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A SPECIFIC ANTIBODY AGAINST THE
EPITHELIUM OF THE SALIVARY DUCTS IN
SERA FROM PATIENTS WITH
SJÖGREN'S SYNDROME¹

By

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Sjögren's syndrome has several pathological, serological and clinical features in common with auto-immune diseases. The pathological findings in Sjögren's syndrome are chronic sialoadenitis and dacryoadenitis. The histological changes characteristic of these conditions have many features in common with the changes found in Hashimoto's thyroiditis in which autoimmune mechanisms seem to be of pathogenic importance. Both diseases are characterized by lymphocytic and plasma cell infiltration, fibrosis and epithelial changes. In Hashimoto's thyroiditis the epithelium is disorganized so that the follicles are substituted by clusters and trabeculae (Fig. 1 A and B). In the initial stages of Sjögren's syndrome the epithelial changes are only seen in the salivary ducts whereas the acini are left intact (12). The epithelium of the salivary ducts becomes disorganized and hyperplastic, the lumen disappears resulting in the formation of characteristic epithelial islets (Fig. 1 C and D). In both diseases the fibrosis is progressive and eventually all the glandular tissue is replaced by fibrotic tissue.

The initial stages of both Hashimoto's and Sjögren's dis-

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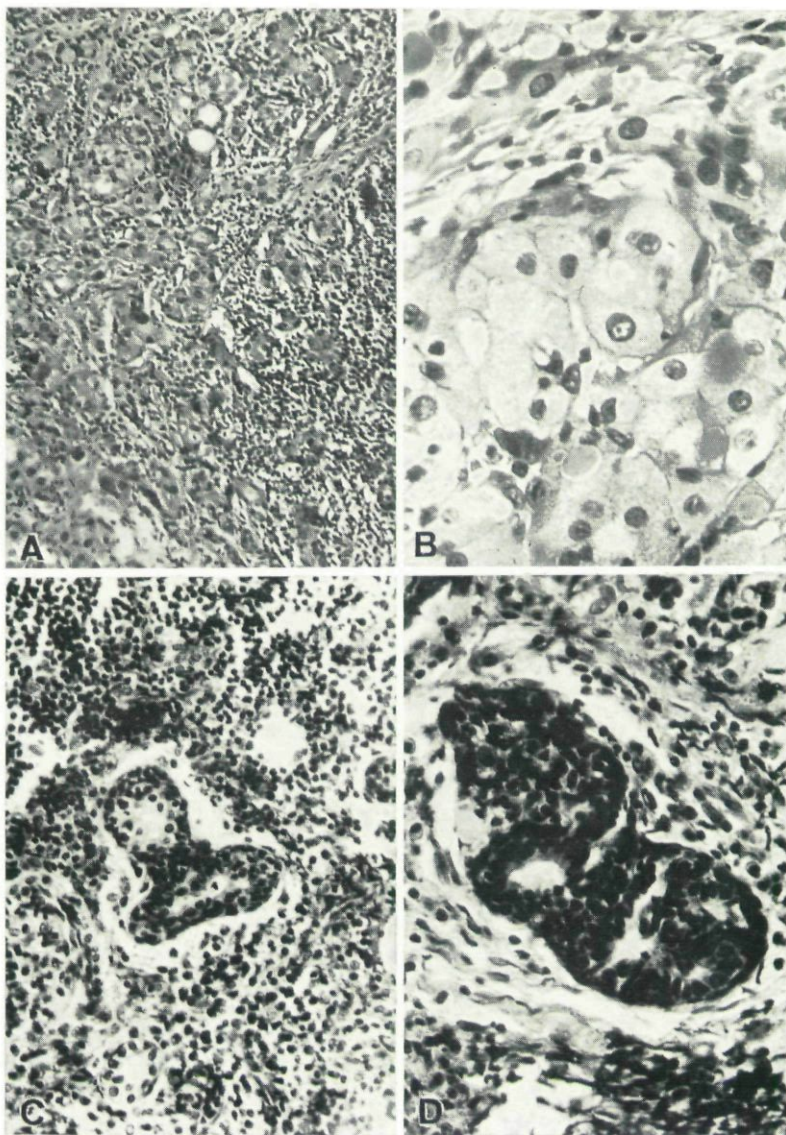


Fig. 1.

