PATHOGENETIC DIFFERENCE BETWEEN COLLAGEN ARTHRITIS AND ADJUVANT ARTHRITIS

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Collagen arthritis can be induced readily in many strains of rats by immunizing them with heterologous or homologous native type II collagen emulsified in incomplete Freund's adjuvant (1). This disease, which is characterized by the development of both cellular and humoral immune response to type II collagen (2–4), can be passively transferred by sensitized spleen and lymph node cells (5) as well as IgG antibodies to type II collagen (6). These findings are consistent with the proposal that collagen arthritis is the result of immunologic hypersensitivity to type II collagen. In a recent report, Trentham et al. (7) showed that rats with adjuvant arthritis exhibited both humoral and cellular sensitivities to homologous type II collagen, and suggested that an autoimmune response to type II collagen is a common feature to both collagen arthritis and adjuvant arthritis. However, subsequent studies (8, 9) have produced conflicting results that shed some doubt on this exciting concept, and the discrepancies need further clarification.

The present study was intended to determine if an autoimmune response to type II collagen is responsible for the induction of both collagen arthritis and adjuvant arthritis by using a relatively new immunosuppressive agent, cyclosporin, which has been shown to be capable of inducing specific immunologic tolerance in several in vivo and in vitro conditions (10, 11).

Materials and Methods

Animals. Outbred female Sprague-Dawley rats were purchased from Japan Charles River Breeding Laboratories, Kanagawa, Japan. They were allowed 1 wk to adapt to their environment and were used at 6 wk old, weighing 130–160 g at the start of the present experiment. All the animals received standard laboratory chow and water ad libitum.

Preparation of Type II Collagen and Production of Collagen Arthritis. Type II collagen was isolated and purified from bovine articular cartilage as previously described (1). The purity was assessed as described elsewhere (12). Lyophilized type II collagen was dissolved in 0.1 M acetic acid at a concentration of 3 mg/ml. Equal volumes of collagen solution and incomplete Freund's adjuvant (Difco Laboratories, Inc., Detroit, MI) were emulsified using a homogenizer (Polytron PT 10-35; Kinematica, Lucerne, Switzerland) and kept cold with an ice bath. Collagen arthritis was produced by intradermal injection of 1 ml of

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the cold emulsion at several sites on the back and at one or two sites into the base of the tail.

Production of Adjuvant Arthritis. Adjuvant arthritis was produced by a single intradermal injection of 0.1 ml of complete Freund's adjuvant (CFA)[1] at the base of the tail. CFA was prepared by adding dried, heat-killed *Mycobacterium butyricum* (Difco Laboratories, Inc.), which was finely powdered with a mortar and pestle, to mineral oil (Sigma Chemical Co., St. Louis, MO) at a concentration of 10 mg/ml.

Treatment with Cyclosporin. Cyclosporin (kindly provided by Sandoz Ltd., Basel, Switzerland) was dissolved in pure olive oil at a concentration of 25 mg/ml by heating in a water bath to 65°C. It was freshly prepared every 3 d. Cyclosporin was given subcutaneously under light ether anesthesia at a dose of 25 mg/kg per day for 14 d beginning on the same day as the injection of type II collagen or CFA. The dose was adjusted according to the daily body weight. Control rats received olive oil alone. Cyclosporin-treated and control rats were handled identically for administration of the agent and the solvent.

Assessment of Arthritis. Rats were examined daily for 6–7 wk after the injection of type II collagen or CFA to record the day of onset and the severity of arthritis. The lesions of the four paws were each graded from 0 to 4 according to the increasing extent of periarticular erythema and swelling as well as joint deformity, as described previously (13). The maximum possible score was 16.

Immune Responses to Type II Collagen. Delayed-type hypersensitivity (DTH) skin testing was performed as described by Griffiths et al. (14) using 50 μg of type II collagen in 0.05 M Tris-HCl buffer, pH 7.2, containing 0.2 M NaCl. The responses were read at 48 h.

