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A Prospective Immunofluorescence Study of Immune Deposits in the Skin of Primary Sjögren's Syndrome

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There are conflicting opinions concerning the epidermal immunofluorescence pattern in primary Sjögren's syndrome. In a prospective study of 12 patients we found a characteristic pattern of epidermal nuclear/cytoplasmic IgG deposits in 8 (67%). This pattern was associated with the presence of antibodies against SSA/Ro and SSB/La in the serum and was also found in 2 out of 5 LE patients with monospecific antibodies against SSA/Ro. There is a resemblance to the pattern of dust-like particles described in the diseased skin of patients with subacute cutaneous LE. In one patient with primary Sjögren's syndrome, IgG deposits were confined to epidermal cell nuclei (in vivo ANA). Instead of antibodies against SSA/Ro or SSB/La, this particular patient had nRNP-antibodies. From this study, we conclude that the epidermal IgG deposits in primary Sjögren's syndrome may represent antibody binding to the sites within epidermal cells where the respective antigens are located. ***Key words: Antinuclear antibodies; SSA/Ro; SSB/La.***

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Features of distinct immunofluorescence (IF) are found in the skin of patients with connective tissue disorders. In systemic lupus erythematosus (SLE), this mainly concerns deposits of immunoglobulins and complement factors at the dermo-epidermal junction. In addition to this 'lupus band', immunoglobulin deposits in epidermal cell nuclei have been observed in SLE, but also in mixed connective tissue disease (MCTD), progressive systemic sclerosis, poly/dermatomyositis and Sjögren's syndrome (1).

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration and destruction of salivary and lachrymal glands (2). The syndrome is considered to be primary if it occurs without, — and secondary if it is associated with — another inflammatory connective tissue disorder, e.g. rheumatoid arthritis, SLE, MCTD, progressive systemic sclerosis or poly/dermatomyositis. In these diseases, as well as in the case of patients with primary SS, serological abnormalities, i.e. hypergammaglobulinemia, rheumatoid factors, circulating immune complexes and antinuclear antibodies often occur. In recent studies published concerning immunofluorescence of the skin in primary SS, 68% of the patients showed intercellular epidermal staining (3, 4), a feature thought to be characteristic to pemphigus vulgaris. In a retrospective study on the frequency of in vivo ANA of the skin, we found speckled type nuclear staining in the epidermis of 14 of the 21 patients (67%) with the primary syndrome (5). To reconcile the discrepancy between these two investigations we designed a prospective study of the clinically normal skin in 12 patients with primary SS.

PATIENTS

Twelve patients (all female) with primary SS were studied (Table I), after the patients had given their informed consent. All patients were suffering from keratoconjunctivitis sicca and xerostomia. The diagnosis was confirmed by a Schirmer test, tear lysozyme content and sublabial biopsy (focus score >1 focus per mm^2 tissue) (6). The diagnoses rheumatoid arthritis, MCTD, SLE, dermato/polymyositis and progressive systemic sclerosis were excluded in all patients, as they did not fulfil the accepted criteria for these diseases. The mean time between the initial manifestations of the disease and the moment of biopsy (follow-up, Table I) was 9 years. Some patients had associated abnormalities: No. 3 had cutaneous vasculitis, No. 7 had renal tubular acidosis, No. 8 had non-toxic multinodular goitre and No. 9 had severe Raynaud's phenomenon. Five LE patients with monospecific antibodies against SSA/Ro served as controls (3 had subacute cutaneous LE, one SLE and one discoid LE).

MATERIALS AND METHODS

Three-millimetre punch biopsies were taken from the clinically normal skin of the extensor surface of the forearm. The biopsies were snapfrozen in liquid nitrogen and stored at −70°C for ultimately one week before processing. Frozen tissue was cut at 4 μm, and sections were air-dried and then

