Oral mycoplasmas in Sjögren's syndrome

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SUMMARY Mycoplasmas were sought in the salivary secretions and minor salivary gland tissue of 26 patients with Sjögren's syndrome or the allied sicca complex. A mycoplasma (*M. orale* type 1) was recovered from the stimulated parotid saliva of only one case. Possible mechanisms of mycoplasmal cell damage in this and allied disorders are considered and some future lines of investigation are suggested.

Sjögren's syndrome consists of the triad of xerostomia, keratoconjunctivitis sicca, and rheumatoid arthritis (Sjögren, 1933; Bloch, Buchanan, Wohl, and Bunim, 1965). The presence of two of the three main components is generally considered sufficient for the diagnosis of the syndrome (Bloch *et al*, 1965) while the term 'sicca syndrome' is used when xerostomia and keratoconjunctivitis sicca only are present. In Sjögren's syndrome, a preponderance of female patients, especially in the postmenopausal age group, is consistently noted. The disorder is of particular interest since there is strong clinical, histopathological, and serological evidence that the disease may have an autoimmune aetiology. Thus, rheumatoid arthritis may be replaced in the triad by another connective tissue disease such as systemic lupus erythematosus, progressive systemic sclerosis, dermatomyositis, or polyarteritis nodosa (Bloch *et al*, 1965; Talal, 1966), while the histopathological changes in the major and minor salivary glands are similar to those observed in Hashimoto's thyroiditis, chronic gastritis and primary adrenocortical atrophy, all of which have specific autoimmune associations (Anderson, 1964). Furthermore, Sjögren's syndrome is characterized by a multiplicity of both organ-specific and non-organ-specific antibodies (Bunim, 1961; Bertram and Halberg, 1965).

Recently, renewed interest has been shown in the role of infective agents including bedsoniae, diphtheroids, and mycoplasmas in association with Reiter's disease, rheumatoid arthritis, systemic lupus erythematosus, and other connective tissue disorders (Bartholomew, 1965; Duthie, Stewart, Alexander, and Dayhoff, 1967; Schachter, 1967; Williams, 1968;

Stewart, Alexander, and Duthie, 1969; Williams, Brostoff, and Roitt, 1970; Sharp, 1970).

The impressive clinical and immunological associations of Sjögren's syndrome with other connective tissue disorders in which mycoplasmas have been found, and the clinical prominence of salivary gland involvement, have prompted us to search for oral mycoplasmas in the saliva and minor salivary gland tissue of patients with this fascinating symptom complex.

Materials and Methods

PATIENTS STUDIED

Twenty-six patients with Sjögren's syndrome were included in the present study. Twenty-one patients had Sjögren's syndrome complicated by rheumatoid arthritis and five had the sicca syndrome alone (Table I). The clinical diagnosis of Sjögren's syn-

<i>Clinical Diagnosis</i>	<i>No.</i>	<i>Sex</i>	<i>Mean Age and Range (yr)</i>
Sicca syndrome	10	9F; 1M	64 (42-78)
Sjögren's syndrome with rheumatoid arthritis	14	12F; 2M	62 (30-76)

Table I *Clinical diagnosis, age, and sex distribution in 24 patients with Sjögren's syndrome*

drome was based on the presence of any two of the three major components of the disease, namely, xerostomia, keratoconjunctivitis sicca, and rheumatoid arthritis or other connective tissue disease (Bloch *et al*, 1965). Ophthalmological examination by the method of Williamson, Cant, Mason, Greig,

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and Boyle (1967) was performed in each patient. The diagnosis of rheumatoid arthritis was based on the criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958).

ORAL EXAMINATION

Each patient was closely examined and questioned for oral and pharyngeal signs and symptoms of Sjögren's syndrome. Parotid salivary flow rates were determined on each patient by the method described by Mason, Harden, Rowan, and Alexander (1966). In addition, sialography, using the hydrostatic techniques of Park and Mason (1966), was performed on all patients. The criteria of Bloch *et al* (1965) were used as a basis for the diagnosis of sialographic abnormality. In addition, sera from all 26 patients were examined for antinuclear specific tissue precipitins (Anderson, Goudie, Gray, and Buchanan, 1961) and salivary duct antibody (MacSween, Goudie, Anderson, Armstrong, Murray, Mason, Jasini, Boyle Buchanan, and Williamson, 1967); the results of these examinations are shown in Tables I and II.

SALIVARY SECRETION AND TISSUE COLLECTION

Parotid saliva was collected using a modified Carlsson-Crittenden cup with an outer chamber diameter of 20 mm and an inner chamber diameter of 10 mm and a depth of 4 mm. Saliva was collected for five minutes under stimulation with lemon juice (Mason *et al*, 1966).

