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The anti-arthritic and immunosuppressive effects of cyclosporine on arthritis induced in the rat by type II collagen

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SUMMARY

The influence of cyclosporine (CsA) on the induction and pathogenesis of type II collagen-induced arthritis has been investigated in inbred and outbred Wistar rats. The proportion of animals developing disease and the severity of disease they developed were both diminished by treatment with CsA. These effects were accompanied by a marked suppression of the antibody response to both the immunizing collagen and also to rat type II collagen. CsA treatment also resulted in a decreased accumulation of lymphocytes in arthritic joints. The results indicate that the anti-arthritic and immunosuppressive effects of CsA probably result from a modification of both systemic antibody-mediated and local cell-mediated immunity.

Keywords arthritis autoimmune diseases collagen type II cyclosporine

INTRODUCTION

A systemic polyarthritis can be induced in rats by injecting them with type II collagen (CII) derived by enzymic digestion from hyaline cartilage (Trentham, Townes & Kang, 1977). The development of arthritis in susceptible strains of rats is accompanied by the development of strong anti-collagen immunity which is, at least in part, demonstrably directed against the animals' own tissues. To what extent this is a model for rheumatoid arthritis is a matter of conjecture (Trentham, 1982).

The arthritis induced by CII can be considered to be a tissue specific autoimmune disease like experimental thyroiditis, encephalomyelitis and many others. Unlike these, however, it does not depend upon administering the inducing antigen in Freund's adjuvant containing mycobacteria. In this regard it is unique but in spite of the probably partial T-independence of the anti-CII antibody response (Fuchs *et al.*, 1974) the involvement of T cells in the disease is implied by specific lymphocyte transformatiom data (Stuart *et al.*, 1979) and the resistance of nude rats to the disease (Klareskog *et al.*, 1983).

Polyarthritis can also be induced in rats by injecting mycobacteria in Freund's adjuvant. This adjuvant arthritis is similar in many regards to CII-induced arthritis but obviously has a different aetiology. Its pathology is very similar and anti-collagen immunity develops as part of its clinical progression (Trentham *et al.*, 1980). The disease can be passively transferred by cloned T cells underlining the importance of T cell-dependent immunity in arthritis (Holoshitz *et al.*, 1983).

Cyclosporine (CsA) is an endecapeptide of fungal origin that has profound immunosuppressive effects (reviewed extensively in White, 1982) in regulating graft versus host disease, organ allograft

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rejection and delayed type hypersensitivities. It was a relatively early observation by Borel and co-workers that CsA inhibited the development of adjuvant arthritis in rats (Borel, Wiesinger & Gubler, 1978). Subsequently several other organ specific autoimmune diseases (listed by Borel, 1982) were shown to be similarly modifiable by the drug.

The immunosuppressive action of the drug appears to depend upon its ability to modify the activation of helper type T cells although its precise mode of action is unclear. Nonetheless, its gross effects in T-dependent immunity are obvious.

Because of the relationship of CII-induced and adjuvant arthritis to each other and to other autoimmune diseases we have tested CsA and have found it effective in modifying the development of CII-induced arthritis in rats.

MATERIALS AND METHODS

Animals. Inbred Wistar (WA/KIR) and outbred Wistar (WA/CH) rats were bred and maintained at Chelsea College.

Induction of arthritis. Animals were immunized with 0·8 mg CII prepared from bovine nasal septum. The method of preparing collagen and the immunization protocol using Freund's incomplete adjuvant are described by Staines *et al.* (1981).

Administration of CsA. CsA was dissolved in olive oil at 60°C by constant stirring. Each animal received 15 mg/kg/day intramuscularly (i.m.) in the upper hind leg muscles; control animals received olive oil only. Ten such i.m. injections were given. In three experiments they commenced on the day of immunization with CII and in one experiment they started 5 days before immunization.

Animals were examined daily for signs of disease. The numbers of limbs involved, degree of inflammation and the relative amount of soft tissue swelling and bony ankylosis were recorded. The flexion of the ankle joint was also noted.

Bleedings. Animals were bled from the tail artery at weekly intervals following injection of CII.

