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INVESTIGATIVE REPORT

Palmoplantar Pustulosis: an Autoimmune Disease Precipitated by Smoking?

EVA HAGFORSEN[1], AWDER MUSTAFA[2], ANN-KARI LEFVERT[2], KLAS NORDLIND[1] and GERD MICHAËLSSON[1]

[1]*Section of Dermatology and Venereology, Department of Medical Sciences, University Hospital, Uppsala and* [2]*Immunological Research Laboratory, Center for Molecular Medicine and Department of Medicine, Karolinska Institutet, Stockholm, Sweden*

Ninety-five percent of patients with palmoplantar pustulosis are smokers at onset of the disease. The aim of this study was to determine whether these patients have serum antibodies to nicotinic acetylcholine receptors (nAChR ab) and if their sera induce a specific immunofluorescence in normal palmar skin. Sera from 45 patients with palmoplantar pustulosis and 23 patients with chronic hand eczema were analysed for muscle nAChR ab, and immunofluorescence was performed on healthy palmar skin. Forty-two percent of the patients with palmoplantar pustulosis but none of the eczema patients had raised levels of nAChR ab. Immunofluorescence showed staining on endothelial cells in the papillary dermis in 47% of all sera from patients with palmoplantar pustulosis and in those with nAChR ab in 68%. On palmar skin from smokers there was also a staining of the sweat duct. Sera from patients with chronic hand eczema were negative. Our findings indicate that palmoplantar pustulosis is an autoimmune disease, possibly induced by smoking. ***Key words: gliadin and thyroid antibodies; immunofluorescence; nicotinic receptor autoantibodies.***

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Eva Hagforsen, Section of Dermatology and Venereology, Department of Medical Sciences, University Hospital, Uppsala, Sweden.
E-mail: eva.hagforsen@medsci.uu.se

Palmoplantar pustulosis (PPP) is a common chronic skin disease with unknown pathogenesis. It more often affects women than men, and 95% of the patients with PPP are smokers at onset of the disease (1). PPP is characterized by sterile pustules and usually also erythematous, scaly skin on the palms and soles; in some patients it is accompanied by psoriasis vulgaris.

We have found that the intense inflammation with massive infiltration of mast cells and lymphocytes in the papillary dermis which is most pronounced below the pustule is associated with migration of large numbers of neutrophil and eosinophil granulocytes outwards in the acrosyringium. This results in the formation of the pustule and a loss of the normal acrosyringium (1). Recently, we have also observed strong immunoreactivity of antibodies against the alpha-7 subunit of the nicotinic acetylcholine receptor (nAChR) in the acrosyringium in palmar skin from both healthy controls and patients with PPP (2). The papillary endothelium in palmar skin also expressed the alpha-3 and alpha-7 nAChR subunits. (In involved skin from PPP patients, the alpha-7 nAChR staining was stronger than in skin from healthy subjects – smokers and non-smokers.)

Autoimmune disease is common in patients with PPP and patients with PPP have an increased prevalence of autoimmune thyroid disease. There is also evidence that coeliac disease is more common among patients with PPP. Thus, in a recent study 6% of the PPP patients had coeliac disease and after exclusion of those with a verified coeliac disease, 20.5% had elevated levels of IgA antibodies to gliadin (3). There was also a high prevalence of both diabetes type 1 (4.8%) and diabetes type 2 (9.8%).

The possibility that PPP itself might be an autoimmune disease, possibly precipitated by smoking, has not previously been discussed. One aim of this study was to screen PPP sera for the presence of the type of antibodies to nicotinic acetylcholine receptors present in myasthenia gravis (a disease which is also often associated with other autoimmune diseases). Another aim was to investigate whether sera from patients with PPP might induce a specific immunofluorescence in palmar skin from healthy non-smokers or smokers and to investigate if there might be a relationship between the immunofluorescence pattern and the presence of nAChR antibodies (nAChR ab) as well as antibodies to thyroid antigens and to gliadin.