In six patients, lobules of minor salivary gland tissue were obtained by dissection from labial minor salivary gland biopsy specimens obtained by the method described by Chisholm and Mason (1968). Samples of saliva and gland tissue were distributed into bottles containing mycoplasma growth medium and were transmitted for laboratory examinations.

Methods

MEDIA FOR CULTIVATION OF MYCOPLASMAS

Liquid mycoplasma medium comprised a basic medium of 70 ml Difco PPLO broth, 20 ml unheated Burroughs Wellcome horse serum no. 6, 10 ml of a 25% aqueous extract of dried yeast (Distillers Company Ltd), 2 ml of a 2.5% aqueous solution of thallium acetate (reduced to 1 ml for T-strain culture

medium), 1 ml of a solution containing 100,000 units of penicillin G, and 2 ml of 0.1% aqueous solution of phenol red. Aliquots of this basic medium were separately supplemented with glucose, arginine, and urea (to a final concentration of 0.1%) for the detection, respectively, of glucose-fermenting, arginine, and urea-splitting mycoplasmas. The glucose medium was adjusted to pH 7.8 and arginine and urea media to pH 6.5. Solid medium was prepared by the addition of 1% agar (Oxoid Ionagar no. 2) to the PPLO broth before the addition of the other constituents.

CULTURAL METHODS

Parotid salivary specimens were inoculated in 1 ml amounts into 10 ml volumes of glucose, arginine, and urea indicator broths and in 0.1 ml amounts on PPLO agar plates. The bottles of medium were incubated at 35°C and observed daily for any colour change, while the solid media were incubated anaerobically at 37°C and examined for mycoplasma colonies every 48 hours. Incubation was continued for at least two weeks before the cultures were discarded. Salivary gland biopsy specimens were homogenized in PPLO broth in a Tenbroek grinder and the homogenate was then treated in the same manner as the saliva.

IDENTIFICATION OF MYCOPLASMAS

The method used was that of growth inhibition using the technique of Clyde (1964).

Results

Apart from one strain of *Mycoplasma orale* recovered from the salivary secretions of one patient, no oral mycoplasmas were recovered from the stimulated parotid saliva of any of the other patients or from any of the minor salivary gland biopsy specimens. This patient was a 65-year-old woman who suffered from the full Sjögren syndrome.

Discussion

Although the precise aetiological basis of Sjögren's syndrome remains unknown, it is generally accepted that the disease has strong autoimmune associations.

Clinical Group	Mean Stimulated Parotid Flow Rate (ml/min)	Sialectasis	Focal Labial Sialadenitis	Salivary Duct Antibody
Sicca syndrome	0.34 (0.0-0.48)	6 (60%)	7 (70%)	2 (20%)
Sjögren's syndrome with rheumatoid arthritis	0.39 (0.0-0.42)	7 (50%)	11 (79%)	8 (57%)

Table II Clinical and laboratory features in 24 patients with Sjögren's syndrome

Recently, an autoantibody specifically reacting with the cytoplasm of salivary duct epithelial cells has been detected by indirect immunofluorescence in patients with Sjögren's syndrome (Bertram and Halberg, 1965; MacSween *et al*, 1967; Feltkamp and van Rossum, 1968). An interesting feature is that this autoantibody is infrequently found in patients with the sicca syndrome and that it is present in 25% of patients with rheumatoid arthritis without clinical evidence of Sjögren's syndrome (MacSween *et al*, 1967). In the latter instance, the presence of the antibody may reflect subclinical focal lymphocytic sialadenitis in the major and minor salivary glands (Waterhouse and Doniach, 1966; Chisholm and Mason, 1968). However, the pathological basis of this finding has not yet been elucidated, though the possibility of an autoantibody being induced by an infective agent must be considered.