Serum antibodies to type II collagen were measured by the enzyme-linked immunoassay technique as described elsewhere (12), which was adapted from the methods of Voller et al. (15). The quantity of IgG anti-type II collagen antibody was expressed as milligrams per 100 ml of serum by comparison with standard curves obtained from a purified rat anti-type II collagen antibody control.

Antibodies to type II collagen were purified by affinity chromatography (16) on cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Briefly, CNBr-activated Sepharose 4B (2 g dry weight) was washed several times with 400 ml of 1 mM HCl on a sintered glass funnel and finally with coupling buffer (0.1 M $NaHCO_3$ buffer, pH 8.3, containing 0.5 M NaCl). The activated Sepharose was added to the collagen solution (35 mg type II collagen in 14 ml of coupling buffer). The suspension was stirred gently at 4°C for 16 h. 2 ml of 1 M ethanolamine was added and incubation was continued for 1 h. The collagen-Sepharose conjugate was washed alternately with phosphate-buffered saline (PBS) and 0.2 M glycine-HCl buffer, pH 2.5, for three cycles. Then the collagen-Sepharose conjugate was equilibrated with PBS and 6 ml of antisera obtained from arthritic rats 4 wk after immunization with type II collagen was added. After incubation overnight at 4°C, the suspension was packed in a 1 × 10-cm column and washed with PBS until no protein could be detected in the wash fluid by spectrophotometry. The antibodies bound to the affinity column was eluted with 0.2 M glycine-HCl buffer, pH 2.5, dialyzed against several changes of cold PBS, and concentrated by pressure dialysis. The quantity of IgG antibody was measured by radial immunodiffusion methods using the Miles rat IgG kit (Miles Laboratories, Inc., Elkhart, IN).

Statistics. Continuous variables were analyzed by their group means (Student's *t* test) and dichotomous variables by their proportionate group frequencies (chi-square test). *P* values <0.05 were considered to be statistically significant.

Results

Effect of Cyclosporin on Collagen Arthritis and Immune Responses to Type II Collagen. Rats were immunized with 1.5 mg of type II collagen on day 0 and were treated with cyclosporin or olive oil for 14 d beginning on the day of immunization. An inflammatory polyarthritis developed in 89% of type II collagen-

[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; CNBr, cyanogen bromide; DTH, delayed-type hypersensitivity; PBS, phosphate-buffered saline.

immunized, non–cyclosporin-treated rats. Daily treatment with cyclosporin at a dose of 25 mg/kg for 14 d gave complete suppression of arthritis induction during an observation period of 45 d (Table I).

Serum antibody levels to type II collagen were measured on day 21 and DTH skin testing was performed on day 25. Olive-oil-treated control rats showed high antibody levels and strong positive skin test responses to type II collagen, whereas very weak antibody responses and negative to weak skin test responses to type II collagen could be detected in the rats treated with cyclosporin. These results are in accord with our previous report (12).

Effect of Cyclosporin on Adjuvant Arthritis and Immune Responses to Type II Collagen. An inflammatory polyarthritis was produced in all CFA-injected, non–cyclosporin-treated rats. None of the cyclosporin-treated animals manifested any evidence of disease, in accord with a previous report by Borel et al. (17).

In adjuvant arthritic rats, serum antibody levels to type II collagen were measured on day 21 or 38 and DTH skin testing was performed on day 25 or 42. Not only cyclosporin-treated rats but also adjuvant arthritic rats showed almost no antibody responses and no DTH skin test responses to type II collagen, even well after the disease onset (Table II).