Table I. *Immunofluorescence and serum studies*

Patient	Follow-up (years)	Immunofluoresence		Serum					
		Epidermal	DEJ	ANA*	ENA	SSA	SSB	nRNP	RA
1	6	nucl/cyt sp	IgG	1 000	+	+	+	–	+
2	9	nucl/cyt sp	IgM/C3	1 000	+	+	+	–	+
3	4	nucl/cyt sp	–	100	+	+	+	–	+
4	6	nucl/cyt sp	–	10	+	+	+	–	–
5	9	nucl/cyt sp	–	10	+	+	+	–	–
6	7	nucl/cyt sp	–	0	–	+	–	–	–
7	11	nucl/cyt sp	–	100	+	–	+	–	+
8	21	nucl/cyt sp	–	0	–	+	–	–	+
9	16	nucl sp**	–	100	+	–	–	+	+
10	13	–	–	10	–	–	–	–	+
11	3	–	–	10	–	–	–	–	–
12	7	–	–	0	–	–	–	–	–

– = negative; + = positive. nucl = nuclear staining. cyt = cytoplasmic staining. sp = speckled staining. * Reciprocal ANA titre is given. ** All speckled type staining-patterns consisted of IgG. ENA/SSA/SSB/nRNP = presence of antibodies against these antigens. RA = RA test.

washed in phosphate-buffered saline (PBS; pH 7.2) for 30 min. From five biopsies (Nos. 1, 3, 4, 6, 12), a second set of slices was cut and prefixed in acetone for 10 min before further processing, to test its effect on the IF pattern. All sections were tested for the presence of IgA, IgG, IgM and C3, using commercially available FITC-conjugates (Dakopatts, Copenhagen) by incubation in a moist chamber at room temperature for 30 min. The sections were then washed again in PBS for 30 min and mounted under glass coverslips with a solution of 10% glycerol in PBS. Specificity of all conjugates was tested by crossed immunoelectrophoresis, and on plasma cells from bone marrow, jejunum and lymph nodes from various subjects. Optimal working dilutions were established by chess-board titrations on frozen skin sections with a known specific IF pattern, and were found to be 1 : 30 in PBS for all conjugates used. All slides were assessed by means of two different Zeiss-microscopes, one equipped for darkfield fluorescence, the other for incident light illumination with an epi-illuminator. With both microscopic systems the immunofluorescence patterns observed were identical.

The presence of IgG class ANA in the serum was tested for by an indirect immunofluorescence technique on mouse liver sections. Titres $\geq 1:100$ were considered positive. Antibodies against extractable nuclear antigens (ENA), native ribonucleoprotein (nRNP), Sm, SSA/Ro and SSB/La were tested for by counter-immunoelectrophoresis with reference sera from CDC (Atlanta). Antibodies against dsDNA were tested for by indirect immunofluorescence on *Crithidia luciliae*. Rheumatoid factors were determined by Latex-RF test (RA-test; BehringWerke AG). Statistical significance was tested for by Fisher's exact test.

RESULTS

Results are summarized in Table I. Eight of the 12 SS patients (67%) showed a distinct epidermal staining

Fig. 1. Patient No. 6: Epidermal IgG deposits scattered throughout nucleus and cytoplasm, with peripheral accentuation in some cells (direct IF method; ×350).

