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Immunology

Involvement of endogenous tumor necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice

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ABSTRACT **Both tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) are found in synovial fluid from arthritic joints of humans and of rodents with experimental arthritis. The role of endogenously produced TGF-β and TNF in the pathogenesis of collagen type II-induced arthritis (CIA) in DBA/1 mice was examined by determining the effect of neutralizing monoclonal antibodies to these factors on the course of the disease. Endogenously produced as well as systemically administered TGF-β_1 and TNF-α had opposite effects, since TGF-β_1 and anti-TNF protected against CIA, whereas anti-TGF-β and TNF-α increased CIA incidence and/or severity. Intraperitoneally injected TGF-β_1 at a dose of 2 μg per day for 14 days significantly ameliorated arthritis, even when started at the time of arthritis development, although it did not reverse established disease. The resistance to CIA induction caused by a prior intravenous injection of collagen type II was not significantly influenced by the simultaneous injection of TGF-β_1, TNF-α, or interleukin 1α. It is concluded that the endogenous production of TNF and TGF-β is important in determining the course of CIA.**

Susceptibility to collagen type II (CII)-induced arthritis (CIA) in DBA/1 and B10.RIII mice is readily modified by immunologically specific interventions, such as the oral (1) or intravenous (i.v.) administration of CII prior to (2, 3) or up to 14 days after (4) immunization with CII in complete Freund's adjuvant (CFA). Immunosuppressive agents, including cyclophosphamide (5), cyclosporin A (CsA) (6), or antibodies either to CD4 (7), the interleukin 2 (IL-2) p55 receptor (8), or Ia (9), cause only partial protection, whereas interferon γ increases the incidence of CIA (9).

Recent studies have demonstrated multiple immunoregulatory effects of transforming growth factor β_1 (TGF-β_1). These include inhibition of T-cell (10, 11) and B-cell (12, 13) proliferation, cytokine production (14–16), antibody or immunoglobulin formation (17–19), natural killer (NK) cell activity (20), and the maintenance of suppressor cell function (19). Many of the inhibitory effects of TGF-β_1 antagonize the stimulatory effects of other cytokines. An antagonistic relationship between tumor necrosis factor α (TNF-α) and TGF-β_1 has been described for the generation of allospecific cytotoxic T lymphocytes (21) and the induction of lymphokine-activated killer cells (22). In addition, TGF-β_1 shares with CsA (23) the ability to counteract the *in vivo* and *in vitro* augmenting effects of IL-1, TNF-α, interferon γ, IL-3, and granulocyte/macrophage-colony-stimulating factor but not IL-2 and IL-6 on Ia expression by epidermal Langerhans cells (24).

In spite of the antagonistic activities of TNF-α and TGF-β_1, both cytokines have been implicated as contributing to local inflammatory responses in arthritis. These cytokines are present in synovial fluid from arthritic joints of humans with rheumatoid arthritis (25, 26) and rodents with experimentally induced arthritis (27, 28). In addition, TNF-α has also been implicated in the induction of experimental autoimmune allergic encephalomyelitis (EAE) (29, 30). However, when administered systemically, TGF-β_1 protects against the development of CIA and the occurrence of relapses in EAE (31). We have extended these findings and examined the role of endogenously produced TGF-β and TNF in CIA. Systemically administered TNF-α and anti-TGF-β were found to increase CIA morbidity, while TGF-β_1 and anti-TNF-α afforded a significant degree of protection.

MATERIALS AND METHODS

Mice. DBA/1J male mice were purchased from The Jackson Laboratory and used at 8–14 weeks of age.

Reagents. Chick CII was purchased from Genzyme. Recombinant murine TNF-α was produced in *Escherichia coli* and had a specific activity of 7×10^7 units/mg as determined by a cytotoxicity assay using the murine cell line L-M (32, 33). Recombinant human TGF-β_1 was produced in Chinese hamster ovary cells (34) and purified to 0.8 mg/ml in 20 mM sodium acetate (pH 4) [contained 8 endotoxin units/ml (*Limulus* amebocyte lysate assay), equivalent to 1 pg of endotoxin per μg of protein]. Recombinant murine IL-1α (lot 1/87; specific activity, 2.5×10^9 units/mg) was a generous gift from P. Lomedico (Hoffmann–La Roche). Monoclonal antibody 2G7 neutralizes the activity of TGF-β_1, TGF-β_2, and TGF-β_3 (35). Monoclonal antibody TN3 19.12 neutralizes the activity of murine TNF-α and TNF-β (36) and was provided by R. D. Schreiber.