Rechallenge Studies of the Cyclosporin-protected Rats. In the preceding sections, it was demonstrated that daily treatment with cyclosporin for the first 14 d gave complete suppression of the arthritogenic reaction produced by type II collagen or CFA. In the next experiment, we investigated the specificity of the cyclosporin-induced immunologic unresponsiveness by rechallenging the cyclosporin-protected rats with either type II collagen or CFA on day 17 in an identical manner as that used for the primary challenge. As shown in Table III, type II collagen-immunized, cyclosporin-protected rats did not develop arthritis in response to reimmunization with type II collagen, whereas they did develop arthritis in response to a subsequent injection of CFA, though the severity of arthritis in these rats was weakened as compared with that of adjuvant arthritic rats in Table

TABLE I
Effect of Cyclosporin on Collagen Arthritis

	Group		*P* values
	Cyclosporin	Olive oil	
Incidence of arthritis	0/14 (0%)	17/19 (89%)	<0.001
Arthritic index*	—	6.7 ± 0.6	
Day of onset‡	—	10.2 ± 0.2	
Antibody level§	0.17 ± 0.08	108.39 ± 14.14	<0.001
DTH skin reaction‖	3.1 ± 0.8	5.6 ± 0.3	<0.005

Rats were immunized with 1.5 mg of type II collagen on day 0 and treated with cyclosporin (25 mg/kg) or olive oil for 14 d.

* Expressed as the mean of maximum arthritic indices ± SEM for arthritic rats only.

‡ Based on arthritic rats only (mean ± SEM).

§ Antibody levels to type II collagen were measured on day 21 using enzyme-linked immunoassay system and expressed as milligrams of IgG anti-type II collagen antibody per 100 ml of serum (mean ± SEM).

‖ Performed on day 25 and expressed as the mean ± SEM diameter of induration (millimeters).

TABLE II
Effect of Cyclosporin on Adjuvant Arthritis

	Group		*P* values
	Cyclosporin	Olive oil	
Incidence of arthritis	0/10 (0%)	20/20 (100%)	<0.001
Arthritic index	—	13.0 ± 0.5	
Day of onset	—	9.7 ± 0.3	
Antibody level			
Day 21	<0.001	0.001 (10)*	
Day 38	ND‡	0.001 (10)	
DTH skin reaction			
Day 25	0	0 (10)	
Day 42	ND	0 (10)	

Rats received an injection of CFA on day 0 and were treated with cyclosporin (25 mg/kg) or olive oil for 14 d. Parameters and units are identical to those described in Table I.
* Parentheses indicate the number of rats used in data calculation if different from total tested.
‡ Not done.

TABLE III
Induction of Antigen-specific Unresponsiveness Mediated by Cyclosporin

	Type II collagen (day 0)		CFA (day 0)	
	Cyclosporin (days 0–13)		Cyclosporin (days 0–13)	
	Type II collagen (day 17)	CFA (day 17)	CFA (day 17)	Type II collagen (day 17)
Incidence of arthritis	0/10 (0%)*	8/10 (80%)	5/16 (31%)‡	10/10 (100%)
Arthritic index	—	5.8 ± 1.4	5.6 ± 1.1	10.4 ± 0.9
Day of onset	—	18.0 ± 1.3	14.2 ± 1.3	10.6 ± 0.2
Antibody level (day 38)	0.99 ± 0.30	1.36 ± 0.59	0.001§	111.62 ± 21.48
DTH skin reaction (day 42)	4.7 ± 0.8	4.0 ± 0.8	0§	7.0 ± 0.3

Groups of rats were injected with type II collagen or CFA on day 0 and treated with cyclosporin from day 0 to 13. Rats in each group received an injection of either type II collagen or CFA on day 17. Parameters and units are identical to those described in Table I, except that the day of onset was calculated from day 17.
* $P < 0.002$.
‡ $P < 0.003$.
§ $P < 0.001$.

II. On the other hand, CFA-injected, cyclosporin-protected rats showed a suppressed arthritogenic reaction in response to rechallenge with CFA, whereas their response to a subsequent immunization with type II collagen remained intact or somewhat enhanced. Thus, the prevention of arthritogenesis produced by a short course of cyclosporin treatment might be explained as specific immunologic unresponsiveness, which can be defined as the situation where an individual displays a functional nonreactivity to certain antigens while preserving reactivity to others.

