Effect of cyclosporin A (CyA) on the immunopathological lesion of the labial minor salivary glands from patients with Sjögren's syndrome

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SUMMARY Labial minor salivary gland biopsy specimens from 14 patients with Sjögren's syndrome treated either with cyclosporin A (CyA) or placebo (5 mg/kg body weight day for six months) were studied to determine T lymphocyte subsets and HLA-DR antigen expression using the avidin-biotin-peroxidase technique. In all CyA treated patients we observed a decrease in the number of T lymphocytes and in the number of T helper cells, while the percentage of T suppressor cells and B cells was the same in both treated and untreated groups. It was also shown that the HLA-DR antigen expression on the epithelial cells was eliminated in the CyA treated patients. These findings suggest that the HLA-DR antigen expression on the epithelial cells is the result rather than the triggering factor of this T cell mediated process and is probably related to decreased lymphokine production by activated T lymphocytes.

Key words: T lymphocytes, T helper cells, T suppressor cells, HLA-DR antigens.

Cyclosporin A (CyA), a new T lymphocyte immunomodulatory agent, has been used for the treatment of different autoimmune diseases, with controversial results.[1]

Sjögren's syndrome is an autoimmune disease characterised by lymphocytic infiltration of the exocrine glands and polyclonal B lymphocyte hyperreactivity.[2] The availability of monoclonal antibodies enabled us and others to investigate the phenotypes of cells infiltrating the labial minor salivary glands of patients with Sjögren's syndrome.[3][4] It is generally accepted that the predominant cell of this infiltrate is an activated T lymphocyte bearing the T helper-inducer phenotype. This finding and the fact that CyA acts on T helper lymphocytes prompted us to study the efficacy of CyA in the treatment of patients with Sjögren's syndrome in a random double blind fashion.[5] This study showed that CyA given in small doses for six months improves xerostomia and seems to retard the histopathological lesion of the labial minor salivary glands.

In the present study we have examined the effect of CyA on the phenotypes of cells infiltrating the minor salivary glands, and the effect on the HLA-DR antigen expression on both infiltrating and resident cells.

Materials and methods

Fourteen minor labial salivary gland biopsy specimens were obtained from 14 patients with primary Sjögren's syndrome after six months of either CyA or placebo treatment. The patients (13 women, one man) were assigned blindly and randomly to receive orally either CyA (5 mg/kg body weight/day) or identical placebo for six months. The mean age and disease duration were similar in both groups.

The specimens were immediately snap frozen in a mixture of dry ice/2-methylbutane ($-75°C$) and stored at $-70°C$ until processed. Frozen sections (4 μm) were stained with monoclonal antibodies against human T lymphocytes (Leu-4), T helper lymphocytes (Leu-3a), T suppressor lymphocytes (Leu-2a), B lymphocytes (Leu-14) (Becton and Dickinson Monoclonal Center Inc, Mountain View, Ca), and HLA-DR antigens (OKIa₁) (Ortho Diagnostic Systems Inc, Raritan, NJ) using the

Table 1 *Effect of CyA or placebo treatment on the histological lesion of minor salivary glands from patients with Sjögren's syndrome (Tarpley's classification[6])*

Patient No	Before treatment	After treatment
CyA treated		
1	1+	1+, fibrosis
3	1+	2+
6	1+	Scattered lymphocytes
7	2+	<1+, fibrosis
9	3+	3+
15	1+, fibrosis	1+, fibrosis
18	1+	4+
Placebo treated		
2	2+	Fibrosis
4	3+	Fibrosis
5	3+	4+
8	1+	1+, fibrosis
10	1+	2+
12	2+	2+
17	1+, fibrosis	3+

Table 2 *Effect of CyA on the infiltrating cells of the minor salivary glands from patients with Sjögren's syndrome*

Treatment regimen	Percentage stained cells			
	Total T cells	T helper cells	T suppressor cells	B cells
Placebo	61·4 (14·6)*	49·2 (18·3)	15·7 (7·8)	18·0 (10·6)
CyA	37·8 (16·5)	23·1 (14·5)	16·7 (13·9)	10·4 (8·7)

*Values are mean (SD).