A. Histological sections from thyroid with chronic thyroiditis. $\times 40$.

B. As 1 A. $\times 160$.

C. Histological sections from parotid gland with chronic sialoadenitis. $\times 100$.

D. As 1 C. $\times 200$.

eases are characterized by swelling of the affected organs; the patients develop goitre and swelling of the salivary and lacrymal glands (Mikulicz's disease). As the diseases progress the swelling disappears and the function of the glands stops *i.e.*, the patients develop myxedema, xerostomia and xerophthalmia.

Other observations suggest that Sjögren's syndrome might be an auto-immune disease. Other diseases of possible auto-immune origin, disseminated lupus erythematosus (D.L.E.) and rheumatoid arthritis, are overrepresented in the families of patients with Sjögren's syndrome (6), and these diseases are frequently found together with Sjögren's syndrome (2), (9).

Serologically several findings suggest an auto-immune pathogenesis. Hypergammaglobulinemia is found in practically all sera from patients with Sjögren's syndrome, anti-nuclear factor is a common occurrence, specific thyroid antibodies are frequently found (10), and Rose-Waaler's test and the RA test are often positive, but as emphasized by us (4) all previous attempts of demonstrating a specific antibody against salivary or lacrymal tissues have been unsuccessful. Jones (11) and Anderson, Gray, Beck & Kinnear (1) found a precipitin against extracts of lacrymal tissue in sera from patients with Sjögren's syndrome, but the phenomenon was not specific since extracts of other organs could give similar reactions. Anderson *et al.* (1), Block & Bunim (6) and Mosbech & Østergaard Kristensen (12) found a complement fixing antibody against extracts of salivary glands in sera from patients with Sjögren's syndrome, but this antibody was not specific either since other organ extracts could also fix complement in presence of these sera. This finding is probably identical with the non specific antibody described by Gajdusek (8).

In the present paper a serological phenomenon is described which might represent a specific antibody against salivary tissue in sera from patients with Sjögren's syndrome.

MATERIAL

Sera from 19 patients with Sjögren's syndrome were examined. 14 of the patients had dryness of the mouth, 15 of the patients had sialometry (5) performed and 11 of them had xerostomia and 2 had hyposialia. Changes in the oral mucous membranes as described by *Bertram, Pindborg, Seedorff & Videbæk* (5) were found in 11 patients. Swelling of the salivary glands (parotid, submaxillary or palatine glands) were found or had previously been present in 16 of the patients. 15 of the patients had sialography performed the dye being injected through Stensen's duct. In 2 cases the examination was unsuccessful because the dye could not pass the obliterated main excretory ducts, and in 11 cases roentgenological changes were found which were compatible with chronic sialoadenitis. In 4 cases a biopsy was made of a salivary gland (3 of parotid gland and 1 of palatine gland) and in these cases histological changes were found as described in the introduction. 8 patients complained of dryness of the eyes, 10 patients had xerophthalmia as determined by Schirmer's test, and 7 patients had conjunctival erosions stainable with Rose Bengal. All of the 19 patients had hypergammaglobulinemia, the concentration of gammaglobulin ranging from 1.37 til 4.64 g per cent.

METHODS

The demonstration of the antibody against salivary tissue was performed by means of an immune fluorescence technique on unfixed sections of parotid, submaxillary and sublingual tissues. The technique was similar to the one described by *Coons & Caplan* (7). Tissue from normal salivary glands removed by operation was frozen within one hour by means of a solid CO₂-alcohol mixture. The tissue was stored in tubes under solid CO₂ for a period of time up to 2½ months. Sections of 3 μ were cut in a cryostat at minus 20 centigrade. After drying by fan, sera were applied in a hygrophor for one hour at 37 centigrade, washed in running buffered saline (Na barbitone 20.6 g, 0.1 N HCl 80.6 ml, NaCl 85.0 g, distilled water to 5 l, diluted with an equal volume of distilled water before use (3)) and left in buffer for 20 minutes after which fluorescein labelled anti human globulin (horse origin) from Progressive Laboratories Inc., U.S. was applied at room temperature for 20 minutes followed by a second wash in buffer for 20 minutes. A drop of 50 per cent glycerin buffer and a cover slip was

placed on the top of the section, and if the cover slip was sealed the sections could be kept for several weeks if stored in darkness.

