

Use of Scale Antibodies for the Detection of Antigens in Psoriatic Lesions

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Extractable IgG from psoriatic scale was purified, labelled with biotin and used in ELISA and immunofluorescence (IF) in an attempt to detect and localize prominent antigens in psoriatic scale extracts and in psoriatic lesions, respectively. Biotinylated immunoglobulins isolated from psoriatic scale from each of 5 patients were used. Scale extracts were fractionated on a Sephacryl S-300 column, and antigens detected by scale IgG were eluted in the void volume and at a K_{av} 0.55. The profile was very similar for each antibody preparation. Antigens recognized by serum IgGs from both healthy controls and psoriatic patients were detected in the void volume only. Antigens recognized by a rabbit antiserum against the psoriasis-associated antigen, pso p27 (6), were restricted to the fractions eluted at a K_{av} 0.55. Furthermore, the binding of scale IgG to the antigens eluted at K_{av} 0.55 was inhibited by purified pso p27 antigen. Two of the scale antibody preparations gave rise to a distinct fluorescence on skin biopsies from psoriatic lesions in indirect immunofluorescence. The antigens recognized were localized to a subfraction of dermal cells and in the endothelial lining of some of the dermal vessels. Double labelling with these scale antibodies and a rabbit anti-pso p27 antiserum showed that both antibody preparations bound to the same cells in the psoriatic lesions, while only a minority of these cells were recognized by a murine monoclonal antibody against human IgG. The observations described indicate that the pso p27 is a major antigen in the immune reactions in psoriasis. **Key words:** Psoriasis; Scale; Antigen; Pso p27.

(Accepted September 21, 1992.)

Acta Derm Venereol (Stockh) 1993; 73: 31–34.

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The main pathological changes in psoriasis are a hyperproliferation of epidermal keratinocytes and an inflammatory reaction in the dermis with infiltration of lymphocytes and macrophages. In established lesions polymorphonuclear leukocytes are regularly noted in the epidermis, often present in the characteristic Munro microabscesses.

The aetiology and the pathogenesis of psoriasis are unknown. Even the causal relationship between the hyperproliferation and the inflammatory reaction is still debated.

Autoantibodies with specificity for a stratum corneum antigen (1) and for nucleoproteins (2, 3) in addition to rheumatoid factors (4) have been detected in psoriatic scales. Recently we described the presence of autoantibodies against a protein, pso p27, isolated from psoriatic scale (5, 6). This antigen shows a serological cross reaction with the major internal protein, p27, of a retrovirus-like particle obtained from the urine of psoriatic patients (7–11).

The aim of the present study was to apply biotinylated scale antibodies in indirect ELISA on scale extracts and in immunofluorescence analyses on biopsies from psoriatic lesions in an attempt to detect and localize antigens and compare these observations with the reactions obtained with a rabbit anti-pso p27 antiserum.

MATERIALS AND METHODS

Fractionation of psoriatic scale

Fractionation of psoriatic scale was performed with extracted material from each of 5 patients.

The scale (300 mg) was sonicated in 10 ml 0.1 M carbonate buffer (pH 10, 8) containing 0.5 M NaCl. The sonicate was centrifuged at 100 000 x g for 1.5 h and the supernate was dialyzed against PBS and passed through a protein A-sepharose CL-4B column (Pharmacia, Uppsala, Sweden) followed by an immunosorbent column with rabbit anti-human light chain antibodies (Dakopatts) bound to CNBr-activated sepharose 4B (Pharmacia). The eluate was further fractionated on a DEAE-sephacel column (Pharmacia) by a NaCl gradient (0.1 M) in 0.02 M tris-HCl (pH 7.9). The effluent and eluted material were diluted 1:2000 in coating buffer and applied in an indirect ELISA using biotinylated scale IgG as described below. Antigens recognized by the scale antibodies were dialyzed against distilled water, lyophilized, redissolved in PBS (1 ml) and fractionated on a sephacryl S-300 column. The fractions were diluted 1:2000 in coating buffer and applied in indirect ELISA using biotinylated serum IgG from psoriatic patients and healthy controls in addition to biotinylated scale IgG and rabbit anti-pso p27 antiserum as described below.

