

## Sera from Patients with Palmoplantar Pustulosis Show Immunoreactivity Against Endothelial Cells

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Sir,

Palmoplantar pustulosis (PPP) is considered to be a variant of psoriasis, although recently it has been shown to be genetically different from psoriasis vulgaris (1). The majority of patients with PPP are women and smokers. The final target for inflammation in this condition is the palmoplantar sweat duct (2, 3), but the mechanisms underlying the inflammation are poorly characterized.

PPP patients have co-morbidity, with an increased prevalence of autoimmune thyroid disease, coeliac disease (4), abnormal calcium homeostasis and a greatly increased risk for diabetes type 2 (5). The possibility that PPP might be an autoimmune disease has recently been suggested by us, as sera from 47% of PPP patients reacted with papillary endothelium in palmar skin from healthy subjects, as assessed by indirect immunofluorescence (IIF) (6).

The main purpose of this study was to determine whether PPP sera also show immunoreactivity against non-palmar skin and non-dermal tissues. In addition, the immunoreactivity of PPP sera against cultured dermal endothelial cell and palmar skin proteins was studied using a Western blot technique.

Sera were obtained from 43 women and 1 man with typical PPP on the palms and/or soles. Twenty-one of these 44 new sera (47.7%) induced IIF staining of the endothelium in the palmar skin from a healthy woman, which is in agreement with our first study (6).

Sera from 18 PPP patients studied previously (6) were obtained 1–6 years after the first samples and were used for follow-up screening of the IIF pattern in palmar skin. With 16 of these 18 PPP sera the staining results were the same as in the first sample (7 were still negative, 9

still positive). The positive staining had the same pattern (chain-like) and intensity as before. Two sera previously giving weak IIF staining were now negative. There had been only mild changes in the clinical conditions in the majority of these patients.

Two patients who had stopped smoking had unchanged, strong endothelial IIF, despite almost total clearance of the PPP lesions in one of them and marked improvement in the other. The IIF pattern induced by PPP sera in palmar skin thus persists over time regardless of variation in clinical activity.

With the intention of screening for IIF in non-palmar skin and non-dermal tissues, sera from 10 PPP patients, 5 healthy non-smokers and 5 patients with eczema on the hands, were used. Five of the PPP sera had previously produced strong endothelial IIF on palmar skin and 5 were negative.

The former positive sera showed endothelial staining also in skin from the fingertips, elbow, forearm and back, but not in scalp skin, from healthy non-smokers. However, the most widespread endothelial immunoreaction was seen in palmar skin.

The most pronounced IIF staining with PPP sera in the non-dermal tissues was seen on the endothelium in the richly vascularized parathyroid gland (Fig. 1a). These PPP sera also induced a staining in the endothelium between the thyroid follicles (Fig. 1d), and in the pancreas strong IIF staining was seen on the endothelial cells lining the lumen of some larger blood vessels (Fig. 1e). All other sera were negative (Fig. 1b). Double staining with endothelial cell antibodies verified the location (Fig. 1c). No specific staining was observed in the liver or duodenum.

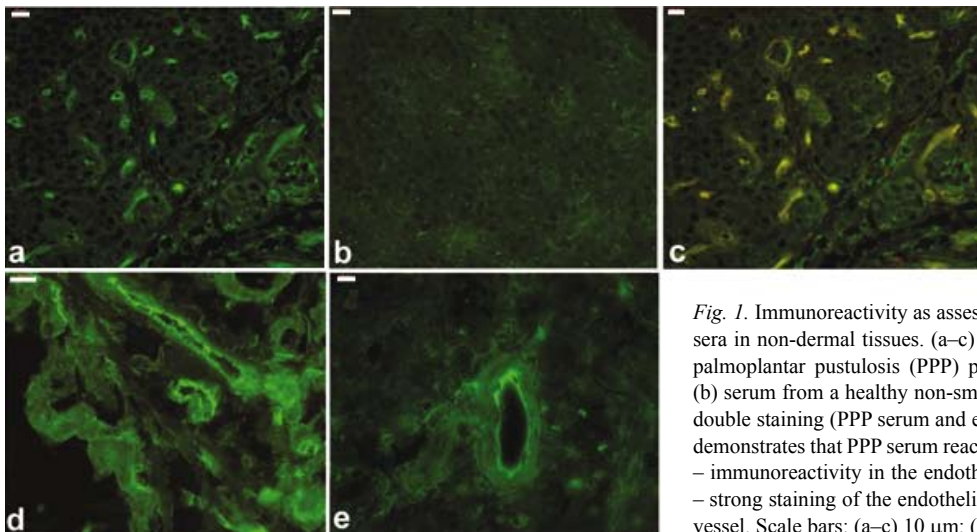


Fig. 1. Immunoreactivity as assessed by indirect immunofluorescence with sera in non-dermal tissues. (a–c) The parathyroid gland: (a) serum from a palmoplantar pustulosis (PPP) patient with endothelial immunoreaction; (b) serum from a healthy non-smoking person, no endothelial staining; (c) double staining (PPP serum and endothelial cell antibodies), yellow colour demonstrates that PPP serum reacts with endothelial cells; (d) thyroid gland – immunoreactivity in the endothelium between the follicles; (e) pancreas – strong staining of the endothelial cells lining the lumen of a larger blood vessel. Scale bars: (a–c) 10 µm; (d) 20 µm; (e) 10 µm.