Table 3 *Effect of CyA on HLA-DR antigen expression*

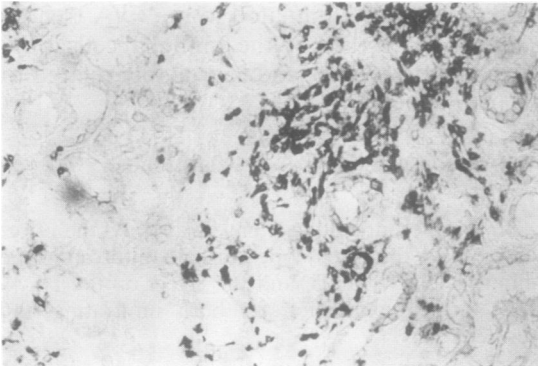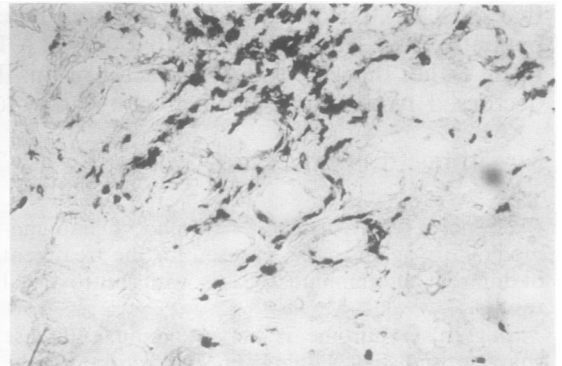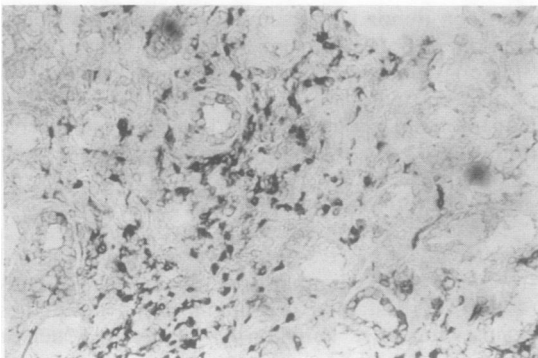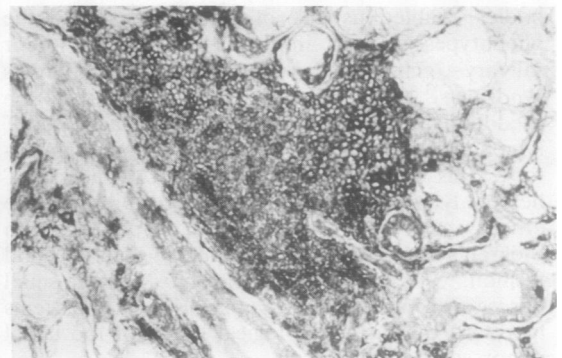| Treatment regimen	HLA-DR antigen expression (No of cases)	
	Infiltrating cells	Epithelial cells
Placebo	7/7	7/7
CyA	7/7	1/7

Fig. 1a

Fig. 1c

Fig. 1b

Fig. 1d

Fig. 1 *Effect of CyA on infiltrating cells and HLA-DR antigen expression. Minor salivary gland biopsy specimen from a CyA treated patient: (a) total T lymphocytes (Leu-4); (b) T helper cells (Leu-3a); (c) T suppressor cells (Leu-2a); (d) HLA-DR antigen expression (OKIa).*

avidin-biotin-peroxidase technique as described elsewhere.[4]

The processed sections were read in a blind fashion and scored independently by two observers using 40× magnification. The numbers of cells labelled with each monoclonal antibody were counted for at least 10 different fields and averaged. Special attention was paid to the staining of the glandular tissue (acini and ducts) by different antibodies. Cells were considered positive if they showed a dense dark black-bluish ring on the cellular membrane. After the cells were counted the results from the two observers were averaged. The variability between the two observers was less than 5%.

Student's *t* test and Wilcoxon analysis were used when indicated.

Results

In 5/7 patients who received CyA the histology of the minor labial salivary gland lesion after haematoxylin and eosin staining was improved or remained unchanged and in 2/7 deterioration was observed. In the placebo treated patients 6/7 showed further deterioration and 1/7 remained unchanged (Table 1).

The total number of T lymphocytes and the number of T helper lymphocytes in the CyA group were significantly smaller than those observed in the placebo group (Student's *t* test, $p<0.015$ and $p<0.012$ respectively), while the numbers of T suppressor and B lymphocytes were similar in both groups (Table 2).

HLA-DR antigen expression on the infiltrating cells was observed in all specimens from both groups, but in only 1/7 specimens of the CyA group did the epithelial cells express HLA-DR antigens, while in 7/7 specimens of the placebo group the epithelial cells were HLA-DR positive (Table 3).

