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Immunology

Protective effect of transforming growth factor β_1 on experimental autoimmune diseases in mice

(experimental allergic encephalomyelitis/collagen-induced arthritis)

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ABSTRACT **Interleukin 1 (IL-1) and tumor necrosis factor α are thought to contribute to the inflammatory response associated with autoimmune diseases. Transforming growth factor β_1 (TGF-β_1) counteracts many effects of these cytokines and has various immunosuppressive properties. In the present study, it is shown that microgram amounts of TGF-β_1, injected daily for 1–2 weeks, protect against collagen-induced arthritis (CIA) and relapsing experimental allergic encephalomyelitis (REAE), the animal models for rheumatoid arthritis and multiple sclerosis, respectively. When administered during induction of the disease, TGF-β_1 prevents CIA but only delays the onset of REAE by 2–3 days. However, when administered during a remission, TGF-β_1 prevents the occurrence of relapses in REAE. The results suggest that TGF-β_1 has powerful anti-inflammatory effects, mimicking in some respects the beneficial effects of immunosuppressive drugs in these experimental models of autoimmune disease, but without discernable adverse effects.**

The treatment of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis is still a clinical challenge. Immunosuppressive and anti-inflammatory substances are only of limited value and have serious side effects (1, 2). In animal models, collagen-induced arthritis (CIA) and relapsing experimental allergic encephalomyelitis (REAE), drugs such as cyclosporin A and cyclophosphamide suppress only when administered during induction of the disease (3–10). Biological agents that can suppress the inflammatory effects of interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α), cytokines that increase the severity of autoimmune diseases in animal models (11), deserve attention as alternative approaches. Use of such agents is also suggested by observations on patients with rheumatoid arthritis. Enhanced IL-1 production has been reported to be linked to disease severity in these patients (12) and their synovial fluid has been shown to contain granulocyte/macrophage colony-stimulating factor (13), γ interferon (IFN-γ), and TNF-α (14). Among the potentially therapeutic substances are IL-1 receptor antagonists (15, 16) and transforming growth factor β_1 (TGF-β_1).

Recent studies have demonstrated multiple immunoregulatory effects of TGF-β_1. These include inhibition of T-cell (17, 18) and B-cell (19, 20) proliferation; cytokine production (21–23); antibody or immunoglobulin formation by murine, human, or chicken lymphoid cells (24–26); and NK cell function (27). In view of the anti-inflammatory and immunosuppressive properties of TGF-β_1, we investigated the effects of this cytokine on both autoimmune disease models CIA and REAE.

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METHODOLOGY

Mice and Induction of Disease. CIA was induced in 8- to 14-week-old male DBA/1 mice, obtained from The Jackson Laboratory, as described (28, 29). Complete Freund's adjuvant (CFA) was prepared by mixing pulverized, lyophilized, heat-killed *Mycobacterium* (strains C, DT, and PN; Ministry of Agriculture, Fisheries and Food, Weybridge, Surrey, England) in incomplete Freund's adjuvant (Difco) at 4 mg/ml. Chicken collagen II (CII) was obtained from Genzyme, dissolved in 0.01 M acetic acid at 1–3 mg/ml, 24 hr prior to use, and stored at 4°C. Each mouse received 100 μg of CII emulsified with CFA in 0.2 ml, divided among four intradermal sites on the back. A booster injection of 100 μg of CII in aqueous solution was given intraperitoneally (i.p.) without adjuvant 4 weeks later.

EAE was induced in 8- to 12-week-old female SJL/J mice obtained from The Jackson Laboratory. Each mouse was injected subcutaneously at three sites on the back with a total of 0.3 ml of an emulsion containing 0.03 mg of *Mycobacterium tuberculosum* H37Ra in 0.15 ml of incomplete Freund's adjuvant (Difco) and 1 mg of lyophilized mouse spinal cord homogenate in 0.15 ml of Dulbecco's phosphate-buffered saline (PBS) (30). This immunization was repeated after 2 weeks and again after 6 weeks.