MATERIAL AND METHODS

Patients

Palmoplantar pustulosis. Serum samples were collected from 45 patients (39 women, 19–71 years old; 6 men, 36–70 years old) with typical PPP of the hands and/or feet. At onset of the PPP, 43 patients were smokers. At the time of the present study, nine had stopped smoking or had reduced the number of cigarettes in recent years, but none of the patients was aware of any influence of their smoking on the PPP symptoms. Five of the patients had previously been diagnosed as having

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hypothyroidism, 2 hyperthyroidism, 4 coeliac disease, 1 manic-depressive disease, 4 had type-1 diabetes and 4 type-2 diabetes. The patients had serum antibodies to: thyroglobulin (6), thyroperoxidase (10), parietal cells (2) and IgA antibodies to gliadin (13). For analysis of these antibodies, routine methods were used as previously reported (1).

No patients were receiving any systemic treatment for their PPP. Most used only emollients, but topical corticosteroids were used during exacerbation of the PPP.

Chronic hand eczema. For comparison with the results from PPP sera, sera were also obtained from 23 patients with chronic hand eczema, but otherwise healthy (15 women, 20–80 years old and 8 men, 27–61 years old). Fifteen of these 23 patients had smoked for many years, but 6 had stopped smoking in recent years. Topical corticosteroids were used intermittently and emollients regularly. No patients had any systemic treatment.

Myasthenia gravis. Sera from seven patients with myasthenia gravis, all of whom had elevated levels of serum antibodies to muscle nicotinic acetylcholine receptors, were also included. None of these patients had any skin disease.

Healthy subjects. Sera from four healthy non-smoking subjects were also included.

Serum samples were frozen within a few hours and stored at -70°C.

The local Medical Ethics Committee at the University Hospital, Uppsala, approved the study.

Methods

Biopsies from healthy subjects. Three-millimetre punch biopsy specimens were taken from the palms (hypothenar region of healthy non-smoking and smoking persons, after intradermal injection of xylocaine-adrenaline). For comparison, biopsy specimens were also taken from the dorsal aspect of the forearm and from the gluteal region.

Antibodies against skeletal muscle nicotinic acetylcholine receptor in serum. Serum nAChR ab were measured in the 45 PPP sera – and for comparison also in 23 hand eczema sera – with a radioimmunoassay used for determination of acetylcholine receptor antibodies in myasthenia gravis as described by Lefvert et al. in 1978 (4). In brief, a preparation of cholinergic receptors from human skeletal muscle was incubated with radiolabelled alpha-bungarotoxin, serum was added and the toxin-receptor-IgG complex was precipitated using anti-human IgG. The precipitate was separated and washed by centrifugation. Radioactivity (counts per minute) was determined and the concentration of receptor antibodies in arbitrary units was calculated.

Immunofluorescence. The specimens taken from healthy subjects (non-smokers and smokers) were frozen directly in isopentane-acetone at -70°C. Sections, 6 μm thick, were fixed in acetone for 10 min and treated with 10% normal rabbit serum (Vector, Burlingame, CA, USA) for 15 min to reduce non-specific staining. The sections were then incubated with serum from the patients (dilution 1/150) overnight at $+4^{\circ}$C. Fluorescein-isothiocyanate (FITC)-anti-human IgG (dilution 1/40; Dakopatts) was used as secondary antibody. Control with FITC anti-human IgG omitting the patient serum was negative. Between each incubation the sections were washed twice for 5 min in phosphate-buffered saline (PBS).

Double staining. Sections, 6 μm thick, were fixed in acetone. Thereafter they were allowed to react with 10% normal horse serum (Vector) for 10 min and then incubated with two mouse monoclonal anti-human endothelial antibodies, Q bend 10 (dilution 1/40; Skybio, Bedfordshire, UK) and CD 31 (dilution 1/40; Dakopatts), overnight at 4°C. Biotinylated horse anti-mouse IgG (dilution 1/200; Vector) was used as secondary antibody. Subsequently, the sections were incubated with Texas Red Streptavidin (dilution 1/100; Vector) for 30 min and then with 10% normal mouse serum (Dakopatts) for 60 min. The sections were then allowed to react with 10% normal rabbit serum for 10 min and thereafter with serum from the patients and FITC-anti-human IgG as above. To rule out non-specific staining, including overlapping between the fluorescence filters, three control stainings were performed: one using mouse IgG of the same isotypes and dilutions as the primary endothelial antibodies plus patient serum, another with mouse IgG and omitting the patient serum; as a third control the endothelial antibodies were used without the patient sera. Between each incubation, the sections were washed twice for 5 min in PBS.