A number of infective agents, including mycoplasmas, have been sought and found in the various connective tissue diseases, especially in rheumatoid arthritis. The view that the latter might have an infective aetiology has been held for many years and recent reviews of the subject have been published (British Medical Journal, 1970; Lancet, 1970). Diphtheroid organisms have been recovered from the synovial tissues of patients with rheumatoid arthritis (Duthie *et al*, 1967). The pathogenic significance of these findings is doubtful (Stewart *et al*, 1969) though it seems possible that they are contaminants (Clasener and Biersteker, 1969). Listeria (Pease, 1968) and L-forms of bacteria (Pease, 1969) have also been isolated from the synovia of rheumatoid cases, of Reiter's disease (Schachter, Barnes, Jones, Engleman, and Meyer, 1966), and established rheumatoid arthritis (Schachter, 1967). A viral aetiology has been sought with negative results (Barnett, Balduzzi, Vaughan, and Morgan, 1966), although further work has suggested the possibility of latent or defective viral infection of synovial cells (Grayzel and Beck, 1969). Warren, Marmor, Liebes, and Hollins (1969) have reported the isolation of an active agent from rheumatoid arthritis synovial membranes. The full significance of these findings remains speculative, but the possibility exists of contamination or the stimulation of latent endogenous infections. Other workers have isolated mycoplasmas from the tissues in rheumatoid arthritis and some allied connective tissue disease (Bartholomew and Himes, 1964; Bartholomew, 1965), the strains belonging to a variety of species including *M. hominis* and *M. hyorhinitis*. Williams (1968), employing techniques designed to exclude contamination, has identified *M. fermentans* in synovial fluids from 39% of rheumatoid arthritis patients and in only 8% of fluids from control subjects. T-strain mycoplasmas

have also been recovered from joint fluids in cases of Reiter's disease (Jonsson, 1961; Bartholomew, 1965). The significance of such isolations remains undecided and, while they may merely indicate contaminants or the presence of secondary 'opportunists' in the tissues of individuals with immunological derangements, it is of interest that mycoplasmas, including *M. agalactiae* and *M. arthritidis*, may produce a chronic polyarticular disease with synovitis during natural infection in rats, while *M. pulmonis* has been shown experimentally to have an arthritogenic effect in mice (Barden and Tully, 1969). A syndrome which included joint manifestations has also been encountered during *M. pneumoniae* infection of man (Lambert, 1968).

In this study, we have investigated the salivary mycoplasmal flora of patients with Sjögren's syndrome. The healthy human oropharynx is colonized by a variety of mycoplasmas, principally *M. salivarium* and *M. orale*, type 1 and, less frequently, *M. orale*, types 2 and 3, *M. hominis* and T-strains (Kundsen and Praznik, 1967; Hendley and Jordan, 1968). However, with the exception of *M. pneumoniae*, which is rarely recovered from the healthy oropharynx, and *M. hominis* which has induced pharyngitis in some human volunteers under experimental conditions (Mufson, Ludwig, Purcell, Cate, Taylor-Robinson, and Chanock, 1965), there is no real evidence so far that any of the naturally occurring oral mycoplasmas are ever pathogenic. These organisms have not been implicated in recurrent aphthous ulceration (Gordon, Dick, Mason, Manderson, and Crichton, 1967), a condition considered by some workers to have an autoimmune basis (Lehner, 1967). The only human species whose pathogenicity is beyond doubt is *M. pneumoniae*. Subsequent to its identification as the cause of Eaton's primary atypical pneumonia (Chanock, Hayflick, and Barile, 1962) this mycoplasma has become widely recognized as a fairly common cause of acute upper and lower respiratory tract infections (Marmion, 1967). It has also been associated with otitis media, bullous myringitis, meningo-encephalitis (including the Guillain-Barre syndrome), polyarthritis, and various mucocutaneous rashes (Marmion, 1967; Lambert, 1969). It has been known for many years that *M. pneumoniae* infection of the respiratory tract is often associated with the development of cold agglutinins, while antibodies with a specificity for lung tissue have been recognized in Eaton agent (*M. pneumoniae*) atypical pneumonia (Thomas, 1964). More recently, it has been found that the cold agglutinin is a red cell autoantibody possessing anti-I specificity arising as a result of a surface alteration by the mycoplasmas of I receptors on the red cell surface (Feizi and Taylor-Robinson, 1967). The

ability of one human pathogenic mycoplasma to induce autoantibody formation during infection has led to considerable speculation about a possible association between mycoplasma infections and other diseases considered to have an autoimmune basis. The recovery of these organisms from the tissues in some of the connective tissue disorders has served to intensify research along such lines, but there is no evidence so far that any of the currently recognized human mycoplasma species other than *M. pneumoniae* is capable of evoking autoantibody formation.