Treatment with TGF-β_1. Recombinant human TGF-β_1 was produced in Chinese hamster ovary cells (31) and stored at 0.8 mg/ml in 20 mM sodium acetate (pH 4) (1 pg of endotoxin per μg of protein). TGF-β_1 was diluted in 0.2 ml of PBS and injected i.p. The various treatment schedules used are detailed in the results.

Assessment of Disease. For assessment of arthritis, mice were observed two or three times each week for distal joint swelling and erythema. Swelling was quantified by measuring the thickness of feet and width of wrist and ankles with a constant-tension caliper (Dyee, Lancaster, PA). Clinical severity of arthritis was assessed by an "arthritic index." Each limb was subjectively graded on a scale of 0–3 (0, absence of arthritis; 1, mild swelling and erythema; 2, swelling and erythema of both tarsus and carpus; 3, ankylosis and bony deformity).

The first attack of EAE occurred ≈7 days after the last immunization. Mice were examined daily for signs of disease and were graded on an increasing severity scale of 0–4 (1, floppy tail with mild hindlimb weakness; 2, floppy tail with moderate hindlimb weakness and difficulty with righting reflex; 3, severe hindlimb weakness and mild forelimb weak-

Abbreviations: CIA, collagen-induced arthritis; REAE, relapsing experimental allergic encephalomyelitis; IL-1, interleukin 1; TNF-α, tumor necrosis factor α; IFN-γ, γ interferon; TGF-β_1, transforming growth factor β_1; CFA, complete Freund's adjuvant; CII, chicken collagen II.

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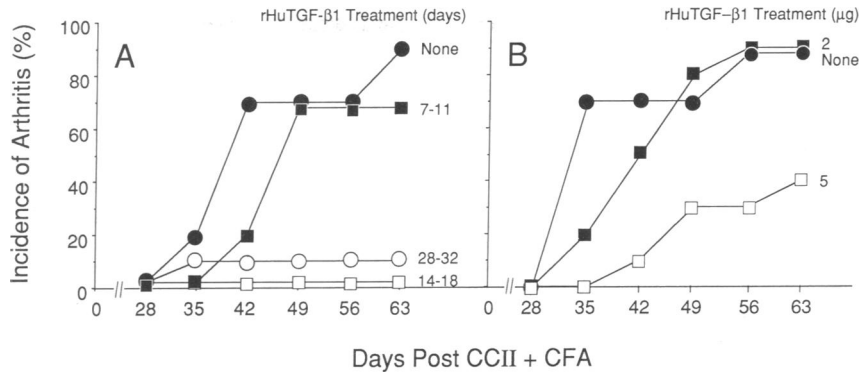

FIG. 1. (*A*) Effect of 2 μg of recombinant human TGF-β_1 (rHuTGF-β_1) given at different time intervals on CIA incidence. Groups of 10 mice were used. The arthritis incidence at the end of the experiment was significantly different in the groups treated with TGF-β_1 on days 14–18 ($P < 0.01$) and on days 28–32 ($P < 0.02$). (*B*) Effects of 2 μg or 5 μg of TGF-β_1 given on days 0–4 on CIA incidence in groups of 10–24 mice. The effect of 5 μg/day was significant ($P = 0.05$).

ness; 4, total paralysis of hind legs that may be associated with moderate forelimb weakness).

For both experimental models, crucial observations were cross-checked by an observer who was unaware of the treatment group distribution.

Histology. For histological evaluation of arthritis, carpal and tarsal joints from all TGF-β_1-treated and untreated animals were evaluated histologically after fixation in 10% neutral buffered formalin, decalcification, and dehydration. Paraffin sections were prepared and stained with hematoxylin and eosin by standard procedures. For evaluation of pathological changes in EAE mice, in one of the experiments (see Table 2) the mice were killed for histopathological analysis on day 72 and sections were prepared of the meninges, brains, and spinal cords. Slides were examined by a pathologist who was unaware of treatment group assignments.