Effect of Cyclosporin Pretreatment on Collagen Arthritis and Adjuvant Arthritis. To exclude the possibility that cyclosporin pretreatment might have some nonspecific effect on the development of collagen arthritis or adjuvant arthritis, we under-

took the following experiment. The rats, which were treated with cyclosporin for 14 d without any prior antigenic challenge, received an injection of CFA or type II collagen after the withdrawal of cyclosporin. As shown in Table IV, cyclosporin-pretreated rats developed arthritis in response to a subsequent injection of CFA or type II collagen after the cessation of cyclosporin treatment, indicating that it had no effect on the development of collagen arthritis and adjuvant arthritis.

Discussion

Cyclosporin is a fungal metabolite with profound immunosuppressive properties. In a variety of experimental animal systems, the immunosuppressive capacity of cyclosporin to inhibit antibody production, allograft rejection, graft-versus-host reactions, and experimental autoimmune diseases has been well documented (17–21). Of interest is the fact that cyclosporin is capable of inducing immunologic tolerance in several animal models. The most remarkable results were obtained with renal allograft across major histocompatibility barriers in rabbits (22, 23) and rats (24), from which cyclosporin was successfully withdrawn without subsequent loss of the graft. Similarly, fully allogeneic bone marrow grafts in rats were accepted indefinitely if recipients were treated for a limited period postgrafting with cyclosporin (25, 26). In addition, Wang et al. (27) demonstrated that lymphocytes from mice sensitized with alloantigens and treated with cyclosporin could not be reactivated upon exposure to the same alloantigen in mixed lymphocyte culture, whereas their response to a third-party antigen remained intact. A similar observation on the nature of the specificity of tolerance induced by cyclosporin in vitro was made by Hess et al. (28), in agreement with our observations in vivo.

On the basis of these findings, it would theoretically be possible to use cyclosporin to determine whether immunity to type II collagen plays a critical role in the pathogenesis of both collagen arthritis and adjuvant arthritis. In our initial experiments, we attempted to induce a state of unresponsiveness in rats receiving either type II collagen or CFA concomitantly with a 14-d course of

TABLE IV

Effect of Cyclosporin Pretreatment on Collagen Arthritis and Adjuvant Arthritis

	Cyclosporin (days 0–13)	
	Type II collagen (day 17)	CFA (day 17)
Incidence of arthritis	8/10 (80%)	10/13 (77%)
Arthritic index	6.5 ± 1.0	8.9 ± 1.6
Day of onset	13.1 ± 1.8	13.3 ± 0.7
Antibody level (day 38)	90.49 ± 19.04	0.001
DTH skin reaction (day 42)	6.8 ± 0.3	0

Rats were treated with cyclosporin from day 0 to 13 without any prior antigenic challenge and received an injection of CFA or type II collagen on day 17. Parameters and units are identical to those described in Table I except that the day of onset was calculated from the day of injection of CFA or type II collagen.

prophylactic treatment with cyclosporin. The results in this paper clearly demonstrate that cyclosporin is able to completely suppress the development of arthritis in rats injected with type II collagen or CFA. This confirms the previous reports by us (12) and Borel et al. (17). Of note is the fact that arthritis remained absent even after cyclosporin was discontinued.

To elucidate the possibility that the immunologic unresponsiveness produced by cyclosporin in the present experiments was antigen specific, we rechallenged the cyclosporin-protected rats with either type II collagen or CFA after cessation of cyclosporin treatment. Type II collagen-immunized, cyclosporin-protected rats did not develop arthritis in response to reimmunization with the same antigen, whereas they did develop arthritis in response to a subsequent injection of CFA. Similarly, CFA-injected, cyclosporin-protected rats showed a suppressed arthritogenic reaction in response to a subsequent injection of CFA. However, they developed arthritis in response to a subsequent immunization with type II collagen. These results indicate that there is no cross-reactivity between type II collagen and the responsible immunogen in the mycobacterial adjuvant, and that immunologic unresponsiveness produced by cyclosporin is antigen specific. The alternative explanation that cyclosporin induces some long-lasting, nonspecific changes in the immune system seems to be unlikely, because our results showed that rats could develop arthritis in response to an injection of CFA or type II collagen after a course of cyclosporin treatment given without any prior antigenic challenge, an effect that cannot be nonspecific. The fact that in the experiments reported here, 5 of 16 rats receiving an injection of CFA and a concomitant prophylactic treatment with cyclosporin developed mild arthritis in response to rechallenge with CFA might be explained that the strong immunologic stimulus provided by CFA have partially overcome the suppressive effect of cyclosporin.

