

Epidermal Langerhans Cells in Patients with Primary Sjögren's Syndrome

P. OXHOLM,^{1,2} A. OXHOLM³ and R. MANTHORPE⁴

¹Laboratory of Medical Immunology, Dept. of Rheumatology TTA, Rigshospitalet, ²Dept. of Pathology, Kommunehospitalet and ³Dept. of Dermatology, Finsen Institute, Copenhagen, Denmark, and ⁴Dept. of Rheumatology, Malmö General Hospital, University of Malmö/Lund, Sweden

IgG-binding to the surface of Langerhans cells (LC) is a feature of primary Sjögren's syndrome and might be associated with qualitative and quantitative defects in LC. Fifteen patients with primary Sjögren's syndrome and 15 healthy controls had a punch biopsy performed from clinically unaffected skin. LC were enumerated in epidermal sheets by an immunoperoxidase technique, as well as demonstrated by immunofluorescence in vertical sections, which were furthermore examined for *in vivo* deposits of immunoglobulins, fibrinogen and C3. A significant ($P < 0.05$) reduction in number of LC, measured on epidermal sheets, was found in patients as compared with controls. Examination of vertical sections did not reveal abnormalities in morphology or localisation of epidermal LC. Intraepidermal IgG deposits were demonstrated in 7/15 patients. *In vivo* deposits were not found in any of the controls. The density of epidermal LC was not correlated to the presence of intraepidermal IgG deposits.

Key words: primary Sjögren's syndrome; epidermal sheets; Langerhans cells; IgG deposits.

Accepted for publication 25 April 1986

CLINICAL ASPECTS

Reduced density of epidermal Langerhans cells (LC) in patients with primary Sjögren's syndrome is most likely not specific for the disease, but little is known about LC in the various other systemic autoimmune diseases. LC enumeration and evaluation of LC morphology and distribution in vertical skin sections and in epidermal sheets may contribute to a better understanding of the pathogenetic mechanisms behind the various skin manifestations seen in patients with systemic autoimmune diseases.

Primary Sjögren's syndrome (primary SS) is a systemic, chronic inflammatory connective tissue disease mainly affecting exocrine glands (11). However, symptoms and signs from other organs are frequently seen (extraglandular manifestations). Lymphocytic infiltration of glandular and extraglandular organs, polyclonal B-cell activation and the presence of multiple autoantibodies indicate that autoimmune processes are involved (11).

Clinically, skin symptoms due to hypofunction of sweat and sebaceous glands are common

(11, 15). Purpura and urticaria caused by vasculitis, as well as various forms of erythema, are other cutaneous manifestations of primary SS (1). Intraepidermal deposits of IgG in clinically unaffected skin have recently been demonstrated in 68 % of patients with primary SS and in only 13 % of patients with SS secondary to other chronic inflammatory connective tissue diseases (12). Deposits of IgG were found in the basal and suprabasal layers of epidermis. Immunohistochemical investigations have further indicated that IgG may be Fc-receptor

bound to the surface of epidermal LC (13). It is so far uncertain whether this interferes with the functions of LC as antigen-presenting cells. However, this might be the case, since delayed cutaneous hypersensitivity to dinitrochlorobenzene (DNCB) is impaired in patients with primary SS (10).

The purpose of the present investigation was to examine the number and localisation of epidermal LC in clinically unaffected skin of patients with primary SS. Further we wanted to correlate the epidermal LC density to the occurrence of intraepidermal IgG deposits.

MATERIAL AND METHODS

Patients and controls

Fifteen consecutive out-patients who fulfilled the criteria (11) for primary SS were examined. Mean age was 57 years (range 37–71). Thirteen were females, mean duration of disease was 11 years (range 1–28). None of the patients had abnormal values of anti-DNA antibodies. The patients did not receive any treatment with glucocorticosteroids or cytostatic drugs.

Fifteen healthy persons without a history of inflammatory connective tissue disease or allergy were examined as controls. Mean age was 47 years (range 28–85), 11 were females.

Informed consent to have a skin biopsy performed was obtained from both patients and controls.

Definitions

Primary SS is defined as the presence of keratoconjunctivitis sicca (KCS) and xerostomia in patients lacking criteria for other chronic inflammatory connective tissue disease (11). KCS and xerostomia were both evaluated by three different objective tests (11) as follows: The diagnostic tests for KCS were Schirmer-I test, break-up time and van Bijsterveld score. At least two of the tests had to be abnormal. In the Schirmer-I test wetting of less than 10 mm of the standardized filter paper in 5 min was considered abnormal. Break-up time of less than 10 s indicates abnormal tear film stability and van

Bijsterveld score above 4 (on a scale of 0–9) for one eye was considered pathological.

