HLA class I and II, interferon, interleukin 2, and the interleukin 2 receptor expression on labial biopsy specimens from patients with Sjögren's syndrome

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SUMMARY Labial biopsy specimens from eight patients with primary Sjögren's syndrome (SS), 10 patients with secondary SS, and three healthy controls were studied with monoclonal antibodies identifying HLA class I and class II antigens; interferon- α , β , and γ ; interleukin 2 (IL2); and the IL2 receptor (Tac) among others. In the normal biopsy specimens there was evidence of HLA class I and, to a lesser extent, class II antigens in both ducts and acini, though this was much less marked than in the Sjögren's biopsy specimens. Interferon- γ staining, but not interferon- α or β , was also considerably enhanced in the biopsy specimens from the patients with Sjögren's syndrome. These data support the view that in Sjögren's syndrome the release of interferon- γ may be involved in the induction of class II determinants. Our observations were broadly similar in both primary and secondary Sjögren's syndrome except that patients with primary Sjögren's syndrome tended to have more diffusely scattered T lymphocytes.

Sjögren's syndrome is an autoimmune disorder characterised histologically by a primarily lymphocytic infiltration of the salivary and lachrymal glands. Plasma cells are also commonly found in the cellular infiltrate which accompanies the coincident destruction of the acinar tissue and proliferation of the ducts. Most patients with Sjögren's syndrome may be divided into those with primary disease, dry eyes and mouth alone, and those with secondary disease, in whom these symptoms are set against a background of another autoimmune rheumatic disease such as systemic lupus erythematosus or rheumatoid arthritis.

In a previous study of labial biopsy specimens from patients with secondary Sjögren's syndrome we showed that most of the infiltrating cells were T lymphocytes, among which those expressing the helper/inducer phenotype predominated.¹ Many of

these cells appeared to co-express HLA class II antigens, suggesting that these T lymphocytes were activated. These observations have been confirmed,² and reports suggest that the same findings are evident among patients with primary Sjögren's syndrome.^{2,3}

We have undertaken the present study to examine in more detail the relation between HLA class I and II antigens on both the glandular structures and the infiltrating cells, and interferon and interleukin expression. In particular we were interested to know whether our observations in muscle tissue from patients with polymyositis would be repeated in this related context.⁴⁻⁶ These observations included the demonstration that normal muscle does not express HLA class I antigens but that in damaged muscle of in fibres adjacent to an inflammatory infiltrate there is abundant HLA class I expression, which is closely matched to the detection of interferon- α , β , and γ . We have suggested that after some initial damage the release of interferons from the infiltrating cells is responsible for the HLA class I expression, which may help to drive the autoimmune process.

Accepted for publication 17 March 1987.

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Table 1 Patients with primary Sjögren's syndrome

Patient No	Age	Sex	Ethnic origin	Treatment at time of biopsy
1	73	M	Caucasian	Navidrex-K 2 tab/day
2	49	M	Indian	Naprosyn 1 g/day, Plaquenil 400 mg/day
3	58	F	Caucasian	—
4	61	F	Caucasian	—
5	68	F	Caucasian	Navidrex-K 2 tab/day
6	56	F	Caucasian	Indocid 75 mg/day
7	51	F	Caucasian	—
8	50	F	Caucasian	—

Patients, materials, and methods

PATIENTS

Eight labial biopsy specimens from patients with primary Sjögren's syndrome and 10 from patients with secondary Sjögren's syndrome were studied. The details of these patients are shown in Tables 1 and 2. In addition, three patients attending University College Dental Hospital to have wisdom teeth removed agreed to have labial biopsies having signed a written consent approved by the hospital ethical committee.

MATERIALS AND METHODS

Frozen tissue sections (6 µm) from patients with Sjögren's syndrome and from normal individuals were stained with a panel of monoclonal antibodies (MoAb). The monoclonal antibodies used were: (a) 2A1: an IgG1 mouse MoAb. It identifies a non-polymorphic determinant of HLA class I antigens; (b) UCHT1: an IgG1 mouse MoAb against the CD3 (T3) antigen present on mature T lymphocytes and

some thymocytes; (c) DA2: an IgG1 MoAb with specificity for a non-polymorphic determinant of HLA class II antigens⁷; (d) 74-3: an IgG1 mouse antibody which binds interferon- α ⁸; (e) 117-1: an IgG1 mouse MoAb which binds interferon- β ⁹; (f) 166-5: an IgG1 mouse MoAb which also binds interferon- γ ¹⁰; (g) DMS2: a MoAb to interleukin 2 (gift of Kendal A Smith); (h) DMS3: a MoAb to interleukin 2 (gift of Kendal A Smith); (i) anti-Tac: MoAb to the IL2 receptor¹¹ (gift of T Waldmann).