Evaluation of the histopathological findings. All evaluations of the immunofluorescence were made on coded sections by the same observer. All parts of the sweat gland apparatus (duct and gland), epidermis and dermis were studied for the presence of staining. The staining intensity was classified as weak (+), medium (++) or strong (+++). The intensity of the immunofluorescence was also evaluated independently in a blind fashion by a second observer and the results compared with those of the main observer.

The specimens were examined under a 40× objective in a Leica DMLB microscope. Colour prints were obtained with a Leica DC 200 and Leica Qwin Image analysis system.

Statistics

The statistical significance of differences was calculated using Fisher's exact test.

RESULTS

Antibodies against skeletal muscle nicotinic acetylcholine receptors

Palmoplantar pustulosis. Elevated concentrations of antibodies against nAChR were present in 19 of the 45 sera (42%) from the patients with PPP. The mean antibody level in the positive sera was 0.75 arbitrary units/l (range 0.2–3.1) (normal values <0.2). No nAChR ab have been found in healthy subjects.

Patients with PPP and nAChR ab had a lower prevalence of antibodies against thyroglobulin and gliadin (6/19) than patients without nAChR ab (18/26) ($p = 0.017$) (Fig. 1).

Of the seven patients without antibodies against nAChR, thyroglobulin and gliadin, five had mild PPP.

Chronic hand eczema. None of the 23 patients with palmar eczema had raised levels of nAChR ab.

Immunofluorescence

The staining results in palmar skin from healthy non-smokers and smokers, obtained with sera from patients with PPP, chronic hand eczema, myasthenia gravis as well as healthy subjects, are summarized in Table I.

Sera from PPP patients and palmar skin from non-smokers. Immunofluorescence with PPP patient sera on

sections from normal palmar skin showed a specific staining pattern on structures in the papillary dermis. Thus, 21 of the 45 sera (46.7%) gave positive immunofluorescence staining on some cells, which often formed an endothelial-like pattern in the papillary dermis (Fig. 2a and Table I). The double staining with the endothelial antibodies showed that the positive immunofluorescence was localized to endothelial cells (Fig. 2b–d).

PPP sera and palmar skin from smokers. The PPP sera that produced the positive immunofluorescence in the skin from non-smokers also gave the same endothelial staining on the palmar skin sections from the smoking controls. In addition, 11 sera that gave an endothelial staining also produced a positive staining of the inner layer of the acrosyringium in the vital epidermis (Fig. 3) and also of dermal sweat ducts and sweat glands.

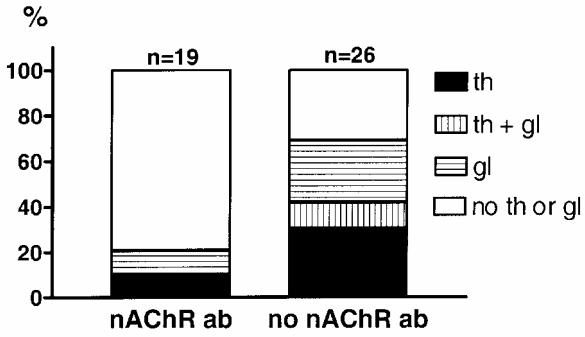

Fig. 1. The proportions of thyroid (th) and gliadin (gl) antibodies in palmoplantar pustulosis sera with and without nicotinic acetylcholine receptor antibodies (nAChR ab). There is a significantly lower prevalence of thyroid and gliadin antibodies in sera with nAChR ab than without ($p = 0.017$).

Staining of the acrosyringium and sweat ducts was not present when palmar skin from non-smokers was used (Table I).

PPP sera and hairy skin from non-smokers. Positive staining was also present in the skin samples from the forearm and the gluteal region from non-smokers, but the pattern differed from that of the palmar skin. The dermal papillary vessels in these areas were shorter than in the palmar skin and they were only stained in the inner layer facing the lumen; thus, the chain-like pattern of endothelial cell was not seen here.

Sera from hand eczema patients and palmar skin from non-smokers. Only two (8.7%) of the 23 sera from patients with palmar eczema produced any positive structures – one with weak (non-smoking man) and one with medium (previously smoking woman) staining intensity – in the papillary dermis and with similar distribution as obtained with PPP sera.

Sera from hand eczema patients and palmar skin from smokers. None of the sera produced any staining of the endothelium or the acrosyringium.