Serum anti-collagen II antibody titers were measured in an ELISA as described (29). In short, Immulon II plates (Dynatech) were coated with 100 μl of collagen II per well (0.01 mg/ml) in PBS. After blocking with 2% bovine serum albumin, serial dilutions of sera were added. After incubation, alkaline phosphatase-conjugated goat anti-mouse IgG (Cappel Laboratories) was used to develop the reaction. *p*-Nitrophenyl phosphate (Sigma) was added as enzyme substrate and the absorbance was determined at 405 nm. Calculation of the anti-collagen II antibody concentration in test samples was achieved by the inclusion of standards containing known amounts of affinity-purified anti-collagen II on each plate.

Statistical Analysis. Comparisons of means were performed by Student's *t* test. Significance of differences between treatment and control incidences of disease were determined by the χ^2 method.

RESULTS AND DISCUSSION

DBA/1 mice were treated with five daily injections of 2 μg of TGF-β_1 i.p. at various intervals after immunization (day 0) with CII in CFA. The results in Fig. 1 show that virtually complete protection was obtained by treatment either on days 14–18 or on days 28–32 (Fig. 1*A*). TGF-β_1 administered earlier during immunization (days 0–4 and 7–11) was ineffective at this dose (Fig. 1), but 5 μg of TGF-β_1 per day (on days 0–4) resulted in partial suppression of CIA (Fig. 1*B*). TGF-β_1 injections started several weeks after the onset of arthritis did not measurably influence the course of the disease. Antibody titers to collagen II were significantly decreased in mice treated with 5 μg of TGF-β_1 per day on days 0–4 (from 1–2 mg/ml in controls to ≈0.4 mg/ml in TGF-β_1-treated mice), but not by TGF-β_1 treatment later during immunization (in one experiment a decrease, and in the other an increase in mean titer was observed; both statistically not significant), although later treatment was more effective in suppressing arthritis. Histopathological changes in the carpal and tarsal joints were dramatically reduced by TGF-β_1 treatment on days 14–18 or 28–32, correlating with clinical observations (Fig. 2). None of the changes seen in control mice, including hyperplasia of synovial lining cells, accumulations of mononuclear inflammatory cells and of fibrous connective tissue, and new exostotic bone formation, were seen in the TGF-β_1-treated animals.

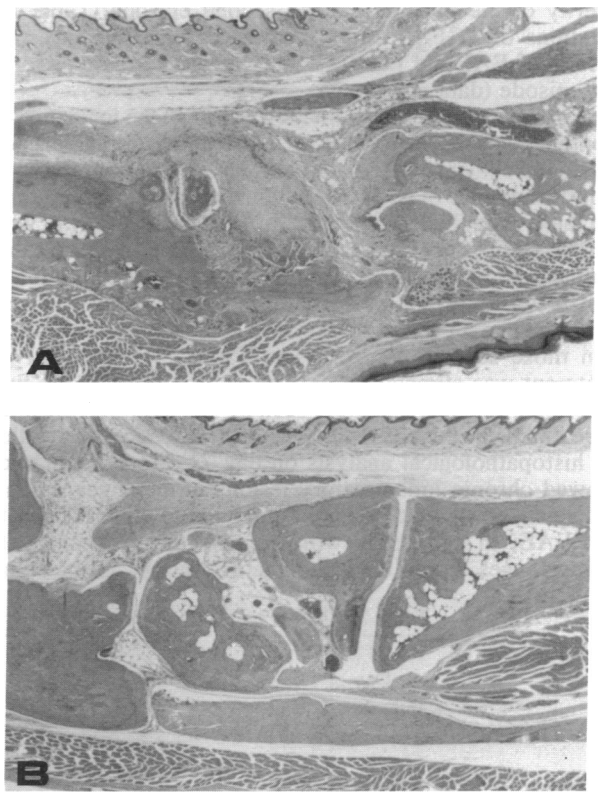

FIG. 2. Tarsal (ankle) joints from mice immunized with CII in CFA and either untreated (*A*) or treated with TGF-β_1 (*B*). The bone and soft tissue elements of the joint from an untreated animal (*A*) are distorted, fused, and effaced by accumulations of mononuclear inflammatory cells, connective tissue, and new bone. In contrast, the joint from a successfully treated animal (*B*) has clearly defined joint spaces and articular and synovial elements with scant to no inflammation. (Hematoxylin and eosin; ×25.)