The diagnostic tests used for xerostomia were lower lip biopsy, unstimulated sialometry and salivary gland scintigraphy (11). At least two of the tests had to be abnormal. The lip biopsy was examined for histopathological changes characteristic of SS (5). Unstimulated sialometry values below 1.5 ml saliva/15 min were considered abnormal (4). In salivary gland scintigraphy the uptake as well as the spontaneous and the stimulated secretion of the isotope were evaluated (14).

Skin biopsies

Biopsy of clinically unaffected skin from the right buttock, anaesthetized with ethyl chloride spray, was obtained by punch technique (4 mm). The tissue was divided (one half for examination of vertical sections and one half for LC enumeration on epidermal sheets), transported in Histocon® (Histo-Lab, Göteborg, S), frozen as soon as possible and stored at -70°C .

Examination of vertical skin sections

Direct immunofluorescence technique for demonstration of in vivo deposits of IgG, IgM, IgA, C3 and fibrinogen. 4–6 μm sections of the skin were cut in a cryostat, air dried for 10 min, washed in phosphate-buffered saline (pH 7.2) (PBS) for 5 min, and in distilled water for 2–3 min and air dried for 5–10 min. The sections were then incubated with diluted conjugate in a humidity chamber for 30 min. Rabbit immunoglobulins (Ig) against human IgG, IgM, IgA (heavy chain specific), C3c, and fibrinogen, labelled with fluorescein isothiocyanate (FITC), were obtained from DAKOPATTS, Copenhagen, Denmark. A 1:10 working dilution was used. Following incubation the sections were washed in PBS (4 $\times$ 5 min), distilled water (2 $\times$ 5 min), dried for 30 min, and finally mounted with Entellan® (Merck, Darmstadt, Germany).

Indirect immunofluorescence technique for demonstration of Langerhans cells. 4–6 μm cryostat sections were air dried for 10 min and incubated with

the mouse monoclonal antibody OKT6 (Ortho Diagnostic Systems, New Jersey, USA) using a 1:100 working dilution. Following incubation for 30 min in a humidity chamber, the sections were washed in PBS (3×5 min), incubated with diluted FITC-labelled rabbit Ig against mouse Ig (DAKOPATTS), previously absorbed with insolubilized human Ig, using a dilution of 1:10. After incubation for 30 min the sections were washed and mounted as above.

Fluorescence microscopy. The slides were read in a Reichert Polyvar Microscope (Reichert, Vienna, Austria), equipped with a 200 W high pressure mercury lamp, and an incident illumination system with interference primary filters, beam splitters and secondary filters adapted for FITC (Reichert).

Autofluorescence was evaluated in specimens without added FITC-conjugated antibody. Non-specific staining in the indirect fluorescence technique was excluded by examination of specimens without added primary layer antibody (OKT6).

Examination of epidermal sheets

Light microscopical enumeration of Langerhans cells. Fat-trimmed biopsies were washed in PBS (pH 7.4, Ca^{2+} - and Mg^{2+} -free) (3×5 min) and immersed in 1.0 mM EDTA for 120 min at 37°C . The epidermis was subsequently teased off the dermis with fine forceps and washed in PBS (3×5 min) prior to immunoperoxidase staining.

For control purposes vertical skin sections prepared for examination of LC were incubated with EDTA for 30 min prior to indirect immunoperoxidase staining. There was no detectable influence of EDTA on the results.

The density of LC within each epidermal sheet was enumerated with light microscope (Leitz Dialux 10 EB) at $\times 1000$ magnification and oil immersion. The slides were read without knowledge of clinical status. Ten randomly chosen, non-overlapping fields (0.0255 mm^2 /field containing 5–15 OKT6 positive cells) were examined, and the number of LC per mm^2 calculated.

Indirect immunoperoxidase technique for demonstration of Langerhans cells. Epidermal sheets were washed in PBS (3×5 min) and incubated at 37°C with OKT6 using a 1:100 working dilution. Following incubation for 60 min, the epidermal sheets were washed in PBS (3×10 min), incubated with horseradish peroxidase labelled rabbit Ig against mouse Ig (DAKOPATTS), absorbed with human serum, using a 1:10 working dilution. After incubation for 60 min the epidermal sheets were washed in PBS (3×10 min) followed by 0.038% 3-amino-9-ethylcarbazol (Sigma, St. Louis, USA) and 0.014% hydrogen peroxide for an additional 10 min. The specimens were then washed in water (3×10 min) and mounted with Gurr® (BDH Chemicals, Poole, England).