Biotinylation of scale and human serum IgG

Psoriatic scale IgG obtained from each of 5 patients (see above) was purified and biotinylated separately.

The scale (500 mg) was extracted in 0.1 M sodium carbonate buffer (pH 10.8), and IgG was isolated by chromatography on a sephacryl S-200 column in the same buffer and identified in an ELISA using peroxidase-conjugated sheep anti-human IgG antibodies. The IgG was further purified by ion exchange chromatography on DEAE sephacel (Pharmacia) (12).

Human serum IgG was isolated from the 5 psoriatic patients and 5 healthy controls by ion exchange chromatography on DEAE sephacel (12). Quantification of IgG was performed by LC-Partigen immunoplates (Behringwerke AG), and the antibodies were labelled with biotin as described elsewhere (12).

Enzyme-linked immunosorbent assay (ELISA)

Microtitre plates (Linbro, Flow Laboratories, Herts, UK) were coated with antigen in coating buffer (0.1 M sodium carbonate buffer, pH 9.6) and blocked with 3% Tween 20 (Sigma Chemical Co., St. Louis, USA) as previously described (6). The wells were then incubated with biotinylated human IgG in PBS (1–5 µg/ml) or rabbit anti-pso p27 antiserum followed by peroxidase-conjugated streptavidin (BRL, Gaithersburg, Md., USA) or peroxidase-conjugated goat anti-rabbit immunoglobulins (Dakopatts, Copenhagen, Denmark) respectively and finally assayed with orthophenylenediamine (13).

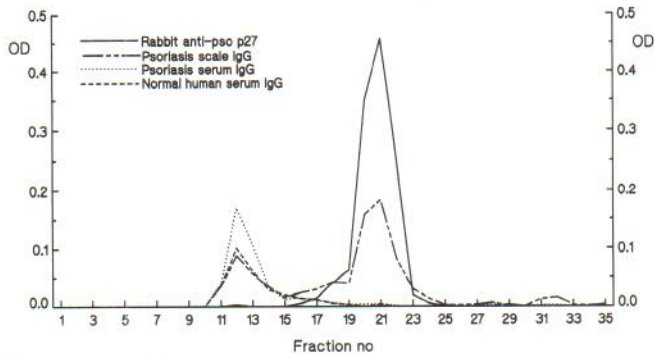


Fig. 1. Indirect ELISA of scale extract fractionated on a sephacryl S-300 column using serum IgG from healthy controls and psoriatic patients and scale IgG from psoriatic lesions in addition to rabbit anti-psy p27 antiserum.

Purification of pso p27 antigen and production of rabbit anti-psy p27 antiserum

The pso p27 antigen was isolated from psoriatic scale as described elsewhere (6, 12). Briefly, psoriatic scale (200 mg) was extracted with 0.5 M glycine-HCl buffer, pH 2.6, containing 1% 2-mercaptoethanol. The extract was centrifuged (1000 x g, 10 min.), the supernate was collected, diluted 1:10 in phosphate buffered saline (PBS), pH 7.2, and applied to an immunosorbent column containing rabbit polyclonal antibodies against p27 bound to CNBr-activated sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) (12). The adsorbed material was eluted with 0.2 M glycine-HCl buffer, pH 2.6, and further purified by gel filtration (twice) on a sephacryl S-300 (Pharmacia) in 6M guanidine-hydrochloride as described elsewhere (8).

The antigen preparations obtained from the sephacryl column were dialysed, concentrated to 1 ml, emulsified with an equal volume of Freund's complete adjuvant and injected subcutaneously in rabbits using multiple injection sites. The rabbits were boosted 6 weeks later with an equal amount of antigen and bled 2 weeks after the last injection.