Serological tests. Sera were tested for the presence of antibodies to bovine and rat CII by solid phase ELISA as described by Staines *et al.* (1981).

Lymphocyte migration. Thoracic duct lymphocytes (TDL) were collected from normal rats and labelled with sodium ^{51}chromate (CJS-1P, Amersham International) at 10 μCi per 10^8 cells, as described by Ford (1978). Eighty million TDL were injected intravenously into arthritic WA/KIR rats 8 weeks after immunization with collagen. Distribution of the radiolabel in the feet, popliteal, cervical and mesenteric lymph nodes and in the spleen was measured 24 h after injection. The results are expressed as the percentage of the injected radiolabel per gram of tissue.

RESULTS

Inhibitory effects of CsA on the induction and severity of arthritis

In four separate experiments CsA had a pronounced inhibitory effect upon the induction and clinical severity of arthritis. Fewer animals developed disease, and in those that did, the disease was less severe (Table 1).

In otherwise unmodified rats, the arthritic disease showed the same progression as reported previously (Staines *et al.*, 1981). Ten to fourteen days after CII injection symptoms of inflammation appeared progressively in the feet, the digits and ankles. This was followed by a progressive loss of joint articulation. Involvement of the front limbs was not seen in these experiments.

By 3 weeks 78% of the unmodified animals were diseased in this way. In contrast, the overall incidence of disease in CsA treated rats was halved to 38% at this time. No further animals developed disease during the 2 month duration of the experiments.

The rats treated with CsA that did become arthritic first showed clinical symptoms at the same time as the unmodified animals. It was observed, however, that the arthritis tended to be unilateral in these animals and bilateral in the controls. The inflammation, swelling, loss of joint flexion and ankylosis in the arthritic feet of CsA treated animals was also less than that seen in controls. Thus the overall severity of the disease was reduced as shown in Table 1.

Table 1. Influence of treatment with CsA on the incidence of induced arthritis and on the severity of disease 3 weeks after induction

Strain	Expt.	Animals with clinical disease		Severity of disease (proportion of hind limbs affected)	
		Cyclosporine	Vehicle	Cyclosporine	Vehicle
WA/KIR (Inbred)	1	3/4 (75%)	4/4 (100%)	3/8 (38%)	8/8 (100%)
	2*	0/5 (0%)	2/4 (50%)	0/10 (0%)	2/8 (25%)
WA/CH (Outbred)	3	1/5 (20%)	4/5 (80%)	2/10 (20%)	7/10 (70%)
	4	3/6 (50%)	5/6 (83%)	3/12 (25%)	6/12 (50%)
Total		7/20 (38%)	15/19 (78%)	8/40 (20%)	23/38 (61%)

* Animals received CsA 5 days prior to immunization and for the following 5 days. Animals in other groups were treated for 10 days prior to immunization with CII.

In three experiments, CsA was first administered at the time of CII injection and in the other from 5 days beforehand. The results suggest that the latter regime of treatment may be more suppressive than the former. This may reflect a stress response of animals injected five times before the challenge with arthritogenic CII. Stress is known to modify induction of disease with CII (Rogers *et al.*, 1980).

Inhibitory effects of CsA on the antibody response to CII

The antibody response against the arthritogenic heterologous CII was severely depressed in animals that were treated with CsA. The average antibody titres were depressed more than 20-fold by 3 weeks in, for example, the fourth experiment (Table 2).

Table 2. Influence of treatment with CsA on the production of antibody reactive with bovine and rat CII in animals immunized with bovine CII

Strain	Expt.	CsA treatment	Antibody reaction with CII	Antibody titres* Weeks after immunization 1	2	3	4	5
WA/KIR (Inbred)	1	+	Bovine	1·98±0·44	3·15±0·18‡	2·75±0·39‡	—	—
		−		1·98±0·48	3·85±0·05	3·91±0·20	—	—
		+	Rat	1·90±0·24	3·41±0·44†	3·37±0·46†	—	—
		−		2·24±0·53	4·28±0·31	4·04±0·13	—	—
	2§	+	Bovine	0·49±0·08	2·09±0·12‡	2·51±0·21†	2·65±0·04	2·40±0·26†
		−		0·62±0·35	3·17±0·26	2·87±0·16	2·79±0·22	2·81±0·13
WA/CH (Outbred)	3	+	Bovine	0·93±0·86	2·66±0·56†	2·51±0·54‡	—	—
		−		1·87±0·45	3·49±0·18	3·51±0·23	—	—
		+	Rat	0·92±0·84†	2·18±0·45‡	2·72±0·51†	—	—
		−		2·12±0·43	3·42±0·22	3·69±0·28	—	—
	4	+	Bovine	1·03±0·06	2·08±0·74‡	2·15±0·81‡	1·82±0·67‡	—
		−		1·10±0·74	3·30±0·53	3·47±0·33	3·23±0·27	—