Sera from myasthenia gravis patients and healthy controls. No sera induced any fluorescence in palmar skin from non-smokers or smokers (Table I).

Immunofluorescence pattern in relation to the presence of serum antibodies against muscle nicotinic receptors, thyroid antigens and gliadin. Among the sera containing nAChR ab, 68% (13/19) produced the positive structures in the papillary dermis (Table I). Among the sera without nAChR ab, the corresponding proportion was 31%

Table I. *Immunofluorescence (IF) of endothelium and acrosyringium on palmar skin from healthy non-smokers and smokers with sera from patients with palmoplantar pustulosis (PPP), chronic hand eczema, myasthenia gravis and healthy controls*

	Papillary endothelium IF		Acrosyringium IF	
	IF positive sera/total (n)	Positive sera (%)	IF positive sera/total (n)	Positive sera (%)
Palmar skin from healthy non-smokers				
PPP sera, all	21/45	46.7	0/45	0
PPP sera without nAChR ab	8/26	30.8 } 1	0/26	0
PPP sera with nAChR ab	13/19	68.4 }	0/19	0
Hand eczema sera	2/23	8.7	0/23	0
Myasthenia gravis sera	0/7	0	0/7	0
Healthy subject sera	0/4	0	0/4	0
Palmar skin from healthy smokers				
PPP sera, all	19/45	42.2	11/45	27.0
PPP sera without nAChR ab	7/26	26.9 } 2	3/26	11.5 } 3
PPP sera with nAChR ab	12/19	63.2 }	8/19	42.1 }
Hand eczema sera	0/23	0	0/23	0
Myasthenia gravis sera	0/7	0	0/7	0
Healthy subject sera	0/4	0	0/4	0

1) $p = 0.017$; 2) $p = 0.031$; 3) $p = 0.033$.
nAChR ab = serum antibodies to muscle nicotinic acetylcholine receptors.

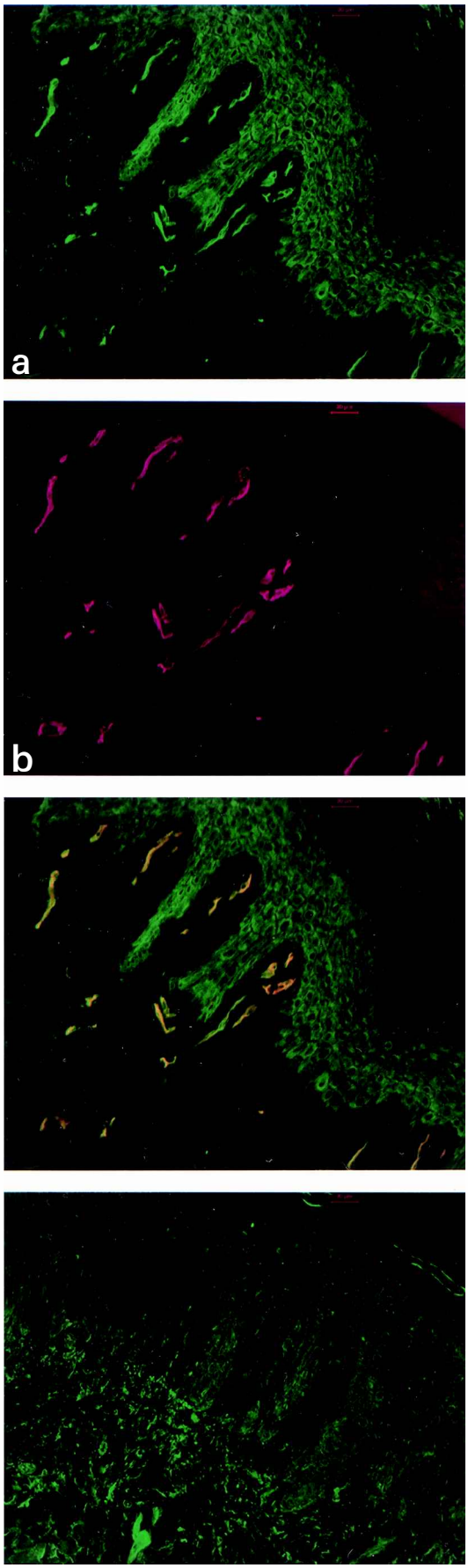

Fig. 2. Double staining on palmar skin from a healthy non-smoker with antibodies against endothelial cells and serum from a patient with palmoplantar pustulosis. (a) The positive pattern with a PPP patient serum. (b) The pattern in papillary dermis with the endothelial antibodies. (c) Double exposure: Yellow visualizes positive staining for both endothelium and PPP serum. (d) Negative control: mouse IgG_1, patient serum omitted. Scale bar: 30 μm.