In order to omit antibodies crossreactive with normal human serum protein, the rabbit anti-psy p27 serum was preabsorbed by chromatography on CNBr-activated sepharose 4B to which serum proteins from 3 healthy donors had been coupled (12).

Immunofluorescence analyses

Punch biopsies (6 mm) were obtained under local anaesthesia from lesions of 6 patients suffering from static chronic plaque psoriasis, including one of the 5 scale IgG donors. After fixation in ethanol, the biopsies were embedded in paraffin as described elsewhere (10). Ul-

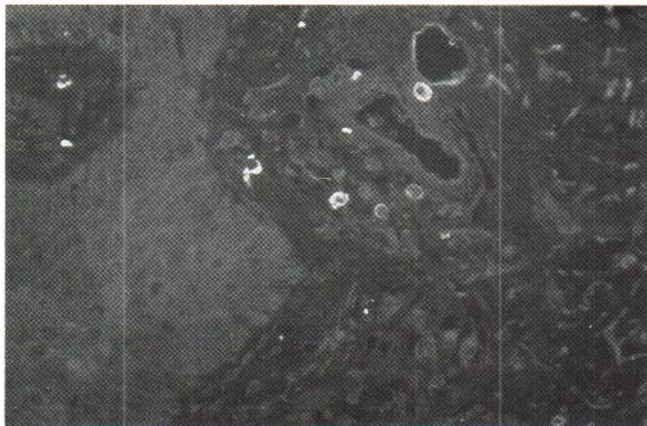


Fig. 2. Indirect immunofluorescence of a psoriatic lesion with biotinylated scale IgG and Texas red conjugated streptavidin.

trathin sections (2–3 um) were then prepared for immunofluorescence (IF) microscopy.

The detection of pso p27 antigen, antigens recognized by the scale antibodies and human IgG were performed on serial sections of skin biopsies from psoriatic lesions utilizing indirect IF. The thin sections were incubated with one or two primary antibodies for 45 min at 37°C. As primary antibodies were used rabbit anti pso p27 antibodies (preabsorbed with human serum proteins), biotinylated scale antibodies (see above) and biotin-labelled goat anti-human IgG (TAGO Inc. Burlingame, CA, USA). After two washes (each 5 min) with PBS containing 0.05% Tween 20 (PBS-T) (Sigma Chemical Co.), the samples were incubated with Texas Red conjugated donkey anti-rabbit immunoglobulins (Amersham International, Amersham, U.K.) and/or fluorescein conjugated streptavidin (BRL, Gaithersburg, Md., USA) for 45 min at 37°C. After two washes, the preparations were mounted under coverslip in glycerol-PBS (9:1) and examined using a Leitz fluorescence microscope.

RESULTS

To identify extractable antigens from psoriatic scale the material was initially passed through a protein A column and an anti-light chain column to remove immunoglobulins (rheumatoid factor, 4). The extract was then applied on a DEAE column and the eluted fractions were assayed for antigens in an indirect ELISA using biotinylated scale immunoglobulins obtained from 6 psoriatic patients. The detectable antigens were eluted at 0.1 to 0.2 M NaCl. The antigens were further fractionated by gel filtration on a sephacryl S-300 column and analysed with respect to antigens. Biotinylated serum IgG from healthy controls and psoriatic patients recognized antigens in the void volume fractions only, while the main antigen detected by the scale IgG was localized at a K_{av} of 0.55. The elution profile is shown in Fig. 1. The elution profile was in principle identical for the patients studied both with respect to antigen and antibodies. The main antigen peak corresponded with the K_{av} of the antigen recognized by the anti-psy p27 antiserum (Fig. 1). Furthermore, the reaction against antigens eluted at the K_{av} of 0.55 was inhibited (more than 70%) by adding purified pso p27 antigen to the scale IgG.