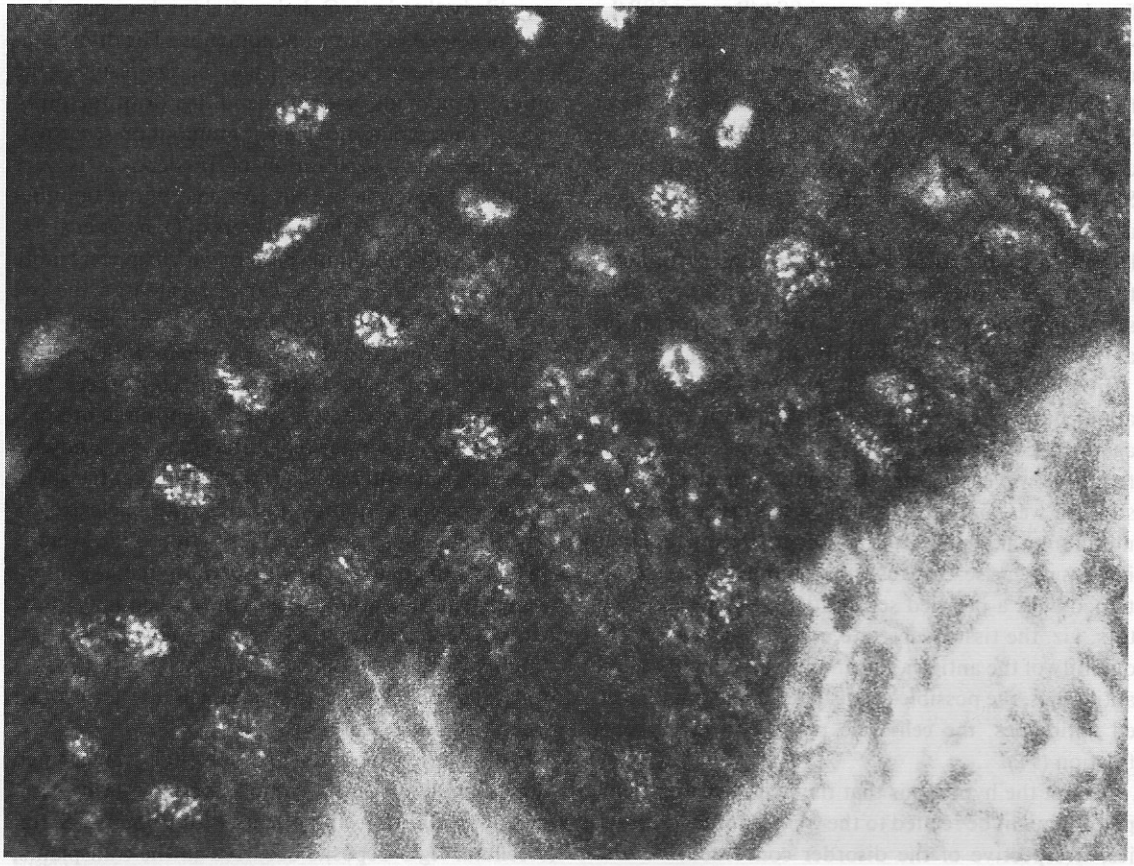

Fig. 2. Patient No. 9: Epidermal IgG deposits confined to cell nuclei (in vivo ANA) (direct IF method; original magnification ×580).

pattern consisting of fine-granular IgG deposits scattered throughout the cytoplasm and the cell nuclei (Fig. 1). Nuclear staining was occasionally absent from parts of the slides. In 2 patients, the staining was accentuated at the periphery of the cells (Fig. 1). This nuclear/cytoplasmic pattern was also observed in 2 out of 5 LE patients with monospecific SSA/Ro antibodies, whereas the other 3 showed no specific IF pattern. In one patient with SS (No. 9), granular IgG deposits were confined entirely to the epidermal cell nuclei (in vivo ANA) (Fig. 2). Continuous granular deposits at the dermo-epidermal junction, forming a small fluorescent band as seen in SLE, were found in 2 patients with primary SS (Table I). Acetone prefixation did not alter the patterns observed. Three patients (Nos. 10–12) did not show any epidermal or dermal staining with the conjugates used.

ANA titres $\geq 1:100$ were found in the serum of 5 patients (42%). SSA/Ro and SSB/La antibodies were found in 7 (58%) and 6 (50%) of the patients, respectively, whereas anti-nRNP was found only in one. No antibodies against dsDNA or Sm were found. Rheumatoid factors occurred in 7 patients (58%). The occurrence of the nuclear/cytoplasmic pattern correlated with serum anti-SSA/Ro and anti-SSB/La ($p<0.05$), but not with high titre serum ANA, the presence of anti-nRNP, or rheumatoid factor.

DISCUSSION

A distinct pattern of fine granular IgG speckles in cytoplasm and nucleus of epidermal cells was seen in 67% of a group of patients with primary SS. This pattern was associated with a high frequency of SSA/Ro and SSB/La-antibodies in the serum of these patients. In one patient with epidermal staining con-

fined to the nuclei, neither anti-SSA/Ro nor SSB/La —but instead nRNP-antibodies—was found. This may suggest that the epidermal staining patterns reflect the cellular localization of either the SSA and/or SSB or nRNP antigens. Native ribonucleoproteins are confined to the nucleus, whilst the SSA/Ro and SSB/La antigens are proteins bound to both nuclear and cytoplasmic RNAs (7). In Wil-2 cells (a human lymphoid line) and in KB cells (a human epithelial tissue culture line) speckled nuclear and cytoplasmic staining was observed, using whole sera containing anti-SSA/Ro and SSB/La antibodies (8).