Induction of CIA. Chick CII was dissolved in 0.01 M acetic acid at 1–3 mg/ml, 8–24 hr prior to use, and stored at 4°C. The chick CII solution was emulsified with CFA, prepared by mixing pulverized, lyophilized, heat-killed *Mycobacteria* (strains C, DT, and PN; Ministry of Agriculture, Fisheries and Food, Weybridge, Surrey, U.K.) in incomplete adjuvant (Difco) at 4 mg/ml (37). Each mouse received either 100 or 150 μg of CII in 0.2 ml, divided among four intradermal sites on the back. A booster injection of 100 μg of CII was given intraperitoneally (i.p.) as an aqueous solution without adjuvant 28 days later. For tolerization, 25–100 μg of CII was injected i.v. Control mice received an i.v. injection of the solvent alone.

Assessment of CIA. Mice were observed two to three times per week for the presence of distal joint swelling and erythema. Swelling was quantitated by measuring the thick-

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Abbreviations: CFA, complete Freund's adjuvant; CII, collagen type II; CIA, CII-induced arthritis; CsA, cyclosporin A; EAE, experimental allergic encephalomyelitis; IL, interleukin; MAI, mean arthritic index; TGF, transforming growth factor; TNF, tumor necrosis factor.

ness of feet and width of wrists and ankles with a constant-tension caliper (Dyee, Lancaster, PA). A mouse was considered arthritic when swelling and erythema in at least one paw were observed on consecutive measurement dates. In addition, clinical severity of CIA was assessed by calculation of an "arthritic index." Each limb was subjectively graded on a scale of 0–3 [0, absence of detectable arthritis; 1, mild swelling (0.1–0.3 mm over a normal thickness of 1.6 mm per foot) and erythema; 2, moderate swelling (≥0.4 mm) and erythema of both tarsus and carpus; 3, ankylosis and bony deformity]. The arthritic index was obtained for each mouse by summing the scores recorded for each limb (0, no detectable disease; 12, highest possible score) (2, 38). Comparisons between groups of mice were performed on the basis of the means ± SD of individual mouse arthritic indices for any given day after immunization. Serum levels of antibody to CII were determined in an enzyme-linked immunosorbent assay (1).

Statistical Analysis. Comparisons of means were performed by Student's *t* test. Arthritic incidences were compared using the χ^2 method on the basis of numbers of animals or on the basis of the number of affected feet.

RESULTS

Effect of TGF-β_1 on CIA. It was of interest to determine when during the induction of CIA TGF-β_1 would be most effective in preventing arthritis development. Treatment with 2 μg of TGF-β_1, either i.p. or i.v., daily for 5 days starting on the day of immunization with CII and CFA, resulted in a slight delay in the onset of CIA but provided no permanent protective effect (data not shown). Increasing the daily dose to 5 μg of TGF-β_1 during the same period caused a significant reduction in arthritis morbidity, lasting throughout the 60-day period of observation (Table 1). In addition, as also shown in previous studies (31), treatment on days 14–18 with 2 μg of TGF-β_1 per day markedly decreased the incidence of CIA (Table 1). A combination of treatment during days 14–18 with several other schedules was no more effective than treatment on these days alone. Since the challenge with CII on day 28 is important in provoking the onset of CIA, we also examined the effectiveness of TGF-β_1 administered after day 28. Treatment on days 28–32 after i.p. challenge with CII was effective in only one of the experiments (compare Exps. 1 and 2, Table 1). This variability is perhaps due to the rate at which the disease develops, which can be slow, as in Exp. 1, or faster, as in Exp. 2. The difference was not due to the difference in TGF-β_1 dose, as in a repeat experiment, neither 2 nor 5 μg/day on days 28–32 caused a significant reduction in the incidence of CIA (data not shown). The results suggest that treatment on days 14–18 after immunization is more effective than treatment after the challenge with soluble CII. The systemic administration of 5 μg of TGF-β1 did not result in any detectable changes in weight compared with controls (Table 1, footnote).