When the scale antibodies were used in indirect immunofluorescence analyses on skin biopsies from psoriatic lesions, two of the five antibody preparations gave rise to a distinct fluorescence localized to a subfraction of dermal cells and at the endothelial lining of some of the dermal vessels (Fig. 2). The reaction was recognized in the skin lesions from each of the 6 patients investigated. Indirect immunofluorescence on biopsies from the skin lesions with rabbit anti-psy p27 serum which had been preabsorbed with normal human serum also gave a bright cytoplasmic fluorescence in a subfraction of dermal cells and at the endothelial lining of some of the dermal vessels, while the preimmune serum was negative. Double labelling of a thin section with anti-psy p27 antiserum and scale antibodies showed that the fluorescence obtained by these two antibody preparations was localized to the same cells (Fig. 3a,b). By increasing the concentration of scale antibody a suppression of the anti-psy p27 fluorescence was observed. Similarly the anti-psy p27 antiserum significantly inhibited the fluorescence brought about by the scale antibodies.

Incubation of the psoriatic skin sections with Texas Red conjugated goat antibodies against human IgG also showed a

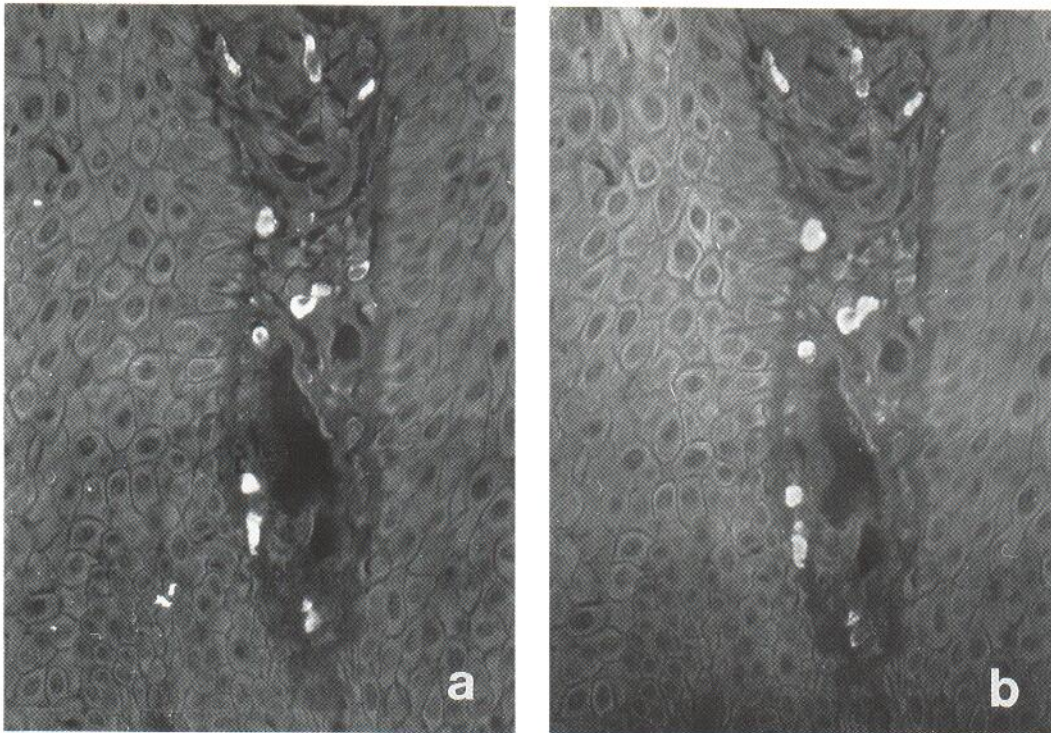


Fig. 3. Double labelling of a psoriatic skin lesion with fluorescein conjugated scale IgG (a) and rabbit anti-psy p27 antibodies visualized by Texas red conjugated donkey anti rabbit-immunoglobulins (b).

bright fluorescence in some of the dermal cells and at the endothelial lining of some of the dermal vessels. However, double labelling of skin biopsies with rabbit anti-psy p27 antiserum and goat anti-human IgG antibodies revealed that the majority of the psy p27 positive cells were negative for human IgG and *vice versa* (Fig. 4).