As demonstrated in the results, the development of collagen arthritis was associated with high levels of both humoral and cellular immunity to type II collagen. Adjuvant arthritic rats showed almost no antibody response and no DTH skin test response to type II collagen even well after the disease onset. Taken together, our results further indicate that immunity to type II collagen plays a critical role in the pathogenesis of collagen arthritis; however, its pathogenetic role in adjuvant arthritis is insignificant.

Although it is difficult to compare the results reported by various investigators using different methods to achieve antigen-specific suppression, our results contradict the findings of Welles and Battisto (29), who showed that the severity of adjuvant arthritis in Lewis rats can be diminished by preimmunization with an alum flocculate of native type II collagen or intraperitoneal administration of antisera to type II collagen. A similar observation was made by Trentham et al. (30), who showed that the intravenous administrations of native type II collagen with syngeneic spleen cells, either exposed or unexposed to coupling reagent, or native type II collagen-coupled rat erythrocytes, can suppress the severity of propanediamine-induced arthritis in Lewis rats. However, no reactivity to collagen was found in propanediamine-injected rats. Therefore they postulated that antibodies to type II collagen are not involved in the initiation of adjuvant arthritis and that host reactions to native type II collagen play some role in the effector functions. Our results agree with those of Iizuka and Chang (9), who

showed that pretreatment of Sprague-Dawley rats with the maximal subarthritogenic dose of CFA prevented the development of arthritis in response to a subsequent injection of an arthritogenic dose of CFA, but had no effect on the development of collagen arthritis when rats were immunized with type II collagen. Although this study might be criticized for lacking proof regarding whether type II collagen-tolerated rats might develop arthritis in response to a subsequent injection of CFA, its conclusion that immunity to type II collagen is not critical to the primary pathogenesis of adjuvant arthritis and that adjuvant arthritis and collagen arthritis are distinctly different diseases is supported by our present data.

The mechanism whereby cyclosporin induces specific immunologic unresponsiveness is not clear. But, recent studies using the in vitro mixed lymphocyte reactions have demonstrated that this agent preferentially inhibits the proliferation of primary helper T cells and the induction of cytotoxic effector lymphocytes while permitting the establishment of an antigen-specific suppressor mechanism (11, 28, 31). These studies support the hypothesis that cyclosporin-induced tolerance may be mediated by suppressor cells. It is also suggested that such a mechanism might be operative in vivo as well by cardiac allografts studies in rats (32).

In conclusion, we have demonstrated that specific immunologic unresponsiveness can be induced by cyclosporin in the two experimental models of polyarthritis, collagen arthritis and adjuvant arthritis. Rechallenge studies suggest that there is no cross-reactivity between type II collagen and the mycobacterial cell wall materials. Taken together, immune response studies indicate that immunity to type II collagen plays a critical role in the pathogenesis of collagen arthritis, but that its pathogenetic role in adjuvant arthritis is insignificant. Unfortunately, our experimental model was unable to define what is the real etiologic immunogen responsible for the initiation of adjuvant arthritis. Further studies will be needed to answer this question.