Control epidermal sheets without adding primary layer antibody (OKT6) were negative.

Statistics

The Mann-Whitney test was used in the statistical analyses. Level of significance, 5%.

RESULTS

Examination of vertical skin sections demonstrated deposits of IgG in the basal and suprabasal layers of epidermis in 7/15 patients (Fig. 1). Deposits of IgG were found in the dermo-epidermal junction zone (DEJ) in one of the patients without intraepidermal IgG deposits. Fibrinogen deposits in the DEJ were demonstrated in one patient with intraepidermal IgG deposits. Intraepidermal and DEJ deposits of IgM, IgA or C3 were not found in any of the patients. In the controls no deposits of IgG, IgM, IgA, C3 or fibrinogen were found.

In vertical skin sections no differences between patients and controls were seen concerning morphology and localisation of epidermal LC (Fig. 2). Dermal LC appeared increased in number in several patients, but exact evaluation of this observation was not performed. In one patient (No. 15) dermal LC were located among mononuclear cells in perivascular cell infiltrates (Fig. 2).

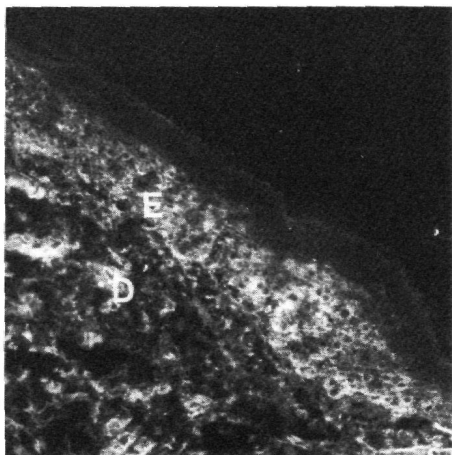


Fig. 1. Intraepidermal IgG deposits in a patient with primary Sjögren's syndrome, demonstrated in vertical section by direct immunofluorescence technique (x 400). E, epidermis; D, dermis.

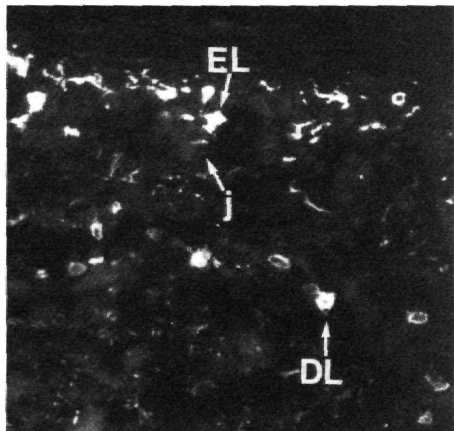


Fig. 2. Cutaneous Langerhans cells (LC) in a patient with primary Sjögren's syndrome, demonstrated in vertical section by the monoclonal antibody OKT6 by indirect immunofluorescence technique (x 400). EL, epidermal Langerhans cell; J, dermoepidermal junction; DL, dermal Langerhans cell.

Enumeration of LC in epidermal sheets (Fig. 3 and Table 1) revealed a reduced number in patients (mean $\pm$ SEM: $409/\text{mm}^2 \pm 35$) compared to controls (mean $\pm$ SEM: $545/\text{mm}^2 \pm$

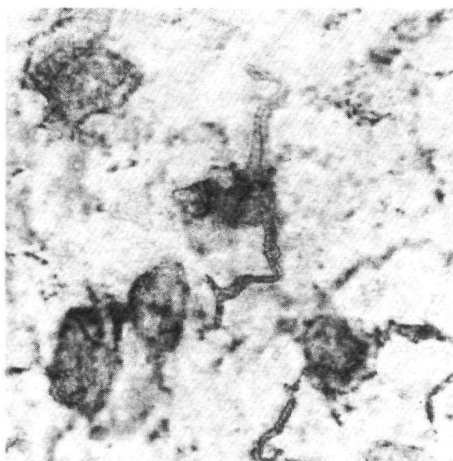


Fig. 3. Epidermal Langerhans cells (LC) in a patient with primary Sjögren's syndrome, demonstrated in epidermal sheet by the monoclonal antibody OKT6 by indirect immunoperoxidase technique (x 1000).

