Impairment of lachrymal and salivary secretion and cellular immune responses to salivary antigens in rheumatoid arthritis

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SUMMARY During a systematic investigation of 100 unselected outpatients with rheumatoid arthritis, 58 were found to have reduced lachrymal or salivary secretion. No correlation could be detected between the presence or absence of secretory abnormalities and the age or sex of the patient, the presence of nodules or salivary duct antibody, or the occurrence of vasculitis. However, there was a significant correlation between diminished salivary or lachrymal flow and the occurrence of cellular immune responses to a protein fraction of normal human saliva, sensitisation being found in 94% of those with impairment of salivary and lachrymal secretion as compared with 33% of those without.

The sicca syndrome (xerostomia or keratoconjunctivitis sicca, or both) is a frequent accompaniment of many autoimmune diseases. When specifically sought, sicca features have been found in scleroderma (Alarcon-Segovia *et al.*, 1974a), polyarteritis nodosa (Ramage and Kinnear, 1956), systemic lupus erythematosus (Alarcon-Segovia *et al.*, 1974b), and rheumatoid arthritis (Thompson and Eadie, 1956), as well as in certain liver diseases, particularly chronic active hepatitis and primary biliary cirrhosis (Golding *et al.*, 1973). Most of these conditions are thought to have an autoimmune origin and the secretory abnormalities of the lachrymal and salivary glands are presumed to arise from immunological damage to these glands. The exact nature of the responsible immune reaction has not been elucidated but both humoral (Bertram and Halberg, 1964; MacSween *et al.*, 1967; Feltkamp and van Rossum, 1968) and cellular (Søberg and Bertram, 1968; Berry *et al.*, 1972; Anderson *et al.*, 1973) mechanisms have been suggested. In the present study, humoral and cellular immune responses to salivary antigens were examined in relation to clinical and subclinical impairment of salivary and lachrymal function in 100 unselected patients with rheumatoid arthritis.

Patients and methods

100 outpatients with definite or classical rheumatoid arthritis (according to the criteria of the American Rheumatoid Association) were evaluated. 92 patients were being treated with nonsteroidal anti-inflammatory drugs, 30 with corticosteroids, 31 with penicillamine, and 11 were receiving gold therapy. Patients on cytotoxic therapy were not included in the study. The patients were specifically questioned about symptoms of dry mouth and dry or gritty eyes. Tear flow was assessed by the Schirmer test. Patients in whom there was less than 5 mm moistening of the filter paper strips in both eyes after 5 minutes were regarded as having xerophthalmia. 14 normal subjects tested under identical conditions all showed greater than 20 mm moistening of the filter paper strips. At the time of testing all patients were adequately hydrated and were not receiving any drugs known to affect lachrymal function. Rose-Bengal staining was performed by introducing a 1% aqueous solution of the dye into the conjunctival sac followed immediately by irrigation with normal saline. Eyes were then examined for corneal or conjunctival staining using a Haag-Streit slit-lamp. Total saliva flow was measured by spitting into a beaker all saliva produced in 10 minutes while chewing mint-flavoured gum. A diagnosis of xerostomia was made when the total saliva

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production was less than 12 ml/10 minutes (2 SD below the mean for 20 normal subjects). Severity of arthritis was assessed by the articular index, grip strength, duration of morning stiffness, and the presence of nodules or vasculitis.

Laboratory investigations included haemoglobin concentration, white blood cell count, erythrocyte sedimentation rate, total serum globulins, and rheumatoid factor. Antinuclear and gastric parietal cell antibodies were detected by immunofluorescence while antithyroid antibodies were detected by immunofluorescence and tanned red cell agglutination.

HUMORAL AND CELLULAR

ANTISALIVA IMMUNE RESPONSES

Antisalivary duct antibody was detected by indirect immunofluorescence of undiluted plasma on human submaxillary gland. Cell-mediated immunity to a salivary antigen preparation was evaluated in 32 patients and 20 controls by the leucocyte migration test (Mitchell *et al.*, 1972). Salivary antigen, prepared from normal human saliva by ammonium sulphate fractionation, was used in the leucocyte migration test at a concentration of 50 µg/ml (McFarlane *et al.*, 1976). Measurements of migration indices in the normal subjects gave a range of 0.74 to 1.02 (mean $\pm$ SD). Indices which fell below or above these limits were regarded as significant inhibition or stimulation of migration respectively.