Summary

Daily treatment with cyclosporin at a dose of 25 mg/kg for 14 d gave complete suppression of the development of collagen arthritis and adjuvant arthritis in Sprague-Dawley rats during an observation period of 45 d. To study whether the immunologic unresponsiveness produced by cyclosporin is antigen specific, we rechallenged the cyclosporin-protected rats with either type II collagen or complete Freund's adjuvant (CFA) after discontinuation of cyclosporin treatment. Type II collagen-immunized, cyclosporin-protected rats did not develop arthritis in response to reimmunization with type II collagen, but, they did develop arthritis in response to a subsequent injection of CFA. Similarly, CFA-injected, cyclosporin-protected rats showed a suppressed arthritogenic reaction in response to reinjection of CFA, whereas their response to a subsequent immunization with type II collagen was unaffected. On the other hand, the rats that were treated with cyclosporin without any prior antigenic challenge could develop arthritis in response to a subsequent injection of CFA or type II collagen after cessation of cyclosporin treatment. These results indicate that specific immunologic unresponsiveness can be induced by cyclosporin in the two exper-

imental models of polyarthritis, collagen arthritis and adjuvant arthritis, and that there is no cross-reactivity between type II collagen and the mycobacterial cell wall components. The results further indicate that immunity to type II collagen plays a critical role in the pathogenesis of collagen arthritis but that its pathogenetic role in adjuvant arthritis is insignificant.

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References

1. Trentham, D. E., A. S. Townes, and A. H. Kang. 1977. Autoimmunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.* 146:857.
2. Trentham, D. E., A. S. Townes, A. H. Kang, and J. R. David. 1978. Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats. *J. Clin. Invest.* 61:89.
3. Stuart, J. M., M. A. Cremer, A. H. Kang, and A. S. Townes. 1979. Collagen-induced arthritis in rats: evaluation of early immunologic events. *Arthritis Rheum.* 22:1344.
4. Morgan, K., R. B. Clague, M. J. Shaw, and P. J. L. Holt. 1980. Native type II collagen-induced arthritis in the rat. I. Incidence and humoral response to collagen. *Ann. Rheum. Dis.* 39:285.
5. Trentham, D. E., R. A. Dynesius, and J. R. David. 1978. Passive transfer by cells of type II collagen-induced arthritis in rats. *J. Clin. Invest.* 62:359.
6. Stuart, J. M., M. A. Cremer, A. S. Townes, and A. H. Kang. 1982. Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J. Exp. Med.* 155:1.
7. Trentham, D. E., W. J. McCune, P. Susman, and J. R. David. 1980. Autoimmunity to collagen in adjuvant arthritis of rat. *J. Clin. Invest.* 66:1109.
8. Cremer, M. A., J. M. Stuart, A. S. Townes, and A. H. Kang. 1980. Collagen-induced polyarthritis in rats: a study of native type II collagen for adjuvant activity. *J. Immunol.* 124:2912.
9. Iizuka, Y., and Y.-H. Chang. 1982. Adjuvant polyarthritis. VII. The role of type II collagen in pathogenesis. *Arthritis Rheum.* 25:1325.
10. Borel, J. F. 1981. Cyclosporin-A—present experimental status. *Transplant. Proc.* 13:344.
11. Hess, A. D., P. J. Tutschka, and G. W. Santos. 1981. Effect of cyclosporin-A on human lymphocyte responses in vitro—induction of tolerance to alloantigens. *Transplant. Proc.* 13:374.
12. Kaibara, N., T. Hotokebuchi, K. Takagishi, and I. Katsuki. 1983. Paradoxical effects of cyclosporin A on collagen arthritis in rats. *J. Exp. Med.* 158:2007.
13. Wood, F. D., C. M. Pearson, and A. Tanaka. 1969. Capacity of mycobacterial wax D and its subfractions to induce adjuvant arthritis in rats. *Int. Arch. Allergy Appl. Immunol.* 35:456.
14. Griffiths, M. M., E. J. Eichwald, J. H. Martin, C. B. Smith, and C. W. DeWitt. 1981. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strain of rats. *Arthritis Rheum.* 24:781.
15. Voller, A., D. E. Bidwell, and A. Bartlett. 1976. Enzyme immunoassays in diagnostic medicine: theory and practice. *Bull. W. H. O.* 53:55.