DISCUSSION

In this report we have analysed scale extracts to identify dominating antigens recognized by scale IgG. The high dilutions of the fractionated extracts used in the indirect ELISA should exclude the possibility of competition between various proteins in the coating of the wells. The analyses show that the most prominent antigen has a molecular weight estimated to about 30 kD based on gel filtration. The inhibiting effect of purified psy p27 antigen in the ELISA strongly suggests that the antigen in question is psy p27. Scale antibodies from 2 of the patients, including one of the patients affording a skin biopsy, bound to a subfraction of dermal cells in psoriatic lesions and to the endothelial linings of some of the blood

vessels in the skin lesions as demonstrated by immunofluorescence. These cells were concomitantly positive for antigen recognized by anti-psy p27 serum. The fact that the scale antibodies and the rabbit anti-psy p27 antibodies mutually suppressed each other indicates that the antibodies are at least partially directed against the same epitopes.

The antigens detected in the dermal cells could be present in the state of phagocytized immune complexes. An increased number of phagocytic cells has been detected both in the dermis and the epidermis of psoriatic lesions (14). The vast majority of these cells are monocytes or macrophages which are believed to function as antigen-presenting cells to activated T-lymphocytes present in the psoriatic lesions (15). However, the fact that only a minority of dermal cells are concomitantly stained with anti-psy p27 serum and anti-human IgG antibodies argues against this possibility and may indicate that the majority of these cells are synthesizing the antigen in question. Although the immunofluorescence described with scale antibodies was obtained with extracts from only 2 of the patients, this does not exclude the possibility that we are focusing on a common antigen of importance in psoriasis. Failure in anti-

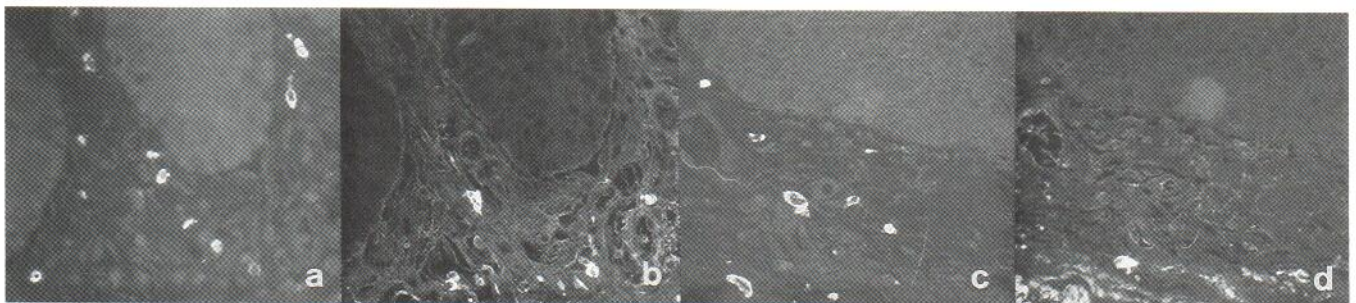


Fig. 4. Double labelling of a psoriatic skin lesion with fluorescein conjugated murine monoclonal antibodies against human IgG (b and d) and rabbit anti-psy p27 antibodies indirectly visualized by Texas red conjugates (a and c).

body binding in the immunofluorescence analyses can be caused by destruction of major epitopes during preparation of the thin sections of the biopsies and may explain the discrepancy observed between ELISA and immunofluorescence.

We have previously suggested that the major internal protein, p27, of a retrovirus-like particle may be an important antigen in the inflammatory reactions in psoriasis (5, 11). The findings of a serological crossreacting antigen in psoriatic scale, pso p27, and antibodies against pso p27 in psoriatic lesions forming complement activating immune complexes strengthen the suggestion of a potential role of such an antigen in the pathogenesis of psoriasis (5, 6). The data presented here indicate that pso p27 antigen is produced by dermal cells in psoriatic lesions and may represent an important antigen in the immune reactions in psoriasis.

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