Table 1. Effect of TGF-β_1 administered during induction of EAE

EAE episode	TGF-β_1 treatment	Mean day of onset ± SD	Mean severity ± SD	Incidence of EAE
First	−	7.5 ± 0.52*	2.5 ± 1.38	11/15
	+	9.5 ± 1.96*	2.2 ± 1.70	10/14
Second	−	7.1 ± 0.64†	3.3 ± 0.85‡	13/13
	+	9.8 ± 1.42†	1.9 ± 1.05‡	17/19

To induce the first episode of EAE, mice were repeatedly injected with 1 mg of lyophilized mouse spinal cord homogenate in CFA on days −44, −30, and 0 (the last day of immunization). The second EAE episode in these experiments was booster induced (in mice that did not exhibit spontaneous relapses) 3 months after the first episode. Daily i.p. injections of 2 μg of TGF-β_1 started 1 day before the last immunization and continued for 2 weeks. Control mice received injections of PBS. Mice were examined daily for signs of disease and were graded on an increasing severity scale of 0–4 (see *Methodology*). Statistical evaluation of differences between similarly labeled values was by Student's *t* test.
*$P < 0.01$.
†$P < 0.0001$.
‡$P = 0.0004$.

As CIA is a progressive disease in mice, additional studies were performed with TGF-β_1 in another autoimmune disease, REAE, in SJL mice, characterized by remissions and exacerbations. Induction of EAE proved more resistant to the effect of TGF-β_1 than did induction of CIA. Daily injections of 2 μg of TGF-β_1 i.p. during the first 2 weeks after immunization delayed, but did not suppress, development of an acute episode of EAE (Table 1). Treatment after the first attack of EAE during a repeat immunization reduced the severity of the booster-induced second episode (Table 1). Injections of TGF-β_1 initiated after the onset of an acute episode of EAE did not noticeably influence the course of that episode (data not shown).

However, TGF-β_1 had a marked effect on the incidence of spontaneous relapses (Table 2). Nine of 20 untreated mice developed clinical exacerbations when left for 2 months after their first episode of EAE without further immunizations, and 2 of these mice died. In contrast, of 18 mice injected i.p. with 2 μg of TGF-β_1 per day, starting 35 days after the beginning of their first episode (i.e., on day 42) and continuing for at least 4 weeks, 17 failed to develop spontaneous relapses. Only 1 mouse showed a mild relapse that occurred much later than those seen in control mice (Table 2). When TGF-β_1 treatment was stopped on day 70, as was done in the first experiment, no relapses were seen by day 80, when the mice were killed. In the second experiment, the mice were killed for histopathological analysis on day 72. Most of the mice showed chronic inflammatory lesions in meninges, brains, and spinal cords that appeared unrelated to clinical severity of REAE, in agreement with previous observations (30). Hemorrhages within the inflammatory lesions in the spinal cord were observed in 1 of the 3 control mice that had exhibited and survived a spontaneous relapse (data not shown).

Thus, TGF-β_1 prevents relapses, but not initial induction of EAE, possibly because the severity of the initial episodes of EAE is greater than the disease severity attained during relapses. This could, therefore, constitute a quantitative rather than a qualitative difference in the effect of TGF-β_1 during different stages of the disease.

