis of PPP as 95% of our patients were smokers before the onset of PPP and we have observed improvement of PPP after cessation of smoking.[17] In addition, nicotinic receptors are expressed in the acrosyringium[18] and because nicotine is excreted in sweat[19] it is able to affect this structure.

The increased number of LCs in PPP skin suggests an antigen-driven inflammation. A remarkable observation was that LCs were gathered around sweat ducts in living epidermis in PPP skin, with the possibility of recognizing antigens excreted through the sweat duct. Our findings of LCs in epidermis and dermis in nonlesional PPP skin and healthy smokers' skin has not been reported before and are in contrast to those reported in psoriasis vulgaris.[20]

More LCs have been found in bronchoalveolar lavage fluid from smokers compared with nonsmokers,[19] and LCs in PPP skin might be more prone to express antigens on their surface as dendritic cells of smokers were characterized by an increased expression of antigen presentation markers.[21]

CD11a, which was strongly expressed in lesional PPP skin, is a subunit of the lymphocyte function-associated antigen-1 expressed by B and T lymphocytes, granulocytes, monocytes and macrophages. There are several reports that treatment with monoclonal antibodies to CD11a has been found to be efficient in patients with PPP while anti-TNF therapy did not give improvement.[22–24] However, after the withdrawal of efalizumab, there is today no treatment that gives total remedy.

pDCs were found mostly in papillary dermis in lesional PPP skin. They are usually found in low numbers in blood and secondary lymphoid organs. In response to viral and some bacterial infections pDCs are recruited to the infection site and produce large numbers of type I IFNs. Recent findings show that pDCs also can infiltrate the basal epidermis and papillary dermis of patients with some noninfectious inflammatory diseases: psoriasis vulgaris, contact dermatitis and systemic lupus erythematosus. The IFN-α produced by the pDCs is known to drive the development of systemic lupus erythematosus and psoriasis.[25,26] The MxA expression implies the presence of biologically active IFN type I in the PPP skin. pDC-derived IFN-α is essential in driving the local activation and expansion of T cells and therefore linking innate immunity with T-cell signalling cascades.[27]

The number of Tregs was greater in PPP skin than in skin from healthy subjects (in epidermis, and papillary and reticular dermis). Tregs are CD4+ T lymphocytes that also express CD25 (the IL-2 receptor α chain) and can suppress immune response and prevent the development of autoimmune diseases. It has been observed that the number of Tregs was normal in peripheral blood and increased in psoriatic plaque,[28] but the suppressor cell activity was deficient, possibly leading to hyperproliferation of psoriatic pathogenic T cells.[29] This might also be relevant for the Tregs in PPP skin.

A possible theory of the pathogenesis of PPP could be that an antigen activates the immune-competent sweat gland and duct, which might lead to the inflammation in the acrosyringium and pustule formation. Nicotine excreted in the eccrine sweat duct might also influence the inflammation in a nonspecific way or facilitate autoimmune reactions. Also, in predisposed persons nicotine exposure might change the expression of the acrosyringia in a way that attracts LCs, and neutrophils are

attracted by the high concentration of IL-8 and IL-17 and migrate into the sweat duct and further outwards in the duct, forming the pustule in the acrosyringium.

In conclusion, our findings point to specific differences in the immunology of PPP and psoriasis vulgaris with a more pronounced innate defence in PPP with a central role for the acrosyringium and an antigen-driven response linked to smoking. In our future studies we will focus on the initial inflammatory event.

What's already known about this topic?

- Earlier findings indicate the acrosyringium as the target for the inflammation in palmoplantar pustulosis (PPP).

What does this study add?

- Our novel findings indicate that the inflammation in PPP is initiated by the 'stand-by' innate immune system at the acrosyingium.
- In seemingly noninvolved palmar skin from patients with PPP there is a significantly larger number of several inflammatory cells, indicating an ongoing inflammation.
- The increased number of Langerhans cells in PPP skin (lesional and nonlesional) suggests an antigen-driven inflammation.

Acknowledgments

This project was supported by grants from the Edvard Welander Foundation, the Swedish Psoriasis Association, the Swedish Medical Society, the Claes Groschinsky Foundation, the Faculty of Medicine, University of Uppsala and the Capio Research Foundation.