In two other studies of the presence of SSA/Ro in adult human epidermis, using monospecific sera against SSA/Ro-antigen, no cytoplasmic staining was found, however, in one study only, speckled nuclear staining was found (9), whilst in the other there was no staining at all (10). It is not surprising that conflicting results arise, because the staining pattern, obtained with a purified serum, depends on many factors, viz. the tissue substrate used, the solubility and quantity of the antigen, the amount and avidity of the antibodies, the possible coexistence of other undetected antibodies, the cell-cycle phase and the type of fixation (11).

To test the hypothesis that the nuclear/cytoplasmic pattern might be related to the type of serum antibodies, irrespective of the disorder concerned, we also studied the normal skin from 5 LE patients with monospecific antibodies against the SSA/Ro-antigen. In 2 of them the nuclear/cytoplasmic pattern was observed. Unfortunately we have not yet been able to study patients with monospecific antibodies against SSB/La. An IF pattern comparable to the nuclear/cytoplasmic presented in this paper, has recently been described in skin lesions of SCLE patients (12): 12 of 35 patients (34%) showed very fine dust-like particles of IgG scattered throughout the cytoplasm of the basal epidermal cells, the subepidermal region and the dermal cellular infiltrates; nuclear staining was found in half of the biopsies. In that study no significant correlation with the presence of anti-SSA/Ro in the serum was found.

In studies on the skin, oral labial mucosa and cervix uteri in patients with primary SS, Oxholm et al. found no nuclear or cytoplasmic staining, but epidermal intercellular IgG deposits in 68% (62 patients) (3, 4), 50% (6 patients) (13) and 24% (17 patients) (14), respectively. The figure of 68% found in skin biopsies is almost identical with the 67% in which we found the nuclear/cytoplasmic IgG pattern. This makes it very likely that both Oxholm et al. and we ourselves have observed the same phenomenon. The discrepancy in the patterns described may be the result of the different methods of tissue handling, or of the problems in interpretating a new immunofluorescent pattern. The exact localization of IF deposits is very difficult to describe in skin sections. In our opinion, the figures presented in the papers of Oxholm et al. (3, 4, 13–16) show a staining pattern which appears more cytoplasmic than intercellular or located on the cell membrane. Using the peroxidase–antiperoxidase complex staining in a study of 5 patients with primary Sjögren's syndrome, Oxholm and co-workers (16) were able to localize in vivo IgG deposits more precisely to the epidermal cell surfaces. In our study we observed some intercellular accentuation of the staining in 2 patients. In the 4 patients with nuclear/cytoplasmic staining in whom prefixation with acetone was performed as well, the pattern remained unchanged, a factor which makes the authenticity of the pattern described in the present study likely. The stability of the phenomenon after prefixation makes it also very likely that the pattern is not an artifact originated during staining.

In an alternative study, Oxholm et al. transplanted human epidermis to athymic nude mice (15). After injections of sera from patients with primary SS, 'intercellular' IgG deposits occurred in the transplants, suggesting epidermal cell binding of IgG-type antibodies. Double-labelling studies of the 'intercellular pattern' in the skin showed that the antibodies were bound to both Langerhans' cells and keratinocytes (16). The epidermal density of Langerhans' cells was found to be decreased in SS, but this was not associated with epidermal IgG deposits (17).

Binding of antibodies to the outer membrane of keratinocytes has been described in cultured neonatal foreskin cells and suspensions prepared from adult skin (18, 19). Binding occurred exclusively with antibodies against the non-histone nucleoproteins (nHNPs) SSA/Ro, SSB/La, Sm, RNP, and not with dsDNA-antibodies. In the skin of patients with connective tissue disorders, a similar association exists between the presence of in vivo ANA in the skin and serum antibodies against nHNP (5). Thus keratinocyte–cell surface binding of nHNP antibodies may occur as a phase in the process, by which antibodies leave the circulation and migrate to their final binding inside the epidermal cell.

We conclude that approximately two-thirds of the patients with primary Sjögren's syndrome show dis-

tinct nuclear and cytoplasmic IgG deposites in the epidermis of the clinically normal skin. These probably represent the localization of the SSA/Ro and/or SSB/La antigen in human keratinocytes.

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