Table 1. Effect of TGF-β_1 treatment schedule on incidence and severity (mean arthritic index, MAI) of CIA

	TGF-β_1 treatment		Response on day 40		Response on day 60		
Exp.	μg	Days	CIA incidence, %	MAI	CIA incidence, %	MAI	*n*
1	None		39	1.7	65[a,b]	3.2	18
	5	0–4	11	0.6	25	1.9	9
	5	28–32	33	2.3	63	4.1	9
	2	3–7; 10–14; 17–21; 31–35	20	0.9	26[a]	2.1	20
	2	0–4; 14–18; 28–32	5	0.3	21[b]	0.8	19
2	None		67[c,d,e,f]	2.2	78[g,h,i,j,k]	4.8	9
	2	7–11	22	0.6	67	2.7	9
	2	14–18	0[c]	0	0[g]	0	8
	2	28–32	10	0.4	10[h]	1.0	9
	2	14–18; 28–32	10[d]	0.6	10[i]	1.4	10
	2	0–4; 28–32	0[e]	0	0[j]	0	9
	2	0–4; 7–11; 14–18; 28–32	10[f]	0.3	10[k]	0.3	10
3	None		57	2.4	71[m]	4.4	14
	5	0–4*	22	1.7	22[m]	2.0	9

Mice (*n* per group) were immunized on day 0 with 150 μg (Exp. 1) or 100 μg (Exp. 2) of CII in CFA subcutaneously and challenged i.p. on day 28 with 100 μg of CII. TGF-β_1 was injected i.p. (Exps. 1 and 2) or i.v. (Exp. 3). χ^2 test (one-tailed) gave the indicated *P* values for differences between similarly designated groups: $P \leq 0.02$, a, d, and f; $P \leq 0.01$, b, c, e, g, h, i, j, and k; $P = 0.04$, m.

*Weight gain of TGF-β_1-treated mice during the week of treatment was 0.29 g per mouse, compared with 0.3 g per mouse in the control group.

As shown previously (39), aged mice were much less responsive to the induction of CIA by bovine CII (29% incidence on day 60) and formed much lower antibody titers to bovine CII than young DBA/1 mice. Treatment with 2 μg of TGF-β_1 on days 14–18 completely prevented CIA development in the aged mice (data not shown).

In additional studies, treatment with 2 μg of TGF-β_1 per day was started at the first symptoms of disease (day 35) and continued until day 49 (Fig. 1). The severity of CIA was much less in TGF-β_1-treated mice, even though the disease incidence was only slightly reduced. After the TGF-β_1 injections were stopped, the disease incidence in the treated animals remained stable until the experiment was terminated on day 60.

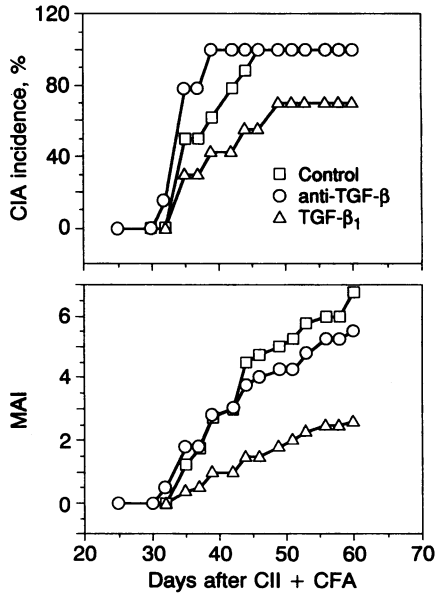

FIG. 1. Effect of TGF-β_1 or anti-TGF-β on CIA. Mice (eight to nine per group) were injected intradermally on day 0 with 100 μg of chick CII in CFA, followed on day 28 by i.p. injection of 100 μg of chick CII. Anti-TGF-β (2G7, 0.25 mg) was injected i.p. on days 14 and 18 and again (0.5 mg) on day 37. TGF-β_1 (2 μg) was injected i.p. on days 35–49. The incidence based on the number of affected feet differed significantly between control and anti-TGF-β-treated mice on days 35 and 42 ($P \leq 0.05$), and between control and TGF-β_1-treated groups on day 39 ($P < 0.05$), day 42 ($P < 0.01$), and thereafter ($P < 0.001$). The MAIs of the latter two groups differed significantly on days 44 ($P < 0.01$) and thereafter ($P < 0.005$). Differences between TGF-β_1 and anti-TGF-β-treated groups were significant for both incidence and severity of arthritis from day 35 to day 49.

