The thermal insulation of test emollients applied on the skin model was minimal. The grade of occlusion caused by different emollients led to varying thermal consequences depending e.g. on the amount of diffusing and evaporating water. On living skin, the emollients did not retard the cooling of the skin in the cold. On the contrary, the applied half was somewhat cooler in a majority of comparisons. However, white petrolatum gave often a subjective perception of a warming effect. False sensation of safety may form the principal cause for the increased risk of frostbite associated with the use of emollients.

When the warning symptoms of cold are weak or absent, the need for protection with efficient methods is neither recognized nor are the necessary protective measures carried out. Other mechanisms for the harmful effect may also be involved.

The moisturizing emollients, sports ointments or cosmetic moisturizers, often used for prevention and treatment of skin dryness during prolonged exposition to the cold, should be applied only when there is no actual risk of frostbite, preferably indoors.

List of original publications

Palmoplantar Pustulosis. Pathogenetic Studies with Special Reference to the Role of Nicotine

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Palmoplantar Pustulosis (PPP) is a chronic skin disease characterised by sterile intraepidermal pustules filled with neutrophils, and red and scaly skin on the palms and soles. It is considered to be a localised form of pustular psoriasis. Mainly women are affected and most patients are smokers. PPP is a rather common, chronic disease and difficult to treat. In spite of that, there are few studies on PPP and the pathogenesis is not known. There is no curative treatment for PPP today. The main purpose of the first study was to characterise the cellular components of the inflammation and, even more, to try to identify a target for the inflammation.

Fifty-nine patients (52 women, 7 men) with typical PPP of the palms and/or soles answered a questionnaire. Their smoking habits over the years were also investigated. Thirty-nine of the 59 patients (35 women, 4 men) were examined clinically.

There was a worsening effect of warm weather and stress in a high proportion of patients that indicated that the sweat gland apparatus might be a possible target for the inflammation. The fact that 95% of the patients were smokers at the onset of the disease (at a mean age of 42 years, range 15-66 years) pointed to nicotine as a possible precipitating factor for the disease. We observed a high prevalence of a number of autoimmune diseases (thyroid disease, coeliac disease, diabetes, vitiligo and alopecia areata) in the PPP patients. The association between autoimmune thyroid disease and PPP is well known, but the increased prevalence of coeliac disease in PPP patients has not been reported previously. The high prevalence of associated autoimmune disease in PPP gave us reason to consider the possibility that PPP itself might be an autoimmune disease affecting the skin and also the joints.

The association between PPP and autoimmune disease was further strengthened by the presence of antibodies to thyroglobulin/thyroperoxidase in 25% of the patients. IgA anti-
bodies to gliadin were present in 25%, compared to 9% in female healthy blood donors.

**Inflammatory cells**

The pustules were found to contain large numbers of eosinophils, an observation not made previously, as well as neutrophils. Furthermore, numerous eosinophils were present in the papillary dermis below the pustules. Another previously unreported feature was the massive infiltrates of mast cells in the upper dermis, especially in specimens with pustules.

There was a massive infiltrate of lymphocytes in the papillary dermis, with a tendency to accumulation below the pustule. The accumulation of the participating inflammatory cells below the pustules may indicate that there is an epidermal target for the inflammation that is not evenly distributed in the epidermis.

**The sweat gland and duct**

Results from immunohistochemistry on specimens from involved PPP skin with a keratin antibody (reported to give staining in the sweat gland apparatus) indicated that the acrosyringium (the intraepithelial duct) may be destroyed in PPP, which might reflect an inflammatory process at this site.

**Nerve fibres in palmoplantar pustulosis skin**

In view of the seemingly strong influence of stress and warm weather on the inflammatory activity in PPP a study of the distribution of nerve fibres and the presence of substance P (SP)- and calcitonin gene-related peptide (CGRP)-like immunoreactivity (LI) in involved skin in PPP patients and in healthy controls was undertaken.

The innervation of the sweat glands was studied with a general nerve marker, protein gene product 9.5 (PGP 9.5). In the patients the nerve fibres around the sweat glands seemed to be more or less fragmented, while in the controls they encircled the sweat gland without any interruptions. Image analysis showed that there were significantly fewer fibres around the sweat glands in the patients than in the controls (p=0.0006).