42) ($P < 0.05$; Fig. 4). Differences in morphology and distribution of LC were not demonstrated by the horizontal examination.

No correlation was found between reduced number of epidermal LC and age, disease duration, or the presence of intraepidermal IgG deposits (Table 1).

DISCUSSION

Demonstration of reduced numbers of LC in epidermal sheets from clinically unaffected skin of patients with primary SS is a new observation.

An extensive amount of information concerning the physiological and pathophysiological role of LC has been gained during the last few years (8). Epidermal LC are dendritic cells; they are not strongly phagocytic but seem to play an important role in cutaneous immune responses. Thus, there is considerable evidence indicating that LC have capacity for antigen presentation (8). It has been established that LC are of mesenchymal origin and that they derive from bone marrow precursor cells; indeterminate cells in the basal layers of epidermis are supposed to be the immediate precursor

Number of epidermal
Langerhans cells
per square millimeter

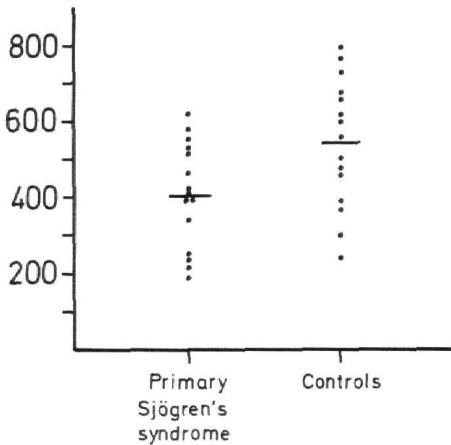


Fig. 4. Number of epidermal Langerhans cells/mm² in 15 patients with primary Sjögren's syndrome and 15 normal controls ($p < 0.05$).

cells (8). LC have the ability to move in the epidermis and to migrate into the dermis and further to regional lymph nodes. This is essential for the presentation of soluble and haptenated antigens to sensitized T cells (8). Beyond

this little is known about the regulation of epidermal LC kinetics.

Ultrastructurally, LC are characterized by the presence of Birbeck granules. By light microscopy they are identified using enzyme histochemical techniques, such as adenosine triphosphatase (ATPase). LC bears surface receptors for the Fc portion of IgG and for C3 and they express HLA-DR/antigens (8). Especially the presence of HLA-DR/antigens has been utilized in immunohistological staining procedures for LC (2). However, the most specific surface marker for human cutaneous LC has been shown to be the thymocyte antigen (HTA-1) defined by the mouse monoclonal antibody OKT6 (6). We therefore applied the OKT6 antibody in indirect immunohistologic techniques in order to visualize cutaneous LC.

Enumeration of epidermal LC can be done on both vertical sections (7) and on epidermal sheets (2). We chose the epidermal sheet method since this allows a more precise quantitation of LC.

The density of epidermal LC has been shown to vary between the different anatomical regions of normal skin (3). It is therefore important to examine skin biopsies from the same area when interindividual comparisons of LC densities are performed. We examined biopsies from the buttock as this area of the skin is not

Table 1

Sex, age, disease duration, density of epidermal Langerhans cells (LC) and epidermal IgG deposits in 15 patients with primary Sjögren's syndrome

Pt. No.	Sex	Age (years)	Disease duration (years)	No. epidermal LC cells/mm ²	Intra epidermal IgG deposits	Dermo-epidermal junction zone IgG deposits
1	F	46	2	396	-	-
2	M	59	3	216	+	-
3	F	37	1	343	-	-
4	F	68	12	196	+	-
5	M	71	14	416	+	-
6	F	63	14	466	-	-
7	F	62	8	245	+	-
8	F	66	5	241	-	-
9	F	57	14	558	-	-
10	F	60	8	582	+	-
11	F	68	28	622	-	-
12	F	38	15	393	+	-
13	F	43	9	425	-	-
14	F	52	16	518	+	-
15	F	68	12	523	-	+

exposed to sunlight. Sunlight, ultraviolet light and X-ray irradiation are known to reduce the number of epidermal LC, when determined by surface antigens (8).

Our sample of patients reflects the sex distribution of primary SS in general, as only two males were included. It is unknown to what degree sex and age influence the density of epidermal LC in normal skin (3). Our data, however, do not indicate any influence of age on LC density.