References

1 Eriksson MO, Hagforsen E, Lundin IP *et al.* Palmoplantar pustulosis: a clinical and immunohistological study. *Br J Dermatol* 1998; **138**:390–8.
2 Wollina U, Hansel G, Koch A *et al.* Tumor necrosis factor-alpha inhibitor-induced psoriasis or psoriasiform exanthemata: first 120 cases from the literature including a series of six new patients. *Am J Clin Dermatol* 2008; **9**:1–14.
3 Hagforsen E, Nordlind K, Michaëlsson G. Skin nerve fibres and their contacts with mast cells in patients with palmoplantar pustulosis. *Arch Dermatol Res* 2000; **292**:269–74.
4 Reitamo S, Anttila HS, Didierjean L, Saurat JH. Immunohistochemical identification of interleukin I alpha and beta in human eccrine sweat-gland apparatus. *Br J Dermatol* 1990; **122**:315–23.
5 Asumalahti K, Ameen M, Suomela S *et al.* Genetic analysis of PSORS1 distinguishes guttate psoriasis and palmoplantar pustulosis. *J Invest Dermatol* 2003; **120**:627–32.
6 Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. *Nature* 2007; **445**:866–73.
7 Steude J, Kulke R, Christophers E. Interleukin-1-stimulated secretion of interleukin-8 and growth-related oncogene-alpha demonstrates greatly enhanced keratinocyte growth in human raft cultured epidermis. *J Invest Dermatol* 2002; **119**:1254–60.
8 Sato K, Sato F. Interleukin-1 alpha in human sweat is functionally active and derived from the eccrine sweat gland. *Am J Physiol* 1994; **266**:R950–9.
9 Sakai A, Sugawara Y, Kuroishi T *et al.* Identification of IL-18 and Th17 cells in salivary glands of patients with Sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. *J Immunol* 2008; **181**:2898–906.
10 Wilson NJ, Boniface K, Chan JR *et al.* Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; **8**:950–7.
11 Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol* 2009; **9**:556–67.
12 Ferretti S, Bonneau O, Dubois GR *et al.* IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol* 2003; **170**:2106–12.
13 Melgert BN, Timens W, Kerstjens HA *et al.* Effects of 4 months of smoking in mice with ovalbumin-induced airway inflammation. *Clin Exp Allergy* 2007; **37**:1798–808.
14 Anttila HS, Reitamo S, Erkko P *et al.* Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis. *J Invest Dermatol* 1992; **98**:96–101.
15 Shelley WB, Wood MG, Beerman H. Pustular psoriasis elicited by streptococcal antigen and localized to the sweat pore. *J Invest Dermatol* 1975; **65**:466–71.
16 Iho S, Tanaka Y, Takauji R *et al.* Nicotine induces human neutrophils to produce IL-8 through the generation of peroxynitrite and subsequent activation of NF-kappaB. *J Leukoc Biol* 2003; **74**:942–51.
17 Michaëlsson G, Gustafsson K, Hagforsen E. The psoriasis variant palmoplantar pustulosis can be improved after cessation of smoking. *J Am Acad Dermatol* 2006; **54**:737–8.
18 Hagforsen E, Edvinsson M, Nordlind K *et al.* Expression of nicotinic receptors in the skin of patients with palmoplantar pustulosis. *Br J Dermatol* 2002; **146**:383–91.
19 Kintz P, Henrich A, Cirimele V *et al.* Nicotine monitoring in sweat with a sweat patch. *J Chromatogr B Biomed Sci Appl* 1998; **705**:357–61.
20 Bieber T, Braun-Falco O. Distribution of CD1a-positive cells in psoriatic skin during the evolution of the lesions. *Acta Derm Venereol (Stockh)* 1989; **69**:175–8.
21 Bratke K, Klug M, Bier A *et al.* Function-associated surface molecules on airway dendritic cells in cigarette smokers. *Am J Respir Cell Mol Biol* 2008; **38**:655–60.
22 Fretzin S, Crowley J, Jones L *et al.* Successful treatment of hand and foot psoriasis with efalizumab therapy. *J Drugs Dermatol* 2006; **5**:838–46.
23 Stinco G, Piccirillo F, Patrone P. Treatment of palmoplantar pustular psoriasis with efalizumab: a quick response with early recurrence. *Eur J Dermatol* 2008; **18**:91–2.
24 Wozel G, Vitez L. Palmoplantar pustular psoriasis: successful therapy with efalizumab after non-response to infliximab. *Acta Derm Venereol (Stockh)* 2008; **88**:169–70.
25 Albanesi C, Scarponi C, Bosisio D *et al.* Immune functions and recruitment of plasmacytoid dendritic cells in psoriasis. *Autoimmunity* 2010; **43**:215–19.
26 Ronnblom L, Pascual V. The innate immune system in SLE: type I interferons and dendritic cells. *Lupus* 2008; **17**:394–9.

27 Nestle FO, Conrad C, Tun-Kyi A *et al.* Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* 2005; **202**:135–43.
28 Bovenschen HJ, van Vlijmen-Willems IM, van de Kerkhof PC *et al.* Identification of lesional CD4+ CD25+ Foxp3+ regulatory T cells in psoriasis. *Dermatology* 2006; **213**:111–17.
29 Sugiyama H, Gyulai R, Toichi E *et al.* Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J Immunol* 2005; **174**:164–73.

Supporting Information

Additional Supporting Information may be found in the online version of the article:

Table S1. Number of specimens used for the different antibodies.

Table S2. The antibodies used in this study together with their dilution and supplier.

Table S3. Inflammatory cells in palmar skin of PPP patients and healthy subjects.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.