Close contacts between nerve fibres and mast cells give an opportunity for neuropeptides to degranulate these cells, and histamine as well as several other degranulation products would be released and further enhance the inflammatory reaction. The itching when new PPP pustules were formed (observed by the PPP patients) might be explained by release of histamine caused by neuropeptides.

The number of tryptase-positive mast cells in the papillary dermis was larger in the lesional palmar skin from PPP patients than in the healthy controls, and the number of contacts between mast cells and nerve fibres with PGP-LI was also significantly larger. As the number of nerve fibres was similar in PPP and control skin, but the number of mast cells was three times larger in the PPP skin than in the controls,
the increased number of contacts may be due to the increase in mast cells.

It has been reported that both the number of mast cells and the number of SP-positive nerve fibres in contact with mast cells are increased in psoriatic lesions. The increased number of contacts in PPP and in psoriatic skin may induce a more pronounced neurogenic influence on the inflammation, than in conditions without a mast cell increase.

Neuropeptide immunoreactivity in granulocytes

PGP 9.5- and SP-LI, but not CGRP-LI, were present in granulocytes. With use of double-staining, neutrophils showed SP-LI, whereas eosinophils were SP-negative. The neutrophils were observed, not only in the pustule or papillary dermis, but also within the sweat duct in the papillary dermis (possibly migrating towards the pustule). Substance P has previously been detected in human peripheral leukocytes from healthy subjects. There has also been found SP-LI in neutrophils in infiltrates in psoriatic lesions. This suggests that neutrophils are another possible source of SP, both in PPP and in psoriatic skin. As there is a massive granulocyte infiltration in PPP, the possible influence of SP may be more pronounced in PPP than in psoriasis, although it is probably of importance in both conditions. SP has been reported to stimulate proliferation of cultured keratinocytes in a dose-dependent manner. Keratinocytes in PPP skin might be influenced in a similar way as in psoriasis, where they have an increased proliferation rate.

The non-neuronal cholinergic system in palmoplantar pustulosis

Sweating and the sweat gland apparatus seem to play an important role in the pathogenesis of PPP. The sympathetic fibres that innervate the sweat glands are cholinergic, and acetylcholine (ACh) is the main inducer of sweating. The ACh-synthesising enzyme, choline acetyltransferase (ChAT), and the ACh-degrading enzyme, acetylcholinesterase (AChE), regulate the ACh level.

In recent years a non-neuronal cholinergic system has been recognised. Human keratinocytes can synthesise, secrete and degrade ACh. In addition to ChAT-like immunoreactivity (ChAT-LI) and AChE-LI in the epidermis, we have found that the eccrine glands and ducts display more intense ChAT and AChE reactivity than the epidermis. There were some differences between the distributions of ChAT-LI and AChE-LI in the epidermis, we have found that the eccrine glands and ducts display more intense ChAT and AChE reactivity than the epidermis. There were some differences between the distributions of ChAT-LI and AChE-LI: In the acrosyringium AChE-LI was most intense in the lower part of the stratum corneum corresponding to the site of the pustule in PPP, whereas no ChAT-LI was present in the acrosyringium at that level. On the other hand, there was marked ChAT-LI in the acrosyringium in the vital part of the epidermis.

Furthermore, the granulocytes displayed ChAT-LI, which was verified with Western blot, while the mast cells showed AChE-LI. In the healthy non-smokers and in the PPP patients AChE-LI was seen in 25% of the mast cells, while only 10% of the mast cells in the smoking controls showed AChE reactivity. This preliminary finding might indicate that smoking can influence the AChE activity. Smoking also seems to influence the ChAT expression since there were fewer ChAT-positive acrosyringia in PPP patients and healthy non-smokers.

The non-neuronal cholinergic system may be involved in inflammation. The presence of ChAT-LI in granulocytes and AChE-LI in mast cells may have implications for inflammatory processes in general in which neutrophil and eosinophil granulocytes are involved and smoking seems to influence the expression of ChAT and AChE but the relevance of these results for the ACh synthesis and degradation and cell differentiation and inflammation is not yet known.