RESULTS

The results are stated in Table 1. A frequent finding was nuclear fluorescence which might be expected since an anti-nuclear factor may often be found in sera from patients with

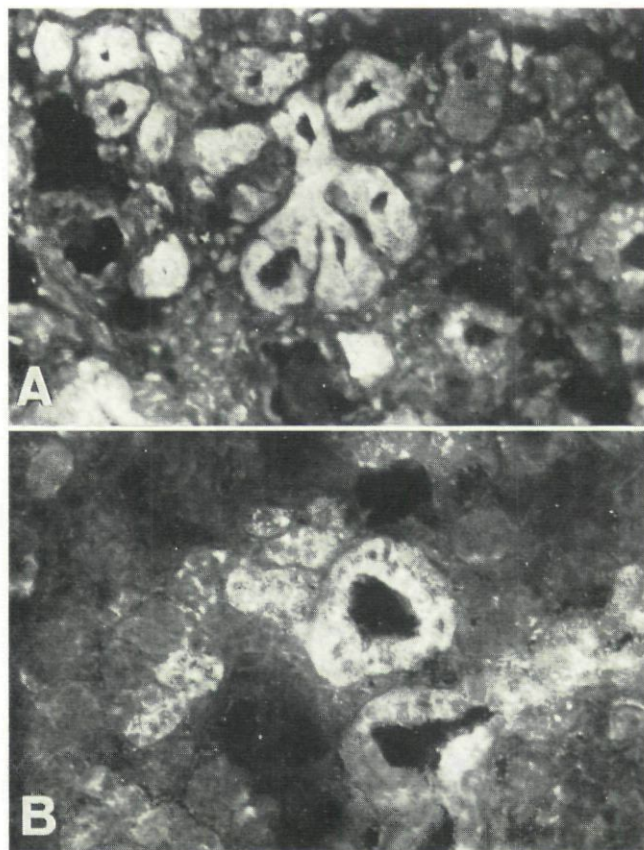


Fig. 2.

Immunofluorescence technique on sections of salivary tissue using sera from patients with Sjögren's syndrome.

- A. Cytoplasmatic fluorescence in salivary ducts and nuclear fluorescence.
- B. Cytoplasmatic fluorescence in salivary ducts, no nuclear fluorescence.

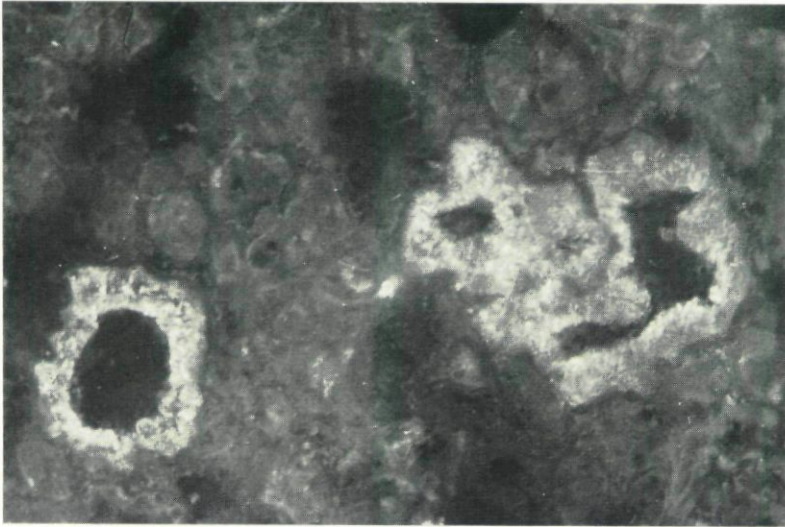


Fig. 3.

As 2 B., notice the granular appearance of the fluorescence.

TABLE I

Results of immunofluorescence technique applied on sections of salivary glands.

Origin of sera	Number of sera	Nuclear fluorescence	Cytoplasmatic fluorescence
Sjögren	19	7	11
Hashimoto	19	0	1
D.L.E.	16	12	4
Blood donors	24	0	1

Sjögren's syndrome. In this material nuclear fluorescence was found in 7 out of 19 sera using the fluorescence technique on sections of salivary tissue.