The method used was as follows: (a) unfixed sections were washed for two minutes in phosphate buffered saline (PBS) and incubated with the first antibody for one hour. (b) They were then incubated in R-anti M Ig (RAM) (concentrations determined by titration, usually around 1/100 in PBS) containing 1/25 pooled normal human serum for 30 min. (c) The sections were then washed in running PBS for 30 s and then for 2 min in a trough. (d) They were incubated for 30 min in an alkaline phosphatase/monoclonal anti alkaline phosphatase complex (APAAP). (e) Sections were again washed in running PBS for 30 s and then for 2 min in a trough. (f) Steps a-e were repeated three times, with incubation in RAM and APAAP reduced to 10 min each. (g) The sections were then incubated in a fast red/naphthol AS-B1 phosphate substrate (Sigma Chemical Co, St Louis, MO, USA) containing 200 µl of 1 mM levamisole per 10 ml substrate solution. The substrate was dissolved in veronal acetate buffer pH 9.2. (h) Sections were washed in distilled water for 2 min. (i) Sections were counterstained in haematoxylin and mounted in Apathy's mounting medium. (j) The biopsy specimens were all assessed 'blind' using a semiquantitative 0 to +++ scale. It has been shown that the APAAP method can detect antigens not seen by more

Table 2 Patients with secondary Sjögren's syndrome

Patient No	Accompanying disease	Age	Sex	Ethnic origin	Treatment at time of biopsy (mg/day)
9	SLE	34	F	Caucasian	Dapsone 50
10	SLE	41	F	Caucasian	Plaquenil 400, Naprosyn 500
11	SLE	40	F	Caucasian	Plaquenil 400
12	SLE	68	F	Caucasian	Feldene 20
13	SLE	40	F	Caucasian	Prednisolone 10
14	SLE	32	F	Caucasian	Plaquenil 400
15	SLE	57	F	Caucasian	Prednisolone 7.5
16	SLE	31	F	Chinese	—
17	RA	56	F	Caucasian	Plaquenil 400, Lederfen 900
18	Polymyositis	32	F	Indian	Prednisolone 7.5, azathioprine 100

SLE=systemic lupus erythematosus.

Table 3 Primary Sjögren's syndrome

Patient No	2AI			UCHT1 DA2			Interferon-α			Interferon-β			Interferon-γ			DMS2 DMS3 Tac		
	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys			
1	+++	+++	+++	+++	+	+++	+	Trace	-	+	+	Trace	+	+	-	Trace	+	+
2	+++	+++	+++	+++	+	+++	Trace	Trace	-	+++	+	-	+++	+++	-	++	++	-
3	++ to +++	+	*	+	0 to +	+	+	+	+	-	Trace	-	-	+	-	-	-	-
4	+++	+++	+++	+++	+	+	0 to +	0 to +	+	-	-	-	+	+	+	-	+	+++
5	+++	+++	+++	+++	+	+	+	+	+	-	+	+	+	+	+	-	+	-
6	-	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-
7	+++	+++	+++	+++	+	+	+	+	-	-	-	-	+	+	-	+	+	-
8	+	+	+++	+	+	+	-	-	-	-	-	-	Trace	+++	-	-	-	-

Staining was recorded on a 0 to +++ scale. A=acini; D=ducts; Lys=lymphocytes. *Indicates structure not present in the section examined.

Table 4 Secondary Sjögren's syndrome

Patient No	2AI			UCHT1 DA2			Interferon-α			Interferon-β			Interferon-γ			DMS2 DMS3 Tac		
	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys			
9	+++	+++	+++	+++	+	+++	+	Trace	+	Trace	Trace	Trace	-	-	-	-	Trace	Trace
10	++	+++	+++	+++	+	+++	-	-	-	-	-	-	+	+	-	+	+	-
11	++	++	+++	+++	+	+++	+	+	-	+	-	-	+++	+++	+	-	+	-
12	+++	+++	+++	+++	+	+++	Trace	Trace	+	-	Trace	Trace	Trace	+++	+	+	Trace	Trace
13	Trace	+++	+++	+	+	+++	+	-	-	Trace	Trace	-	+	Trace	-	+	-	-
14	+	+++	+++	+	+	+++	+	-	-	-	-	-	+	+++	+	+	-	-
15	+	+	+++	+	+	+	-	-	-	+	-	-	+	+	+	+	-	-
16	+++	+++	+++	+++	+	+++	+	+	+	+	+	+	-	+	+	+	+	+
17	+++	+++	+++	+++	+	+++	+	+	+	-	-	-	+	+	+	+	+	+
18	+++	+++	+++	+++	+	+	+	+	-	-	-	-	+	+	-	-	ND	ND

Staining was recorded on a 0 to +++ scale. Lys=lymphocytes; ND=not done. *Indicates structure not present in the section examined.