* Antibody titres are expressed as the $\log_{10}$ of the reciprocal of the interpolated dilution giving 50% of the maximum absorbance in the peroxidase titration curve (mean±s.d.).

Suppression of antibody response $P<0{\cdot}05$† or $P<0{\cdot}005$‡ (Student's *t*-test).

§ See footnote to Table 1.

In the experiments where CsA was administered from the time of challenge with the arthritogen the anti-bovine CII antibody titres tended to be higher in the inbred than in the outbred animals. The factor of suppression was the same, however, in both groups of animals.

The production of autoreactive anti-CII antibodies is a consistent feature of the disease. The production of autoreactive anti-CII antibody was depressed to an extent similar to that of the antibody reactive with the bovine CII (Table 2).

In the one experiment where CsA administration was started 5 days before challenge with CII a different pattern was observed. The factor of suppression was greatest at 2 weeks but declined thereafter so that the depression of antibody levels in drug treated animals was apparently less. The disease incidence was lower in the control group for this regime of drug treatment and the low antibody titres reflected that as well.

Effects of CsA on the migration of lymphocytes into arthritic lesions

Radiochromium labelled syngeneic thoracic duct lymphocytes were injected intravenously into inbred WA/KIR rats (experiment 1 animals, Table 1) 8 weeks after their immunization with CII. All unmodified animals were chronically arthritic at this time although the clinical symptoms of the CsA treated rats indicated that the disease was largely resolved in three animals and the fourth had never displayed signs of disease. The accumulation of radiolabel (per unit tissue weight) in the hind feet of CsA treated rats was approximately half that found in the unmodified arthritic rats (Table 3).

A reduction in disease severity was accompanied by a reduction in lymphocyte accumulation in the arthritic feet of the rats. There were no significant changes in cell accumulation in lymphoid tissues of the drug treated animals indicating that major changes in the overall lymphocyte migration pattern had not been caused by the CsA.

These results show that the accumulation of cells per unit weight of lymphoid tissue was unaltered by CsA treatment, but in absolute terms the accumulation of cells in the nodes draining the arthritic feet of drug modified animals was reduced. The weights of the spleen, the cervical and mesenteric lymph nodes were the same in both groups but the popliteal lymph node size was significantly reduced from $11{\cdot}2 \pm 3{\cdot}4$ mg in unmodified rats to $6{\cdot}3 \pm 3{\cdot}7$ mg ($P < 0{\cdot}05$) in CsA treated rats, the latter value being within the normal range.

DISCUSSION

This study shows that CsA diminishes the severity of the clinical symptoms of CII-induced arthritis in rats. This is accompanied by extensive changes in immune reactivity against CII. Daily i.m.

Table 3. The effect of treatment with CsA on the migration of lymphocytes to the feet and lymphoid tissues in collagen immunized animals with arthritis

Tissue	Percentage injected ^{51}chromium per gram tissue	
	CsA	Vehicle
Left foot	$0{\cdot}046 \pm 0{\cdot}01$*	$0{\cdot}094 \pm 0{\cdot}015$
Right foot	$0{\cdot}035 \pm 0{\cdot}006$†	$0{\cdot}09 \pm 0{\cdot}016$
Left popliteal lymph node	$16{\cdot}4 \pm 2{\cdot}7$	$15{\cdot}7 \pm 2{\cdot}0$
Right popliteal lymph node	$12{\cdot}5 \pm 5{\cdot}9$	$15{\cdot}9 \pm 2{\cdot}2$
Spleen	$22{\cdot}1 \pm 2{\cdot}4$	$20{\cdot}6 \pm 2{\cdot}6$
Cervical lymph node	$16{\cdot}8 \pm 2{\cdot}3$	$15{\cdot}2 \pm 2{\cdot}4$
Mesenteric lymph node	$20{\cdot}2 \pm 4{\cdot}8$	$21{\cdot}3 \pm 2{\cdot}5$

Results expressed as Mean ± s.d. (four animals in each group).