When PPP sera were used for IIF on cultured endothelial cells some of them seemed to stain the nucleus. Therefore, 34 sera from PPP patients were analysed for the presence of anti-nuclear antibodies by a standardized routine method (IF on HEp-2 cells). Three sera (with nucleus IIF) were anti-nuclear antibodies positive, but none of them showed any antibodies to native DNA or to the extractable nuclear antigens Sm, RNP, SSA, SSB, Scl-70 and Jo-1.

Endothelial cell proteins were separated on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the numbers of sera used for Western blot were: PPP patients with IIF 18; PPP without IIF 6; healthy non-smokers 6; healthy smokers 3; eczema patients 7. A larger number of bands and also stronger bands were seen with sera from PPP patients than with sera from healthy controls and patients with eczema of the hand (Fig. 2a). One band of 59 kDa was seen with sera from 8 of 24 PPP patients (33%). Six of these 8 sera produced the endothelial IIF staining pattern. Only one of 16 sera from healthy control subjects showed reactivity with an endothelial antigen of 59 kDa ( $p$ -value = 0.044,  $\chi^2$ -test).

When palmar skin proteins were similarly studied, a heterogeneous pattern with bands of different molecular weights (between 32 and 47 kDa) was seen with sera from PPP patients but not with control sera (Fig. 2b).

## DISCUSSION

These IIF results show that sera from patients with PPP have reactivity in particular against endothelial cells in palmar skin, but also against endothelium in non-palmar skin and in some non-dermal tissues. The fact that PPP sera induce stronger and more widespread

endothelial IIF staining in normal palmar skin than in skin from other areas may reflect a different antigenic profile, which may be of pathogenetic relevance. None of the sera from patients with hand eczema showed IIF, which rules out the possibility of a reaction from PPP sera associated with an inflammation in palmar skin.

The pronounced IIF staining of the parathyroid gland endothelium was of particular interest as we have found an abnormal calcium homeostasis in PPP (5). This finding indicates that palmoplantar and parathyroid endothelium might have some properties in common. The clinical relevance of the endothelial staining in the thyroid gland and pancreas is not yet known.

The Western blot results show that patients with PPP have antibodies against several endothelial and palmar skin antigens and that the antigens to which they react vary between the patients. Analogously, several studies of systemic lupus erythematosus using Western blotting and immunoprecipitation have shown that endothelial reactive sera recognize a number of proteins present in endothelial cell preparations (7–9).

Although the clinical relevance of the reactivity against endothelium is not yet known, these results indicate that PPP is a complex autoimmune disease involving not only the skin but also other organs. As most patients with PPP are smokers, the possible link between smoking, reactivity against endothelial components and inflammation in PPP deserves further investigation.

## REFERENCES

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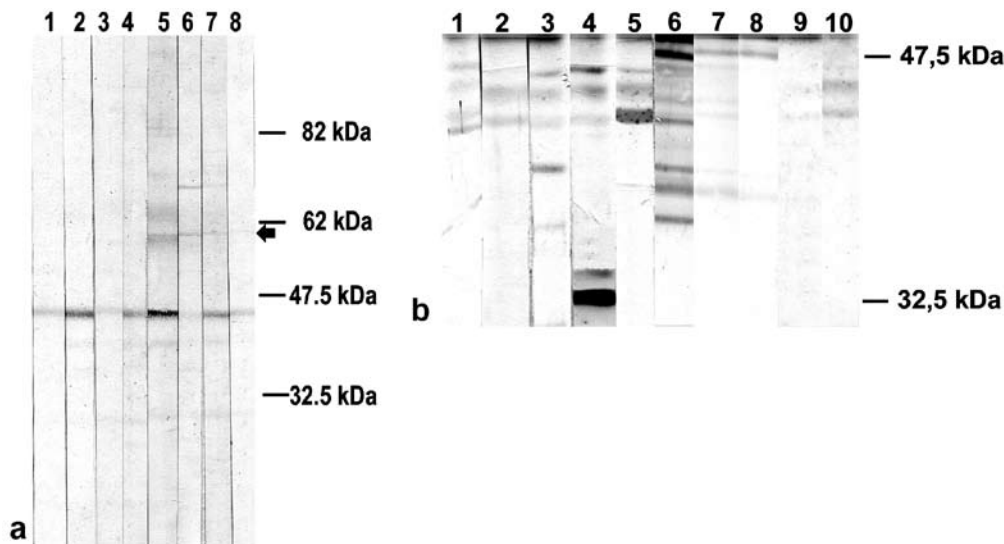


Fig. 2. (a) Western blot on endothelial cell lysate, separated on 7.5–12.5% gradient sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showing a larger number of bands and also stronger bands with sera from palmoplantar pustulosis (PPP) patients (lanes 5–8) than with sera from controls (lanes 1–2) and patients with hand eczema (lanes 3–4). Arrow indicates a 59 kDa band seen with 33% of the PPP sera. (b) Western blot on palmar skin homogenates from a healthy smoker, separated on 10% SDS-PAGE, showing several bands of different molecular weights with PPP sera (lanes 1–6), which were not observed with control sera (lanes 7–8) or with sera from patients with hand eczema (lanes 9–10).

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