16. Fuchs, S., and M. Sela. 1978. Immunoadsorbents. *In* Handbook of Experimental Immunology. Vol. 1. Immunochemistry. D. M. Weir, editor. Blackwell Scientific Publications, Oxford. 10.1.
17. Borel, J. F., C. Feurer, H. U. Gubler, and H. Stähelin. 1976. Biological effects of cyclosporin A: a new antilymphocytic agent. *Agents Actions.* 6:468.
18. Nussenblatt, R. B., M. M. Rodrigues, M. C. Salinas-Carmona, I. Gery, S. Cevario, and W. Wacher. 1982. Modulation of experimental autoimmune uveitis with cyclosporin A. *Arch. Ophthalmol.* 100:1146.
19. Wick, G., P.-U. Müller, and S. Schwarz. 1982. Effect of cyclosporin A on spontaneous autoimmune thyroiditis of Obese strains (OS) chickens. *Eur. J. Immunol.* 12:877.
20. Vladutiu, A. O. 1983. Effect of cyclosporin on experimental autoimmune thyroiditis in mice. *Transplanation (Baltimore).* 35:518.
21. McGregor, A. M., D. P. Rennie, A. P. Weetman, R. A. Hassman, S. M. Foord, C. Dieguez, and R. Hall. 1983. The influence of cyclosporin A on experimental autoimmune thyroid disease in the rat. *Life Sci.* 32:97.
22. Green, C. J., and A. C. Allison. 1978. Extensive prolongation of rabbit kidney allograft survival after short-term cyclosporin-A treatment. *Lancet.* I:1182.
23. Green, C. J., A. C. Allison, and S. Precious. 1979. Induction of specific tolerance in rabbits by kidney allografting and short periods of cyclosporin-A treatment. *Lancet* I:123.
24. Homan, W. P., J. W. Fabre, K. A. Williams, P. R. Millard, and P. J. Morris. 1980. Studies on the immunosuppressive properties of cyclosporin A in rats receiving renal allografts. *Transplantation (Baltimore).* 29:361.
25. Tutschka, P. J., W. E. Beschorner, A. C. Allison, W. H. Burns, and G. W. Santos. 1979. Use of cyclosporin A in allogeneic bone marrow transplantation in the rat. *Nature (Lond.).* 280:148.
26. Tutschka, P. J., W. E. Beschorner, and A. D. Hess. 1980. Use of cyclosporin A (CsA) in a rat model of allogeneic marrow transplantation *Blut.* 25(Suppl.):241.
27. Wang, B. S., E. H. Heacock, Z. Chang-Xue, N. L. Tilney, T. B. Strom, and J. A. Mannick, 1982. Evidence for the presence of suppressor T lymphocytes in animals treated with cyclosporin A. *J. Immunol.* 128:1382.
28. Hess, A. D., P. J. Tutschka, and G. W. Santos. 1981. Effect of cyclosporin A on human lymphocyte responses in vitro. II. Induction of specific alloantigen unresponsiveness mediated by a nylon wool adherent suppressor cell. *J. Immunol.* 126:961.
29. Welles, W. L., and J. R. Battisto. 1981. Suppression of adjuvant arthritis by antibodies specific for collagen type II. *Immunol. Commun.* 10:673.
30. Trentham, D. E., and R. A. Dynesius-Trentham. 1983. Attenuation of an adjuvant arthritis by type II collagen. *J. Immunol.* 130:2689.
31. Hess, A. D., P. J. Tutschka, and G. W. Santos. 1983. Cyclosporin-induced tolerance—mechanism of maintaining specific immunologic unresponsiveness. *Transplant. Proc.* 15:539.
32. Bordes-Aznar, J., P. A. Lear, T. B. Strom, N. L. Tilney, and J. W. Kupiec-Weglinksi. 1983. Kinetics of cyclosporin-A-induced unresponsiveness to cardiac allografts in rats. *Transplant. Proc.* 15:500.