In this investigation, we have produced no evidence to implicate naturally occurring human oral mycoplasmas in Sjögren's syndrome or the sicca syndrome, but it is interesting to speculate on possible mechanisms of autoantibody formation locally or more generally in this and allied disorders. Experimental work with *M. pneumoniae* has led to a greater understanding of the mechanism of adsorption of mycoplasmas to cell surfaces. *M. pneumoniae* strongly adsorbs to red cells, spermatozoa, and to tracheal epithelial cells by means of neuraminic acid receptors (Sobeslavsky, Prescott, and Chanock, 1968) and this affinity for respiratory tract epithelium provides an excellent opportunity for hydrogen peroxide secreted by the mycoplasma to inflict oxidative damage on the tissue cell membrane without being rapidly destroyed by catalase or peroxidase enzymes present in the extracellular fluids (Cohen and Somerson, 1967). Some other human mycoplasmas also produce hydrogen peroxide but to a much lesser degree than *M. pneumoniae*, while they are not known to adsorb to epithelial cells to the same extent as *M. pneumoniae* (Cole, Ward, and Martin, 1968). Nevertheless, by analogy with some virus-host cell relationships, prolonged contact of an oral mycoplasma with salivary epithelial cells in the form of a latent or persistent infection might conceivably result in antigenic alteration on or within such cells with subsequent loss of 'self' recognition and the development of an autoantibody. Alternatively, the occurrence of salivary duct antibody in Sjögren's syndrome might be explained by a sharing of antigenic determinants between components of salivary duct epithelial and mycoplasmal antigens. This unproven concept might gain support by consideration of other examples of immunological cross-reactions in other biological systems. This phenomenon has long been recognized in relation to the appearance of Streptococcus MG agglutinins in atypical pneumonia patients, and recent studies by Plackett, Marmion, Shaw, and Lemcke (1969) have indicated that this is due to the occurrence of a common diglucosyldiglyceride antigenic component in *M. pneumoniae* and Streptococcus MG. Shifrine and Moulton (1968) have demon-

strated the antigenic relationship between the galactan of *M. mycoides* and galactan-containing carbohydrates in bovine lung tissues, which may well explain the occurrence of anti-lung antibodies during bovine pulmonary infection by this species. A similar sharing of antigenic determinants between *M. pneumoniae* and human lung tissue might explain the known development of anti-lung antibodies in mycoplasmal pneumonia (Thomas, 1964). It is not yet known whether human oral mycoplasmas share antigens with salivary tissue components, though this possibility deserves attention.

The prime object of this preliminary study was to search for overt mycoplasmal infection of salivary tissue and related secretions in Sjögren's syndrome employing conventional cultured methods. Our failure to demonstrate these organisms in salivary tissue from a limited number of classical Sjögren's cases, though disappointing, does not of course exclude the possibility of the occurrence of hitherto uncultivable agents or of 'latent' mycoplasmal infection in this and allied conditions. It would be logical to continue the search for mycoplasma elementary bodies or their antigens by means of the newer tools of electron microscopy or immunofluorescence, although their detection in affected tissues would not itself resolve the question of whether these agents were present as primary aetiological agents or merely as 'passengers' or 'opportunists' secondary to the pathological and immunological derangements which are such cardinal features of the syndrome. We did not examine the sera of these patients for mycoplasmal or humoral antibodies against the common oropharyngeal species by routine serological techniques. While this might have been of interest, we did not consider that it would have been a particularly rewarding exercise. Normal serological tests for mycoplasma antibodies did not allow Williams (1967) clearly to differentiate rheumatoid arthritis sera from controls, while Sharp (1970), employing growth inhibition tests, obtained no evidence of a humoral mycoplasmal immune response in individuals with various arthritides, including rheumatoid disease. Furthermore, recent work (Williams *et al*, 1970) has given further stimulus to the concept that the development of arthritic lesions in rheumatoid disease may represent a delayed-type hypersensitivity reaction to mycoplasma infection, a mechanism which would not be reflected by the results of conventional serological tests for humoral antibody. Employing a leucocyte migration inhibition test (Bendixen and Soborg, 1969), which is thought to indicate a state of cell-mediated hypersensitivity (Soborg, 1967; Brostoff and Roitt, 1969), Williams *et al* (1970) have recently observed that *M. fermentans* membranes inhibited

leucocyte migration in 29 of 43 (67%) patients with rheumatoid arthritis, but not in osteoarthritis cases or in healthy controls. It has, therefore, been suggested that rheumatoid arthritis patients are hypersensitive to antigens of *M. fermentans*, a serotype which has not infrequently been recovered from the synovial fluids of such individuals (Williams, 1967, 1968), though it is not often present in the human oropharynx. The part played by hypersensitivity mechanisms in the aetiology of Sjögren's syndrome has been considered by others (Leventhal, Waldorf, and Talal, 1967), but we are unaware of reports applying tests of cell-mediated hypersensitivity to this problem employing mycoplasma antigens. In view of the intriguing results recently obtained with rheumatoid arthritis patients, it would be of extreme interest to utilize the leucocyte migration inhibition test for examination of the sera and saliva of patients with Sjögren's syndrome employing antigens prepared from *M. orale*, *M. salivarium*, or T-strains. Investigations along these lines may cast further light on a disease, the pathogenesis of which remains inadequately understood.

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