In mice treated earlier during the course of immunization, before arthritis developed, the severity of CIA in the TGF-β_1-treated mice, as judged by the arthritic indices, was reduced to the same extent as the incidence. Severely affected joints from untreated animals were characterized histologically by (*i*) synovial hypercellularity due to hyperplasia of synovial lining cells; (*ii*) accumulation of mononuclear inflammatory cells within synovia, joint spaces, and periarticular regions; and (*iii*) excess accumulations of fibrous connective tissue and new, exostotic bone, particularly in the vicinity of the synovial attachment. Joints were commonly bridged by excessive accumulations of new bone and articular surfaces damaged by tissue overgrowth (pannus) and chondrolysis. Joints taken from animals responsive to TGF-β_1 therapy were histologically normal or, if involved, had substantially less inflammatory cell accumulation, reduced synovial hyperplasia, limited new bone development, and scant articular surface injury. There was virtually complete agreement between clinical and histological evaluation of arthritis severity and incidence (data not shown and ref. 31).

Serum antibody titers to CII were not affected by treatment with 2 μg of TGF-β_1 on days 0–5 after immunization with CII in CFA, but after treatment with 5 μg of TGF-β_1 daily during the same period antibody levels were approximately half to one-third those seen in the other groups (data not shown). The results from Exps. 1 and 2 (Table 1) suggest that TGF-β_1 can have a protective effect against CIA without exerting a major effect on the production of antibodies to CII. None of the groups treated on days 7–11 or later during the induction period had a statistically significant change in anti-CII serum titers on day 40, although the mean antibody titers in TGF-β_1-treated mice in Exp. 2 were lower than in control groups (data not shown). As shown previously, when arthritic mice and nonarthritic mice in sufficiently large control groups are compared, the antibody titers in the arthritic mice are significantly higher (4). Similarly, in the present experiments, when the antibody titers in nonarthritic TGF-β_1-treated mice were compared with those in arthritic control mice, the differences were frequently significant but did not correlate with the effectiveness of TGF-β_1 in reducing the incidence of CIA in the individual treatment groups.

Effect of Anti-TGF-β on CIA. Since treatment with TGF-β_1 on days 14–18 after the initial immunization with CII and CFA had a protective effect against the development of CIA, the effect of anti-TGF-β administered during that period was also examined. CIA development after the challenge with CII on day 28 was significantly accelerated in anti-TGF-β-treated mice (Fig. 1).

Effects of TNF-α and Anti-TNF on CIA. Initial experiments examined the effects of TNF-α (1 μg) or IL-1α (0.4 ng) administered on days 0–4 after immunization with CII in CFA (data not shown). Although there was a tendency toward increased morbidity, particularly with IL-1α, the effects on arthritis incidence and serum anti-CII levels were not statistically significant. Prolonged treatment of mice with 1 μg of TNF-α per day can cause weight loss (40). Therefore, the effect of TNF-α at 0.2 μg per injection, starting on day −7 and continuing for five injections per week during the first, second, third, and fifth weeks after immunization with CII in CFA, was examined (Fig. 2*A*). In the TNF-α-treated mice there was an increase in the CIA incidence and disease severity above control levels. In contrast, repeated injections of TGF-β_1 caused a reduced incidence and disease severity.

The effects of TNF-α and anti-TNF on days 14–18 after the initial immunization with CII in CFA were further examined. Anti-TNF caused a significant delay in the development of CIA, whereas TNF-α increased both the incidence and the severity of the disease (Fig. 2*B*). Simultaneous treatment with TGF-β_1 and TNF-α resulted in the same increased incidence in disease as treatment with TNF-α alone, and only a slightly lower severity (data not shown), suggesting that TGF-β_1 could not abolish the effect of injected TNF-α.