Animals received either CsA in olive oil or olive oil (vehicle) i.m. for 10 days following immunization with CII.

Significance of results: *$P < 0{\cdot}05$ and †$P < 0{\cdot}01$.

injections of CsA given over a 10 day period starting either 5 days before or at the time of immunization with CII were found to lower the incidence of disease. In four separate experiments the incidence of arthritis in groups of unmodified control animals was between 50% and 100% (mean = 78%). In contrast, in groups of animals receiving CsA the incidence of disease ranged from 0% to 75% (mean = 38%). Thus administration of CsA was associated with a halving of the incidence of arthritis. In addition to this decrease in the incidence of disease the arthritis which developed in animals receiving CsA was less severe than that found in controls.

It has been observed widely that the serum levels of anti-CII antibody in this disease are positively correlated with the severity of the disease (for example, Trentham *et al.*, 1978; Clague *et al.*, 1980). We have shown previously that the suppression of arthritis caused by hyperimmune anti-CII antisera (passively transfered from arthritic rats) is accompanied by a corresponding drop in anti-CII antibody production (Staines *et al.*, 1981).

Thus the suppression of arthritis and immunity appears very similar in the CsA modified disease and in the immune serum modified (or passively immunologically enhanced) disease. Although in both situations these two effects could have a common origin rather than a causal relationship the latter explanation would be favoured because of the observations that anti-CII serum antibody alone can induce arthritic disease after passive transfer into rats (Stuart *et al.*, 1982) and mice (Stuart & Dixon, 1983). The fact that unfractionated lymphoid cells from arthritic animals can also be used successfully in the passive transfer of the disease itself (Trentham, Dynesius & David, 1978) or of resistance to disease induction support the notion that the arthritis is a direct consequence of anti-collagen immunity. The present results underline the parallelism of the two effects of CII immunization. It is likely, however, that this is too simple an explanation for the pathogenesis of the disease. In adjuvant arthritis, disease can be induced by cloned T cells, some of which are reactive with CII (Holoshitz *et al.*, 1983) and although anti-CII antibodies can be found in adjuvant arthritic rats (Trentham *et al.*, 1980) anti-CII immunity is not uniformly found in such animals (Trentham & Dynesius-Trentham, 1983). It is perhaps illusory to seek a simple or common aetiology for these experimental arthritides.

In CII-induced arthritis the production of antibodies against the heterologous arthritogenic CII is accompanied by the production of antibodies autoreactive with rat CII (Staines *et al.*, 1981). Whether these are separate or partially cross-reactive sets is not known. In the present study the immunosupressive effect of CsA was such that antibody titres against both bovine and rat CII were correspondingly reduced.

The parallel reduction in arthritis and anti-CII immunity caused by CsA treatment is in contrast to the effects of the drug in at least one other experimental autoimmune system. Vladutiu (1983) found that although CsA had a suppressive effect on experimental autoimmune thyroiditis in mice without abrogating the disease completely, it did not cause a decrease in the serum anti-thyroglobulin antibody titre. The reasons for this difference between the two diseases is not known.

The anti-arthritic effects of CsA have only been examined in these experiments by administering it at the time of disease induction. Thus its therapeutic effectiveness on established disease is unknown but these experiments do show that its prophylactic effects are long lasting. At all stages the severity of disease was lower after drug treatment. Even in the relatively mildly arthritic feet of CsA treated rats 8 weeks after disease induction, the extent of lymphocyte infiltration was more than halved and the enlargement of the draining lymph node was also halved.

We conclude therefore that the anti-arthritic action of CsA is mediated by systemic and local effects of the drug on the immune system. Depression of disease is associated with a significant decrease in serum antibody against CII and a decrease in lymphocyte accumulation in arthritic joints.

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