Student's *t* test or χ^2 test with Yates's correction (where appropriate) were used for statistical analyses.

Results

Fifty-eight of the 100 patients showed evidence of diminished salivary or lachrymal function (Table). 18 patients had xerostomia, 26 had xerophthalmia, and 14 had both. As part of a separate study, 32 of these patients were also investigated using the Rose-Bengal test. 11 (65%) of 17 patients with diminished tear flow were found to have positive Rose-Bengal staining. By comparison, all of the remaining 15 who had normal Schirmer's tests were also normal on slit-lamp examination.

Only 19 of those with secretory abnormalities admitted to having dry eyes or mouths and these symptoms were as common in those with apparently normal secretion. Similarly, there were no apparent differences between those with and without impairment of secretion with respect to age, sex, duration or severity of disease, occurrence of vasculitis, or the presence of nodules; nor did they differ with respect to haemoglobin, white cell count, erythrocyte sedimentation rate, total globulins, presence of autoantibodies, or rheumatoid factor.

HUMORAL IMMUNITY

Salivary duct antibodies were detected in 26 patients (Table). A comparison of those with and without

Table Comparison of clinical and laboratory data of patients with and without xerostomia/xerophthalmia and salivary duct antibody

	Xerostomia or xerophthalmia		Salivary duct antibody	
	Present (n=58)	Absent (n=42)	Present (n=26)	Absent (n=74)
Male: female ratio	20 : 38	13 : 29	11 : 15	22 : 52
Age (years)	59 $\pm$ 10	57 $\pm$ 10	60 $\pm$ 8	58 $\pm$ 11
Duration of arthritis (years)	13 $\pm$ 11	9 $\pm$ 9	10 $\pm$ 10	12 $\pm$ 10
Xerostomia or xerophthalmia				
Symptoms	11 (19%)	8 (19%)	6 (23%)	12 (16%)
Signs			12 (46%)	46 (62%)
				$\chi^2=2.73$
Morning stiffness (h)	0.64 $\pm$ 0.62	0.70 $\pm$ 0.72	0.69 $\pm$ 0.67	0.69 $\pm$ 0.71
Articular index	9 $\pm$ 5	9 $\pm$ 7	9 $\pm$ 5	9 $\pm$ 5
Grip strength (mmHg)	107 $\pm$ 72	99 $\pm$ 62	93 $\pm$ 55	107 $\pm$ 70
Nodules	13 (22%)	12 (29%)	8 (31%)	17 (23%)
Vasculitis	2	1	1	2
Haemoglobin (g/dl)	12.9 $\pm$ 1.4	12.8 $\pm$ 1.4	12.8 $\pm$ 1.6	12.8 $\pm$ 1.3
White blood count ($\times 10^9/l$)	7.9 $\pm$ 1.9	8.1 $\pm$ 1.9	8.8 $\pm$ 1.5	7.7 $\pm$ 1.9
				$P < 0.01$
ESR (mm/h)	40 $\pm$ 26	40 $\pm$ 30	44 $\pm$ 28	39 $\pm$ 27
Globulin (g/l)	34 $\pm$ 7	32 $\pm$ 6	35 $\pm$ 6	32 $\pm$ 6
Rheumatoid factor	39 (67%)	28 (67%)	20 (77%)	47 (64%)
Antinuclear antibody	8 (14%)	11 (26%)	6 (23%)	13 (18%)
		$\chi^2=3.30$		
Antithyroid antibody	14 (24%)	8 (19%)	9 (25%)	13 (18%)
				$\chi^2=2.34$
Parietal cell antibody	5 (9%)	4 (10%)	6 (23%)	3 (4%)
				$\chi^2=6.33$
Salivary duct antibody	12 (21%)	14 (33%)		
		$\chi^2=2.73$		

Note: Means $\pm$ SD given where applicable or number of patients with percentages of total numbers in parentheses.

salivary duct antibody showed no difference with respect to age, sex, duration or severity of arthritis. The white blood count in those patients with the antibody was significantly higher than in those without ($8.8 \pm 15 \times 10^9/l$ compared with $7.7 \pm 1.9 \times 10^9/l$), but this difference could not be explained. Surprisingly, no correlation could be detected between the presence of salivary duct antibody and the occurrence or severity of xerostomia or xerophthalmia. There was, however, a statistically significant association between salivary duct antibodies and the presence of gastric parietal cell antibodies ($P < 0.025$). The frequency of anti-thyroid antibodies was also increased in those patients with salivary duct antibodies, but this increase was not statistically significant.