Further studies are needed to determine the mechanism by which TGF-β_1 protects against these autoimmune diseases. It is of interest to note that IL-1 (11) and TNF-α (R.S., M.A.P., A.P.K., H.D.L., A. M. Hardison, and G.J.T., unpublished data) have been shown to increase the severity of CIA. Moreover, increased expression of Ia antigen on synovial cells (32) and on astrocytes (33) is thought to be important in the pathogenesis of CIA and REAE, respectively. TGF-β_1 has been reported to inhibit production of IL-1 (23) and TNF-α, to be antagonistic to many of the effects of TNF-α (34, 35), and to be a potent inhibitor of IL-1 receptor expression (36). In other studies, we have shown that TGF-β_1 prevents the increased expression of Ia antigen on epidermal Langerhans cells induced by IL-1, TNF-α, IFN-γ, and granulocyte/macrophage colony-stimulating factor *in vivo* and *in vitro*; however, it does not down-regulate the normal Ia antigen expression on these cells (37).

A similar antagonistic effect of TGF-β_1 on other cytokines has been noted for Ia expression on human melanoma cells (38). Thus, regulation of Ia expression on target cells may be an important aspect of the effect of TGF-β_1 on CIA and REAE. In addition, the deactivating effect of TGF-β_1 on macrophages, recently described by Tsunawaki *et al.* (39), could make an important contribution to its anti-inflammatory effect in autoimmune diseases.

Although TGF-β_1 has inhibitory effects on B cells (19, 20), inhibition of antibody production by TGF-β_1 is not likely to have played an important role in affecting either of these diseases. In fact, TGF-β_1 injected late during the immunization period, when it does not affect antibody titers (CIA), or after immunization (EAE), is more effective in suppressing disease than treatment early during induction of the diseases. These observations are in complete agreement with our earlier findings that daily injections of 2 μg of TGF-β_1 inhibit the expression, but not the induction, of contact sensitization in mice (37).

TGF-β_1 can also maintain or enhance suppressor cell activity, as shown recently with chicken spleen cells *in vitro* (26). In this respect it may differ from immunosuppressive drugs. Cyclophosphamide can suppress the development of EAE or CIA, when given early during the immunization (4, 5), but can provoke relapses in rats that have recovered from EAE (40). Moreover, cyclosporin A suppresses CIA when given early but augments the disease when given late during

Table 2. Effect of TGF-β_1 on incidence of spontaneous relapses of EAE

Day of immunization	Day of TGF-β1 injections	Incidence of relapses (%)	Mean day of relapse ± SD	Mean change in severity ± SD
−77, −70, 0	None	4/8 (50)	59.0 ± 5.1	1.0 ± 1.1
	+42 to +70	0/7 (0)	—	−0.3 ± 0.5
−120, −90, 0	None	5/12 (42)	56.8 ± 5.1	1.3 ± 1.7
	+42 to +70	1/11 (9)	70	0.1 ± 0.3

Day 0 is the last day of immunization with spinal cord in CFA (see Table 1). The first attack of EAE occurred approximately on day 7. Daily i.p. injections of 2 μg of TGF-β_1 were given on the days indicated. Control mice received injections of PBS. The difference in incidence between control and TGF-β_1-treated groups is statistically significant (χ^2 two-tailed test: $P < 0.01$) for both experiments taken together. Change in severity is expressed as the difference between maximum disease severity during exacerbation and disease severity before the relapse. Disease severity was assessed as described in *Methodology*.

the induction phase (10). It has been suggested that, in both multiple sclerosis (41) and rheumatoid arthritis (42) patients, the "suppressor inducer" T-cell subset is defective. If, indeed, the balance between T-cell subsets is important in the determination of the nature of the autoimmune response, it could be that cyclophosphamide and cyclosporin A preferentially inactivate suppressor effector or inducer cells, whereas TGF-β_1 might promote their activation.

There is an interesting analogy between the effects of TGF-β_1 and IFN-β on two different aspects of the immune system that should be pointed out here. IFN-β has been reported to augment Con A-induced suppressor cell function in peripheral blood cells from multiple sclerosis patients (43), while in our laboratories TGF-β_1 was found to augment CD8 expression in Con A-activated murine spleen cells (I. R. Katz and G.J.T., unpublished observations). In addition, both IFN-β and TGF-β_1 counteract the up-regulation of Ia antigen on macrophages and other cells by IFN-γ (37, 38, 44, 45). Since IFN-β has been reported to have a beneficial effect in the treatment of multiple sclerosis patients (46, 47), it seems appropriate to pay special attention to these similarities in the effects of TGF-β_1 and IFN-β.