Figs 1 and 2 show the results obtained by staining minor salivary gland sections from patients with Sjögren's syndrome treated with CyA or placebo, for T lymphocytes, T helper lymphocytes, T suppressor lymphocytes, and HLA-DR antigens.
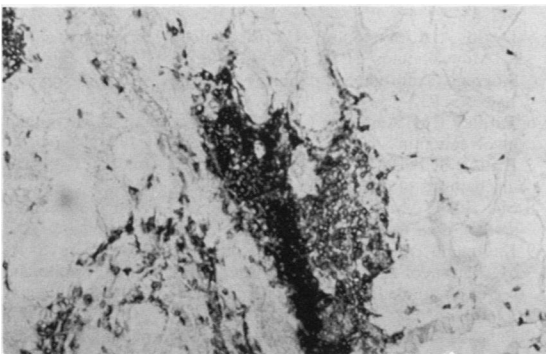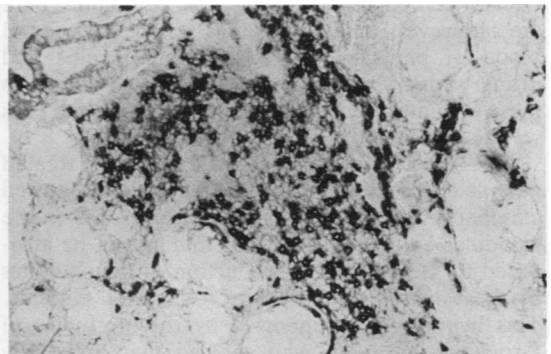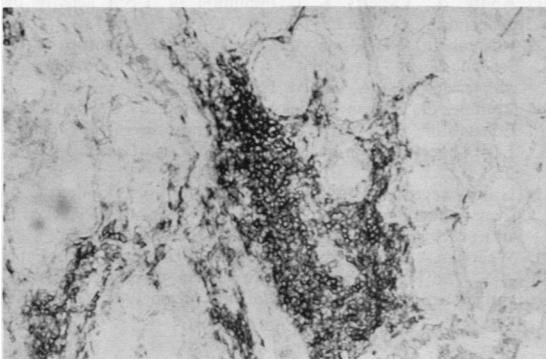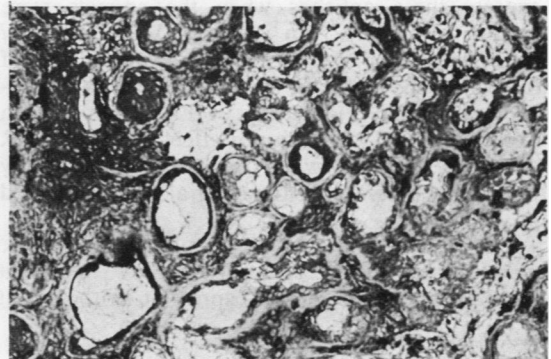

Fig. 2a

Fig. 2c

Fig. 2b

Fig. 2d

Fig. 2 *Effect of CyA on infiltrating cells and HLA-DR antigen expression. Minor salivary gland biopsy specimen from a placebo treated patient: (a) total T lymphocytes (Leu-4); (b) T helper cells (Leu-3a); (c) T suppressor cells (Leu-2a); (d) HLA-DR antigen expression (OKIa).*

Discussion

CyA is a new immunomodulatory agent which interferes mainly with T lymphocyte function. It inhibits T lymphocyte response to mitogens or antigens and lymphokine production by activated T lymphocytes.[7][8] Furthermore, it has been shown that CyA blocks the production of interleukin 2 by activated T lymphocytes.[9] These events result in a decrease in the number of T helper lymphocytes generated during an immune mediated process. In contrast, CyA has no effect on the T suppressor lymphocytes.[8] Finally, CyA can control HLA-DR antigen expression through inhibition of lymphokine production.[10][11]

In this study we observed a decrease in the total number of T lymphocytes and T helper lymphocytes in the minor labial salivary gland lesions of patients with Sjögren's syndrome treated with CyA. Furthermore, the ratio of T helper/T suppressor lymphocytes was decreased. To our knowledge there has been no previous study showing that CyA treatment of an autoimmune disease acts on the immune effector cell population in the affected organ. There are, however, similar findings in the peripheral blood of patients with autoimmune uveitis and primary biliary cirrhosis treated with CyA.[12][13] In these patients a decrease of circulating T helper cells was also observed.

In addition, in contrast with placebo treated patients, most of our CyA treated patients showed elimination of HLA-DR antigen expression on the glandular epithelial cells. This finding can be attributed to the decreased production of lymphokines by the T lymphocytes of the CyA treated patients. This hypothesis is supported by recent studies of Groenewegen *et al*, who showed that previously HLA-DR positive canine endothelial cells failed to express these antigens when the animals were treated with CyA.[14] In addition, Weetman *et al* showed that the presence of CyA in cultures of follicular thyroid cells stimulated with a phytohaemagglutinin inhibited HLA-DR antigen expression.[15] CyA had no effect on HLA-DR antigen expression when exogenous interferon-γ was added, however, suggesting a T cell mediated process.[15]

This elimination of MHC class II antigens of the glandular epithelial cells after CyA treatment strongly suggests that this expression is the result rather than the triggering factor of the T mediated inflammatory process.

Despite the fact that CyA treatment of patients with Sjögren's syndrome resulted in histological and immunopathological improvement, the clinical benefit was minimal. It is possible that continuation of the treatment for a longer period of time might show a significant therapeutic effect on this disease for which at present there is no available treatment.

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