In addition to the nuclear fluorescence cytoplasmatic fluorescence could also be seen in many cases. This phenomenon occurred in the epithelium of the salivary ducts but never in the acini (Fig. 2 A and B). In several cases the cytoplasmatic fluorescence was very intense and it could easily be compared to the cytoplasmatic fluorescence found when testing Hashi-

moto' sera by means of the same technique on sections of thyroid tissue. By high magnification it appeared that the fluorescence was localized in granules which were more numerous in the luminal than in the basal parts of the cells (Fig. 3).

Using low magnification it looked as the intense fluorescence of the salivary ducts might be due to nuclear fluorescence since the nuclei are situated closer to one another in the ducts than the acini. A high magnification easily revealed both nuclear and cytoplasmatic fluorescence and in some cases cytoplasmatic fluorescence was found without nuclear fluorescence.

Cytoplasmatic fluorescence in salivary ducts was found testing 11 out of 19 Sjögren sera by immune fluorescence technique on tissues from parotid, submaxillary and sublingual glands. The phenomenon seems to be specific. 24 sera from random blood donors were tested in the same way. One of these sera, and one of 19 sera from patients with Hashimoto's thyroiditis caused cytoplasmatic fluorescence in the salivary ducts. Sera from 16 patients with disseminated lupus erythematosus were also tested; 4 of these caused cytoplasmatic fluorescence in the salivary ducts.

The 19 Sjögren sera were also tested on sections of thyroid tissue. Nuclear fluorescence was found when testing the same sera which caused nuclear fluorescence in salivary tissues. 2 additional sera caused nuclear fluorescence but cytoplasmatic fluorescence was never found in thyroid tissue.

DISCUSSION

Specific antibodies against salivary tissue in sera from patients with Sjögren's syndrome have not previously been described. The phenomenon described above might represent an antibody of this kind. The corresponding antigen seemed to be localized exclusively in the cytoplasm of the epithelial cells lining the salivary ducts whereas no antigenicity could be demonstrated in the acini. The antigen could be found in the excretory ducts of parotid, submaxillary as well as sublingual

glands. The localization of the antigen in the salivary ducts only, corresponds well with the histological observations in chronic sialoadenitis that the epithelial changes in the initial stages are only found in the excretory ducts whereas the acini at this times seem to be still intact.

In two thirds of Sjögren sera tested an antibody was found against an antigen in the salivary ducts. The same phenomenon was found in a serum from a blood donor. Unfortunately it was not possible to trace this donor. One of 19 Hashimoto sera contained the same antibody. This serum came from a patient who had myxoedema and Hashimoto's thyroiditis verified by biopsy and had also acquired hemolytic anemia. The other 18 patients with Hashimoto's thyroiditis had no signs of additional diseases of a possible auto-immune nature. Of 16 sera from patients with D.L.E. 4 contained the antibody against salivary ducts though none of these 4 patients presented signs of Sjögren's syndrome. A serological overlap between groups of patients with D.L.E. and Sjögren's syndrome may, however, be expected. Thyroid antibodies are frequently found in patients with D.L.E. without signs of thyroid disease (10), 7 of our patients with Sjögren's syndrome had symptoms characteristic of D.L.E. (cirrhosis of the liver, rheumatoid arthritis, thrombocytopenia and hemolytic anemia), L.E. cells were found in 4 and ANF in 9 of the 19 patients.

SUMMARY

Sjögren's syndrome has many features in common with auto-immune diseases both clinically, pathologically and serologically. However, all previous attempts to demonstrate a specific antibody against salivary tissue in sera from patients with Sjögren's syndrome have been unsuccessful. In this paper a serological phenomenon is presented which might represent a specific antibody against an antigen localized in the cytoplasm of the epithelial cells of the salivary ducts of parotid, submaxillary and sublingual glands, demonstrated by means of an immune fluorescence technique. No antigenicity was found

in the acini. The antibody was found in sera from 11 out of 19 patients with Sjögren's syndrome, in one of 24 control sera, in one out of 19 sera from patients with Hashimoto's disease and in 4 of 16 sera from patients with disseminated lupus erythematosus.

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