TGF-β_1 is a molecule extremely well-conserved in evolution, suggesting that its role in humans and animals may be quite similar. Its immunosuppressive properties have thus far been studied primarily *in vitro* and have been found to be quite similar with human and animal cells. Our findings may therefore point to the possible clinical usefulness of TGF-β_1 for the control of autoimmune diseases.

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1. Myers, L. W. (1990) *Autoimmun. Forum Neurol.* **2**, 3–7.
2. Trentham, D. E. (1989) *Rheum. Dis. Clin. N. Am.* **15**, 407–412.
3. Takagashi, K., Kaibara, N., Hotokebuchi, T., Arita, C., Morinaga, M. & Arai, K. (1986) *Ann. Rheum. Dis.* **45**, 339–344.
4. Paterson, P. Y. & Drobish, D. G. (1969) *Science* **165**, 191–192.
5. Sloboda, A. E., Birnbaum, J. E., Oronsky, A. L. & Kerwar, S. S. (1981) *Arthritis Rheum.* **24**, 616–624.
6. Inamura, N., Hashimoto, M., Nakahara, K., Nakajima, Y., Nishio, M., Aoki, H., Yamaguchi, I. & Kohsaka, M. (1988) *Int. J. Immunopharmacol.* **10**, 991–995.
7. Inamura, N., Hashimoto, M., Nakahara, K., Aoki, H., Yamaguchi, I. & Kohsaka, M. (1988) *Clin. Immunol. Immunopathol.* **46**, 82–90.
8. McPhee, I. A. M., Antoni, F. A. & Mason, D. W. (1989) *J. Exp. Med.* **169**, 431–445.
9. Phadke, K., Fouts, R. L., Parrish, J. E. & Butler, L. D. (1985) *Immunopharmacology* **10**, 51–60.
10. Kaibara, N., Hotokebuchi, T., Takagishi, K. & Katsuki, I. (1983) *J. Exp. Med.* **158**, 2007–2105.
11. Hom, J. T., Bendele, A. M. & Carlson, D. G. (1988) *J. Immunol.* **141**, 834–841.
12. Shore, A., Jaglal, S. & Keystone, E. C. (1986) *Clin. Exp. Immunol.* **65**, 293–302.
13. Xu, W. D., Firestein, G. S., Taetle, R., Kaushansky, K. & Zvaifler, N. J. (1989) *J. Clin. Invest.* **83**, 876–882.
14. Hopkins, S. J. & Meager, A. (1988) *Clin. Exp. Immunol.* **73**, 88–92.
15. Arend, W. P., Weigus, H. G., Thompson, R. C. & Eisenberg, S. P. (1990) *J. Clin. Invest.* **85**, 1694–1697.
16. Ohisson, K., Bjork, P., Bergenfeldt, M., Hageman, R. & Thompson, R. C. (1990) *Nature (London)* **348**, 550–552.
17. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163**, 1037–1050.
18. Ristow, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5531–5535.
19. Kehrl, J. H., Roberts, A. B., Wakefield, L. M., Jakowlew, S., Sporn, M. B. & Fauci, A. S. (1986) *J. Immunol.* **137**, 3855–3860.
20. Kehrl, J. H., Taylor, A. S., Delsing, G. A., Roberts, A. B., Sporn, M. B. & Fauci, A. S. (1989) *J. Immunol.* **143**, 1868–1874.
21. Palladino, M. A., Czarniecki, C. W., Chiu, H. H., McCabe, S. M., Figari, I. S. & Ammann, A. J. (1987) *J. Cell. Biochem. Suppl.* **11A**, 10 (abstr.).
22. Espevik, T., Figari, I. S., Shalaby, M. R., Lackides, G. A., Lewis, G. D., Shepard, H. M. & Palladino, M. A., Jr. (1987) *J. Exp. Med.* **166**, 571–576.