Table 5 Normal controls

Control No	2AI			UCHT1 DA2			Interferon-α			Interferon-β			Interferon-γ			DMS2 DMS3 Tac		
	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys	Acini	Ducts	Lys			
1	Trace	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-
2	+	+	+	+	+	+	-	-	-	-	-	-	-	Trace	-	-	-	-
3	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-

Staining was recorded on a 0 to +++ scale. *Indicates structure not present in the section examined.

conventional methods when multiple layers of APAAP complexes are used.⁵

Results

The results using the nine monoclonal antibodies are shown in Tables 3, 4, and 5. With the monoclonal antibody identifying the HLA class I determinants (2A1) considerable staining was seen in the acini, ducts, and lymphocytes of the patients with both primary and secondary Sjögren's syndrome (Fig. 1). There were few infiltrating lymphocytes seen in the biopsy specimens from the healthy controls, but some staining of the ducts and acini was found, though this was clearly less than that found in patients with Sjögren's syndrome (Fig. 2).

UCHT1 was used to ascertain the presence of T lymphocytes and confirmed that focal collections of these cells were frequently present in the biopsy specimens from patients with secondary Sjögren's syndrome. In contrast, patients with primary Sjögren's syndrome tended to have diffusely scattered T lymphocytes. T lymphocytes were rarely identified in the normal controls.

Although the presence of class II antigens in the ducts and acini varied in both intensity and distribution in different patients, most of the lymphocytes were stained in all patients, indicating that these cells were activated (Fig. 3). At least 60% of cells in the lymphocyte infiltrates in all patients were stained. Strong (+++) staining was seen in 4/8 patients with primary Sjögren's syndrome, and in 6/8 patients with secondary Sjögren's syndrome with SLE. In the remainder of the biopsy specimens from patients with secondary Sjögren's syndrome (and SLE) at least ++ staining of lymphocytes was seen.

Fig. 2 Normal control stained for HLA class I using the APAAP technique counterstained with haematoxylin. Some positive cells are arrowed. The ducts and acini are predominantly negative.

Overall, there was much less staining with HLA class II (DA2) in the three normal controls, though some ductal and acinar cells were found to be clearly positive in each case (Fig. 4).

The monoclonal antibody identifying interferon- α showed some strong staining (+++) in two of the patients with primary Sjögren's syndrome. This staining was confined largely to the acini. Much less staining (maximum +) was seen in the acini and ducts of the patients with secondary Sjögren's syndrome with SLE. On plasma cells in two patients with secondary Sjögren's syndrome, however, grade ++ was recorded. In the normal controls no staining was seen.

There was little evidence of interferon- β , with the exception of one patient with primary Sjögren's

Fig. 1 Patient with secondary Sjögren's syndrome stained for HLA class I using the APAAP technique counterstained with haematoxylin. Most of the cells in the duct and surrounding infiltrate are strongly positive.

Fig. 3 Patient with secondary Sjögren's syndrome stained for HLA class II using the APAAP technique counterstained with haematoxylin. Ducts and acini are both positively stained.

Fig. 4 Normal control stained for HLA class II using the APAAP technique counterstained with haematoxylin. Only a few isolated cells are stained.

Fig. 6 Normal control stained for interferon- γ using the APAAP technique counterstained with haematoxylin. A few cells in the duct (arrowed) are weakly stained.

syndrome in whom epithelial cells in some ducts were strongly stained. It was negative on all the normal controls.

The monoclonal antibody identifying interferon- γ showed a notable pattern of ductal staining. In two of the patients with primary Sjögren's syndrome, ductal epithelial staining was identified, whereas secretory cells were specifically stained in two of the remaining patients. Among the patients with secondary Sjögren's syndrome (and SLE) strong ductal staining was seen in three patients and most, if not all, of the ducts were stained (Fig. 5). The staining was only confined to secretory cells in one patient. In two out of three of the normal controls no ductal staining was seen. In one, isolated positive ductal epithelial cells within ducts were seen but not the positive secretory population seen in the patients

with Sjögren's syndrome (Fig. 6). This individual had a periductal lymphocytic infiltrate, although this was not surrounding the ducts in which stained cells were seen.

Anti-IL2 (DMS2) showed very little staining. Very weak ductal staining, not exceeding +, was seen in one patient with primary Sjögren's syndrome and in one patient with secondary Sjögren's syndrome. Focal acinar staining was also seen in one patient with primary Sjögren's syndrome. Some very weak staining of plasma cells was seen in one patient with secondary Sjögren's syndrome. Unidentified spindle shaped cells were also stained in another patient with secondary Sjögren's syndrome. No staining was seen in the normal controls, except for isolated focal deposits previously reported.⁶

The other anti-IL2 antibody, DMS3, showed only extremely weak staining on a few patients, this being mainly in the acini with ductal staining on two patients. There was no staining of the normal biopsy specimens.