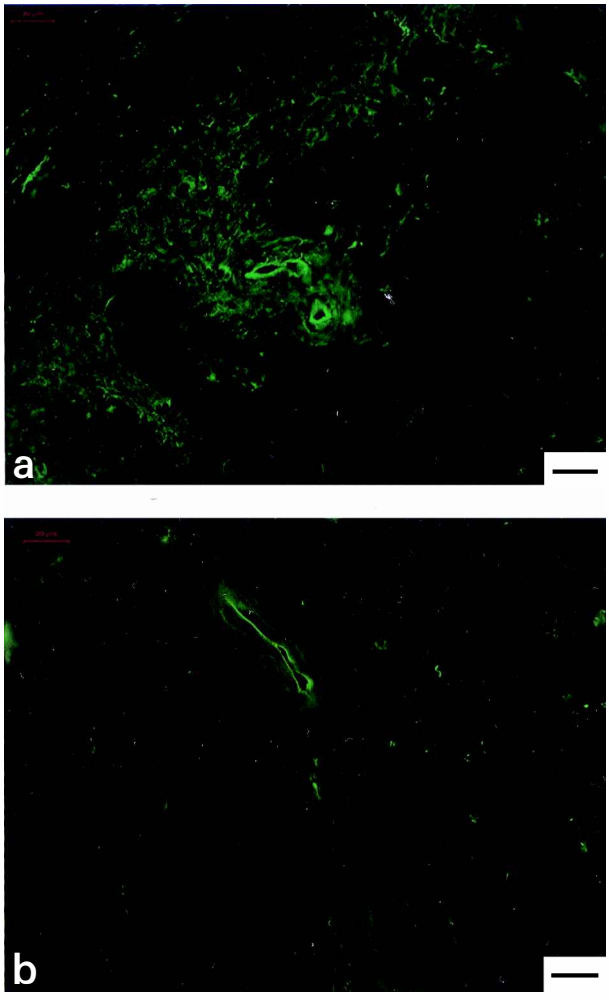

Fig. 3. Immunofluorescence on palmar skin from a healthy smoker and palmoplantar pustulosis serum (same serum as in Fig. 2). In addition to staining of endothelium there is a luminal staining of (a) the acrosyringium and (b) the dermal sweat duct. Scale bars: (a) 30 μm; (b) 20 μm.

(8/26; $p = 0.017$). Sera from PPP patients with nAChR ab gave stronger immunofluorescence staining than those without nAChR ab ($p = 0.026$) (Fig. 4).

Double-staining controls did not show any unspecific staining such as overlapping between the fluorescence filters. Both observers obtained the same results when grading the intensity of the fluorescence of the specimens selected for comparison.

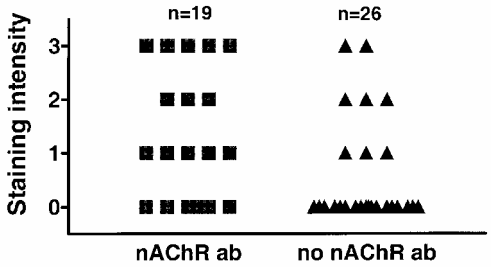

Fig. 4. Endothelial staining intensities of palmoplantar pustulosis sera with and without nicotinic acetylcholine receptor (nAChR) antibodies on palmar skin from healthy non-smokers. Sera from patients with nAChR antibodies more often gave stronger staining (median value 1) than those without nAChR antibodies (median value 0; $p = 0.026$).

DISCUSSION

The results of this study indicate that patients with PPP have autoimmune reactions against structures on the papillary endothelium and the acrosyringium. The putative autoantigen(s) seem to be upregulated by smoking, as the reaction against the acrosyringium was observed only in palmar skin from smokers. Sera from patients with PPP had a high prevalence of antibodies to skeletal muscle nAChR and sera with such antibodies more often induced an immunofluorescence on endothelial cells in the papillary dermis and – using palmar skin from smokers – also of the sweat ducts. This reactivity was less common using sera from PPP patients without antibodies against the nAChR.