Acetylcholine acts on cells through two different classes of receptors, nicotinic (nAChRs) and muscarinic acetylcholine receptors. Acetylcholine can alter a variety of cellular functions, such as cell adhesion and motility, by regulating Ca²⁺ influx via the nicotinic receptors. Nicotinic AChRs are ligand-gated ion channels, formed by various combinations of transmembrane subunits. Nicotine acts as an agonist on nAChRs and is thus able to reproduce the same effects on cells expressing these receptors as ACh, but in contrast to ACh, nicotine is not degraded by AChE. Nicotine is present in high concentrations in the blood.
of smokers and might contribute to desensitisation of the nAChRs and in this way influence their normal function.

The ion channels on keratinocytes can be composed of different combinations of α-3, α-5, β-2 and β-4 subunits or by α-7 subunits that can form functional nAChRs of their own.

With immunohistochemistry we have seen that eccrine glands and ducts are rich in nicotinic receptors. In healthy subjects the epidermis and, to an even greater extent, the eccrine sweat gland and its duct expressed both the α-3 and α-7 subunits of the nAChR. It was also evident that smoking influenced the staining intensity - but not the distribution - in the healthy controls.

The strongest staining of the α-7 subunit in the healthy controls (smokers and non-smokers) was noted in the keratinocytes in the stratum granulosum, with the most pronounced intensity in the acrosyringium. In the involved PPP skin, in which the stratum granulosum was abolished, there was a remarkably different pattern, especially around the acrosyringium and closest to the pustule, where the surface of the keratinocytes was strongly stained, with a fishnet-like appearance. The abnormal distribution of the α-7 nAChR in the keratinocytes in PPP skin may affect the differentiation, since keratinocytes are able to synthesise, release and degrade ACh. The highly abnormal α-7 nAChR distribution in PPP skin may indicate a possible association with smoking with an abnormal response to nicotine resulting in the inflammation.

**Palmoplantar pustulosis - an autoimmune disease?**

With regard to the massive inflammatory reaction in PPP skin and the high prevalence of autoimmune disease, it might be suspected that PPP could be an expression of one or several autoimmune reactions induced by smoking.

A first step in the testing of this hypothesis was to determine whether antibodies (ab) against nAChR could be detected in PPP serum with the method usually used in Sweden when diagnosing myasthenia gravis.

Increased concentrations of such antibodies, though less increased than in myasthenia gravis, was found in 19/45 (42%) of the PPP patients. Nicotinic AChR ab could not be detected in serum from controls with palmar eczema.

Immunofluorescence (IF) on palmar skin from both healthy smokers and non-smokers showed characteristic staining on endothelial cells in the papillary dermis in 68% of the PPP patients with nAChR ab and in 31% of the PPP patients without such antibodies (although mostly with lower intensity). Patients with ab against both nAChR and thyroid and/or gliadin showed the strongest IF intensity. Two of the 23 sera from patients with palmar eczema showed weak IF on the endothelial cells.

Furthermore, when the palmar skin from a non-smoker was replaced by skin from a healthy smoker there was a staining of the acrosyringium in addition to that of the endothelial cells. This illustrates the important role of nicotine, since the acrosyringium is the main target of the inflammation. These results strengthens our theory of a possible up-regulation of an autoantigen by smoking. The strong IF staining with sera from patients with several types of autoantibodies might be due to an “overlap” between these different antigens. This might explain the association between smoking and PPP and possibly also between PPP and autoimmune thyroid disease and coeliac disease.

**List of original publications**


IV. Hagforsen E, Edvinsson M, Nordlind K, Michaëllson G. Expression of α-3 and α-7 subunits of nicotinic acetylcholine receptors in the skin of patients with palmoplantar pustulosis. (submitted)

V. Hagforsen E, Mustafa A, Lefvert AK, Nordlind K, Michaëllson G. Antibodies against nicotinic receptors in serum from patients with palmoplantar pustulosis. (submitted)