Very little staining was seen with anti-Ta (CD25). One of the patients with primary Sjögren's syndrome showed strong staining of one acinus, the significance of which is unclear. Weak staining of some ducts and acini was seen on another patient but this showed weak generalised staining and was probably not significant. In the patients with secondary Sjögren's syndrome, weak staining was seen of macrophages and ducts of one patient and very weak staining on ducts and lymphocytes of another. No other staining was seen in normal controls.

Having assessed the biopsies as described, we reanalysed serial sections from four patients taken at random and the controls, to try to correlate the expression of the class I and II antigens with interferon- γ . Compared with the normals, HLA

Fig. 5 Patient with secondary Sjögren's syndrome stained for interferon- γ using the APAAP technique counterstained with haematoxylin. Strongly stained cells can be seen at intervals in the duct.

class I antigens were much more strongly expressed on the acini, ducts, and infiltrating lymphocytes. There was a similar increase in HLA class II expression on the acini and lymphocytes, but the increase in duct staining was far more variable. In contrast, increased interferon- γ expression was most marked in the ducts, rather less on the acini, and little increased on the lymphocytes.

Discussion

Evidence has recently accumulated which emphasises the potential role of class II MHC molecules in the initiation of autoimmune phenomena. For example, Bottazzo and colleagues have shown that interferon- γ can induce the expression of class II molecules on B lymphocytes, macrophages, and a variety of tissues which do not usually express these molecules.^{12 13} These tissues include thyroid epithelial cells, bile duct epithelium, synovial cells, and islet cells. The co-expression of these HLA D/DR determinants together with other self molecules on the surface of a given tissue may activate adjacent T lymphocytes and induce an autoimmune process.

In this study we have examined tissue from labial glands which bear the brunt of the autoimmune process in Sjögren's syndrome. We and others have shown that many of the infiltrating cells detectable in labial biopsy specimens are T lymphocytes, predominantly those carrying the helper/inducer phenotype, and that many also express HLA class II antigens, suggesting that they are in the activated state.^{1 2 14 15} In the study by Fox *et al* it was claimed that no HLA class II determinants were present on epithelial cells from 'normal subjects'.¹⁵ We have seen staining of a few acini, but this is much less marked than in the biopsy specimens from the patients with Sjögren's syndrome. This relatively minor discrepancy may be due to their use of necropsy material and tissue from 'histologically normal margins of glands removed because of tumours' to form their normal control material. In contrast, our normal tissue was taken from healthy, living volunteers.

The presence of lymphocytic infiltrates in labial biopsy specimens is not disease specific. Lindahl and colleagues have recently shown that eight of 11 labial biopsy specimens from patients with myasthenia gravis had focal lymphocytic infiltrates with similar helper/inducer phenotypic predominance.¹⁶ It is noteworthy that despite the infiltrates these patients had a paucity of the usual sicca complex symptomatology, implying that lymphocytic infiltration cannot be the only cause of impaired exocrine glandular dysfunction. This suggestion is supported by our observation that one of our healthy controls had a lymphocytic infiltrate.

We were interested to examine the relation between the HLA class I and II expression with interferon and, IL2 and the IL2 receptor detection. In our studies of polymyositis we were not able to detect HLA class I antigens in normal muscle.⁵ It was found only in areas of myofibre damage or along the sarcolemma/basement membrane of normal looking fibres which were adjacent to the inflammatory infiltrate. Furthermore, interferon- α , β , and γ expression was closely matched to that of the HLA class I antigens.⁶

In labial biopsy specimens from the healthy controls there was evidence of both HLA class I and class II expression in both ducts and acini, though it was less marked than in the specimen from patients with either primary or secondary Sjögren's syndrome. This suggests that the mechanisms of tissue damage may be different in Sjögren's syndrome than in myositis, though increased HLA expression may potentiate other mechanisms.

Analysis of the results with the monoclonal antibodies identifying the interferons showed some major differences when compared with our studies in polymyositis. Instead of the rather similar patterns that we found in myositis with each of the three interferons, our results in this study showed obvious differences between them. For example, there was very little evidence of any interferon- β expression in any of the biopsy specimens. There was generally weak but persistent interferon- α expression in the ducts and acini in both primary and secondary Sjögren's syndrome, though this was more obvious in the former. Interferon- γ staining in the ducts was very striking, especially in the patients with primary Sjögren's syndrome. This evidence lends some support to the view expressed by Fox *et al* that interferon- γ is produced locally and induces class II determinants.¹⁵ Anatomically, however, it is difficult to be certain that the same cells showing increased HLA class I and II expression are also those which show increased expression of interferon- γ . Further studies using double labelling will answer this point.

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