Forty-seven percent of the sera from patients with PPP induced an immunofluorescence of the papillary endothelium of palmar skin from healthy controls in contrast to only 2/23 sera from patients with long-standing palmar hand eczema. None of the sera from eczema patients stained structures in the palmar skin from a smoker. This shows that the reaction with the PPP sera is not an unspecific reactivity linked to inflammation.

The final target for the inflammation in PPP seems to be the acrosyringium, and, using skin from otherwise healthy smokers but not skin from non-smokers, 27% of all PPP sera showed a reactivity with the acrosyringia and also of dermal ducts in addition to the endothelial reaction. This indicates that smoking increases the reactivity, possibly by up-regulating the (auto)antigen(s).

Antibodies against the skeletal muscle nAChR were present in 42% of the patients with PPP, but in none with chronic hand eczema. Thus, these antibodies were even more prevalent than antibodies to thyroid antigens and gliadin in PPP.

Antibodies against skeletal muscle nAChR are present in the majority of patients with generalized myasthenia gravis (93% of Swedish patients, mean value 3.60 ± 2.20 arbitrary units) (4), but are not found in healthy subjects. The concentrations of nAChR ab in patients with PPP were generally lower than in myasthenia gravis. Myasthenia gravis is not associated with smoking or skin disease. Low concentrations of antibodies against the nAChR are present also in patients with primary biliary cirrhosis (16 of 17 patients) (5) and in systemic lupus erythematosus (6), and both these diseases are influenced by smoking. Howel et al. (7) found that primary biliary cirrhosis had a highly significant association both with past smoking and with psoriasis.

The assay for antibodies against the nAChR in PPP was based on antigen preparation from skeletal muscle. Two types of nAChR, $\alpha_1\beta_1\gamma\delta$ (foetal form) and $\alpha_1\beta_1\gamma\varepsilon$ (adult form), are specific for skeletal muscles, and in adult life expressed on denervated and innervated muscles, respectively. The natural agonists and the ligand α-bungarotoxin bind to these receptors and also to the α-7 nAChR (8), which is expressed on endothelial cells (9) and on the acrosyringium (2). Since the different forms of nAChRs share some ligand binding properties, it is reasonable to assume that antibodies primarily directed against the nAChR of skeletal muscle might partially cross-react with the α-7 nAChR. That sera from patients with myasthenia gravis and high concentrations of antibodies against the nAChR did not bind to structures of the papillary dermis further indicates that the autoantigen(s) in PPP and myasthenia gravis are not identical.

It is reasonable to assume a relationship between the positive immunofluorescence staining and presence of nAChR ab, since the immunofluorescence staining was found in a greater prevalence (68%) with sera containing muscle nAChR ab than with those without. The immunofluorescence staining of endothelial cells was stronger using sera from patients with PPP with muscle nAChR. Moreover, the immunofluorescence staining was more intense using sera from PPP patients with both muscle nAChR ab and antibodies against thyroglobulin or gliadin. This might indicate cross-reactivity between the autoantigen(s), or, alternatively, several autoantibody populations. Thyroglobulin shows partial homology with both acetylcholinesterase (which is strongly expressed in the acrosyringium) (10) and the α-subunit of the nAChR (11).

The results of this study indicate that PPP is an autoimmune disease which may be precipitated by smoking. The antibodies that bind to endothelial cells and to the sweat gland duct may play a pathogenetic role in PPP by activating endothelial cells and enhancing the massive infiltration of inflammatory cells in the papillary dermis and inducing the migration of granulocytes outwards in the acrosyringium, resulting in the pustule formation in the lowest part of the stratum corneum. One putative autoantigen might be components of the α-7 nAChR, since sera from patients with PPP react with the skeletal muscle nAChR, which shares some ligand binding properties with the α-7 nAChR. However, the possible role of α-7 nAChRs or other nAChRs as autoantigens cannot be proved until PPP sera have been

shown to react with any of the nAChRs present in the palmar endothelium. It cannot be excluded that smoking can induce the production of relevant antibodies to other antigens than nAChRs. The nAChR antibodies in PPP sera might be present in parallel with other antibodies against hitherto unknown antigens induced by smoking.

Further investigations of the clinical significance of the studied antibodies and attempts to characterize the antigen(s) are of importance.

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