23. Chantry, D., Turner, M., Abney, E. & Feldmann, M. (1989) *J. Immunol.* **142**, 4295–4300.
24. Straub, C. & Zubler, R. H. (1989) *J. Immunol.* **142**, 87–93.
25. Shalaby, M. R. & Ammann, A. J. (1988) *Cell. Immunol.* **112**, 343–350.
26. Quere, P. & Thorbecke, G. J. (1990) *Cell. Immunol.* **129**, 468–477.
27. Rook, A. H., Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Sporn, M. B., Burlington, D. B., Lane, H. C. & Fauci, A. S. (1986) *J. Immunol.* **136**, 3916–3920.
28. Cremer, M. A., Hernandez, A. D., Townes, A. S., Stuart, J. M. & Kang, A. H. (1983) *J. Immunol.* **131**, 2995–3000.
29. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, G. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7443–7446.
30. Brown, A. M., McFarlin, D. E. & Raine, C. S. (1982) *Lab. Invest.* **46**, 171–185.
31. Derynck, R., Jarret, J. A., Chen, E. Y., Eaton, D. H., Bell, J. R., Assoian, R. K., Roberts, A. B., Sporn, M. B. & Goeddel, D. V. (1985) *Nature (London)* **316**, 701–705.
32. Lindblad, S., Klareskog, L., Hedfors, E., Forsum, U. & Sundstrom, C. (1983) *Arthritis Rheum.* **26**, 1321–1332.
33. Sobel, R. A., Blanchette, B. W., Bhan, A. K. & Colvin, R. B. (1984) *J. Immunol.* **132**, 2393–2407.
34. Ranges, G. E., Figari, I. S., Espevik, T. & Palladino, M. A. (1987) *J. Exp. Med.* **166**, 991–998.
35. Espevik, T., Figari, I. S., Ranges, G. E. & Palladino, M. A. (1988) *J. Immunol.* **140**, 2312–2316.
36. Dubois, C. M., Ruscetti, F. W., Palaszynski, E. W., Falk, L. A., Oppenheim, J. J. & Keller, J. R. (1990) *J. Exp. Med.* **172**, 737–744.
37. Epstein, S. P., Baer, R. L., Thorbecke, G. J. & Belsito, D. V. *J. Invest. Dermatol.*, in press.
38. Czarniecki, C. W., Chiu, H. H., Wong, H. W., McCabe, S. M. & Palladino, M. A. (1988) *J. Immunol.* **140**, 4217–4223.
39. Tsunawaki, S., Sporn, M., Ding, A. & Nathan, C. (1988) *Nature (London)* **334**, 260–262.
40. Minagawa, H., Takenaka, A., Itoyama, Y. & Mori, R. (1987) *J. Neurol. Sci.* **78**, 225–230.
41. Morimoto, C., Hafler, D. A., Weiner, H. L., Letvin, N. L., Hagan, M., Daley, J. & Schlossman, S. F. (1987) *N. Engl. J. Med.* **316**, 67–72.
42. Goto, M., Miyamoto, T., Nishioka, K. & Okumura, K. (1986) *J. Rheumatol.* **13**, 853–857.
43. Noronha, A., Toscas, A. & Jensen, M. A. (1990) *Ann. Neurol.* **27**, 207–210.
44. Ling, P. D., Warren, M. K. & Vogel, S. N. (1985) *J. Immunol.* **135**, 1857–1863.
45. Inaba, K., Kitaura, M., Kato, T., Watanabe, Y., Kawade, Y. & Muramatsu, S. (1986) *J. Exp. Med.* **163**, 1030–1035.
46. Jacobs, L., Salazar, A. M. & Herndon, R. (1987) *Arch. Neurol.* **44**, 589–595.
47. Knobler, R. L., Panitch, H. S. & Braheny, S. L. (1984) *Neurology* **34**, 1273–1279.