Few studies have dealt with LC in chronic inflammatory connective tissue diseases. In Behcet's disease epidermal LC have been shown to exist in a more active state, and located in the middle and upper parts of the epidermis (9). In our patients with primary SS, LC were located in the basal and suprabasal layers of epidermis, similar to what was observed in the controls. Examination of epidermal sheets revealed no differences in morphology or distribution of LC between patients and controls.

Intraepithelial deposits of IgG were demonstrated in 7/15 patients, in accordance with previous findings (12). As it has been hypothesized that IgG is attached to epidermal LC, it was of interest to compare these findings with the results from LC enumeration. However, no correlation was demonstrated. The cause of the reduced number of epidermal LC in clinically unaffected skin of patients with primary SS is at present unknown.

The impaired DNCB reactivity found in these patients (10) might be associated with decreased capacity for antigen presentation due to reduced density of epidermal LC.

Future studies must deal with the possibility of functional abnormalities of epidermal LC as well as with the possible involvement of LC in the various skin manifestations seen in patients with primary SS.

ACKNOWLEDGEMENT

We acknowledge the skilful technical assistance provided by Miss Jette Pedersen.

REFERENCES

- Alexander, E. L. & Provost, T. T.: Cutaneous manifestation of primary Sjögren's syndrome: A reflection of vasculitis and association with anti-RO (SSA) antibodies. *J. Invest. Dermatol.* **80**, 386-391, 1983.
- Belsito, D. V., Sanchez, M. R., Baer, R. L., Valentine, F. & Thorbecke, G. J.: Reduced Langerhans' cell Ia antigen and ATPase activity in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **310**, 1279-1282, 1984.
- Berman, B., Chen, V. L., France, D. S., Dotz, W. I. & Petroni, G.: Anatomical mapping of epidermal Langerhans cell densities in adults. *Br. J. Dermatol.* **109**, 553-558, 1983.
- Bertram, U.: Xerostomia. Clinical aspects, pathology and pathogenesis. *Acta. Odont. Scand.* **25**, (Suppl 49), 1-126, 1967.
- Chisholm, D. M. & Mason, D. J.: Labial salivary gland biopsy in Sjögren's disease. *J. Clin. Pathol.* **21**, 656-660, 1968.
- De Panfilis, G.: ATPase, antigens detected by monoclonal antibodies, ultrastructural characters: Which is the best marker for Langerhans cells? *Arch. Dermatol. Res.* **275**, 407-411, 1983.
- Horton, J. J., Allen, M. H. & MacDonald, D. M.: An assessment of Langerhans cell quantification in tissue sections. *J. Am. Acad. Dermatol.* **11**, 591-593, 1984.
- Hunter, J. A. A.: The Langerhans cell: from gold to glitter. *Clin. Exp. Dermatol.* **8**, 569-592, 1983.
- Kohn, S., Haim, S., Gilhar, A., Friedman-Birnbaum, R. & Nir, L.: Epidermal Langerhans' cells in Behcet's disease. *J. Clin. Pathol.* **37**, 616-619, 1984.
- Leventhal, B. G., Waldorf, D. S. & Talal, N.: Impaired lymphocyte transformation and delayed hypersensitivity in Sjögren's syndrome. *J. Clin. Invest.* **46**, 1338-1345, 1967.
- Manthorpe, R., Frost-Larsen, K., Isager, H. & Prause, J. U.: Sjögren's syndrome. A review with emphasis on immunological features. *Allergy* **36**, 139-153, 1981.
- Oxholm, A., Manthorpe, R. & Oxholm, P.: Immunoglobulin deposits in the epidermis of patients with primary Sjögren's syndrome. *Rheumatol. Int.* **4**, 9-12, 1984.
- Oxholm, P., Manthorpe, R. & Oxholm, A.: IgG attachment to the Langerhans cell in the epidermis of patients with primary Sjögren's syndrome. In Machtey, I. (ed): *Progr. Rheumatol.* Vol II. Rheumatology Service. Hashron Hospital, Israel, 135-139, 1984.
- Schall, G. L., Anderson, L. G., Wolf, R. O. et al.: Xerostomia in Sjögren's syndrome. Evaluation by sequential salivary scintigraphy. *J. A. M. A.* **216**, 2109-2116, 1971.
- Strand, V. & Talal, N.: Advances in the diagnosis and concepts of Sjögren's syndrome (autoimmune exocrinopathy). *Bull. Rheum. Dis.* **30A**, 1046-1049, 1981.

Address

Peter Oxholm, M.D.

Laboratory of Medical Immunology

Department of Rheumatology TTA, Rigshospitalet

DK-2200 Copenhagen N,

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.