CELLULAR IMMUNITY

Inhibition of leucocyte migration in response to the salivary antigen was observed in 13 and stimulation in 3 of 17 patients with impaired secretion (Fig.). By comparison, inhibition was found in only 4 and stimulation in 1 of 15 patients with apparently

Fig. Results of leucocyte migration test using salivary antigen in control subjects and in patients with normal or diminished saliva or tear flow. Hatched area shows the normal range (mean $\pm$ 2 SD of indices in normal subjects).

normal secretions. The difference between those with and those without secretory abnormalities was statistically significant, whether inhibition alone ($\chi^2 = 6.06$, $P < 0.025$) or both inhibition and stimulation ($\chi^2 = 10.4$, $P < 0.005$) are regarded as indicative of cellular hypersensitivity. Sensitisation did not appear to be dependent upon whether the impairment of secretion was salivary or lachrymal.

Discussion

The finding that 58% of our patients had abnormal Schirmer's tests or reduced saliva flow indicates that impaired salivary or lachrymal secretion occurs far more frequently in rheumatoid arthritis than the symptoms of dry eyes or dry mouths might suggest. Indeed, only 19% of our patients with xerostomia or xerophthalmia had suggestive symptoms and these were as common in those with secretory impairment as in those without. A similar poor correlation of symptoms with objective measurements has been noted by MacSween *et al.* (1967).

While a diagnosis of keratoconjunctivitis sicca cannot be made only on the results of a Schirmer's test, it is of interest that 11 of 17 patients with abnormal Schirmer's tests also had positive Rose-Bengal staining. This finding indicates that the Schirmer's test is a reliable method of screening for keratoconjunctivitis sicca, particularly when the criterion of abnormality is 5 mm rather than the customary 10 mm moistening of the filter paper strips in 5 minutes. Moreover, since in the complete series 40 patients were diagnosed as having xerophthalmia, these findings suggest that keratoconjunctivitis sicca may be much more common in rheumatoid arthritis than the frequently quoted figure of 14.3% (Thompson and Eadie, 1956), although fortunately the abnormalities are often not sufficiently severe to cause clinical symptoms.

In contrast to other studies, we did not find that impaired secretion was more common in women than in men (Bloch *et al.*, 1965) and there seemed to be no correlation with the severity or duration of the rheumatoid disease or with associated immunological features such as rheumatoid factor (Whaley *et al.*, 1973). Similarly, salivary duct antibody was no more common in rheumatoid arthritis with xerostomia or xerophthalmia than in rheumatoid arthritis alone. This suggests that the production of salivary duct antibodies is not primarily related to the development of xerostomia but merely reflects the propensity of some of these patients to produce autoantibodies (Whaley *et al.*, 1969). This is supported by the finding of a significantly greater incidence of gastric parietal cell antibodies in these patients with salivary duct antibodies.

The only immunological factor which distinguished the patients with impaired lachrymal or salivary secretion was the finding of cellular immune responses to salivary antigens in 94% of these cases. The finding of sensitisation to salivary antigens in 33% of patients with apparently normal salivary and lachrymal secretion is not surprising. Both the Schirmer's test and saliva flow measurements are relatively insensitive measures of glandular function and the immunological abnormalities would be expected to occur before secretory abnormalities become demonstrable. Indeed, Berry *et al.* (1972) have documented progression to clinically detectable keratoconjunctivitis sicca in a small number of asymptomatic patients with antisalivary gland cell-mediated immunity.

The occurrence of cellular immune responses to salivary gland antigens in patients with the sicca syndrome has also been found by Søberg and Bertram (1968) and is in accordance with the demonstration of a periductal lymphocytic infiltrate in the affected glands (Chisholm and Mason, 1968; Anderson *et al.*, 1973). The exact nature of the target antigen(s) is obscure because these investigators have used relatively crude extracts of whole salivary glands. In this study we have used a partially purified protein fraction of normal human saliva which we have previously shown (McFarlane *et al.*, 1976) contains at least four antigens, two of which are also present in homogenates of whole salivary glands. Whether one of these antigens is derived from the salivary duct epithelium will remain unknown until the relevant antigens have been further purified.

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