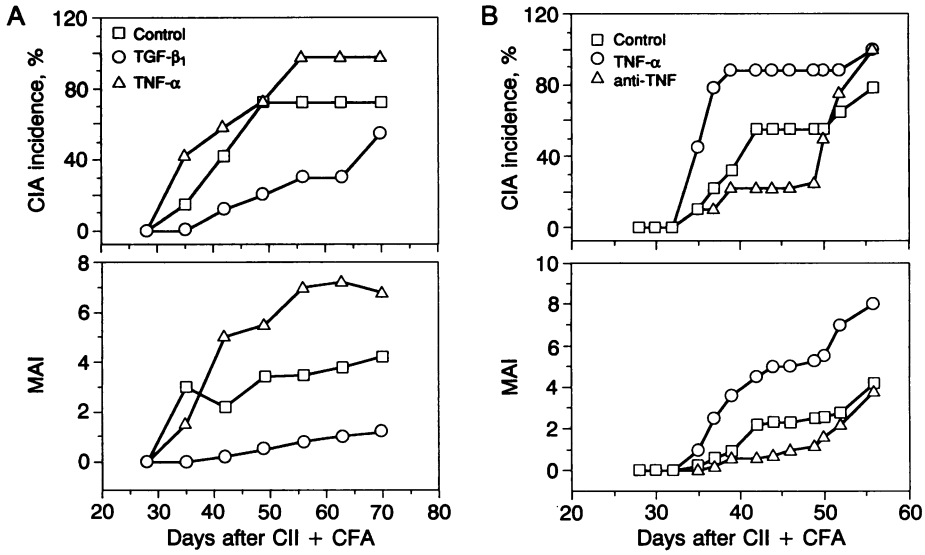

FIG. 2. (*A*) Effect of TNF-α or TGF-β_1 on CIA. Groups of 7–10 DBA/1 mice were injected i.v. on day −7 with 10 μg of chick CII, followed on day 0 with 100 μg of chick CII in CFA and on day 28 by injection of 100 μg of chick CII i.p. (The 10 μg of CII i.v. on day −7 was given in an attempt to induce resistance to CIA induction, but we later found ≥25 μg to be needed to obtain any effect on disease incidence.) TNF-α (0.2 μg) or TGF-β_1 (2 μg) injections were given i.p. on days −7, 0–4, 7–11, 14–18, and 28–32. Measurements of arthritis severity (MAI) and incidence were recorded at weekly intervals. On day 49 after injection of CII in CFA, the difference between the arthritis incidences in the control and the TGF-β_1-treated groups was significant ($P = 0.05$). (*B*) Effect of TNF-α and anti-TNF on CIA. Groups of 9 mice were injected on day 0 with 100 μg of CII in CFA, followed on day 28 by i.p. injection of 100 μg of CII. Hamster anti-TNF (0.3 mg) or hamster immunoglobulin (0.3 mg, control) was injected i.p. on days 14 and 16. TNF-α (0.5 μg) was injected i.p. on days 14, 16, and 18. As judged by the number of arthritic feet, the difference between the control and the anti-TNF-injected groups was significant on days 42 and 49 ($P < 0.05$). The overall arthritis incidence differed significantly between control and TNF-α-injected mice on days 37 and 39 ($P < 0.05$). The MAI differed significantly between control and TNF-α-treated groups on days 37, 39, and 52 ($P < 0.05$).

Effect of Cytokines on Induction of Tolerance to CII. The i.v. injection of 25 μg of chick CII one week prior to immunization with CII in CFA caused a high degree of resistance to the induction of CIA (Table 2). A single simultaneous injection of TGF-β_1 did not appear to enhance or prolong significantly the effect of 25 μg of CII (data not shown). The protective effect of i.v. administration of 100 μg, but not 25 μg, of CII, was accompanied by a sustained lowering of the serum antibody response to CII on days 40 and 60 after immunization with CII in CFA (Table 2 and ref. 4).

Contrary to what has been described for tolerance induction to i.v. injected human gamma globulin (41), at the doses tested, TNF-α, 0.5 μg (Table 2), or IL-1α, 0.8 ng i.v., day −7 (data not shown), did not prevent the protective effect of 25 μg of i.v. injected CII, particularly when the cytokine was given simultaneously with i.v. CII. When TNF-α was administered repeatedly prior to and after injection of CII in CFA, a minor, nonsignificant effect was seen (Table 2). Paradoxically, TNF-α treatment lowered anti-CII antibody responses when the treatment was limited to the first week after the i.v. injection of CII.

DISCUSSION

A protective effect of TGF-β_1 on CIA was observed, even when administration was initiated >2 weeks after immunization with CII in CFA, but before the appearance of symptoms. Daily treatment with TGF-β_1 during the time at which symptoms were beginning to be expressed (days 35 and 49) still caused an amelioration of the disease. However, TGF-β_1 was ineffective when administered after arthritis had been present for a few weeks (data not shown). It is not clear whether the symptoms associated with CIA, which include ankylosis, once they have developed, are reversible by immunosuppression (42).

The mechanisms by which TGF-β_1 modulates CIA need further study. TGF-β_1 treatment *in vivo* inhibits the expression of contact sensitivity in previously sensitized mice (24). In this effect it is similar to CsA, but unlike CsA it does not prevent sensitization to a contact sensitizer when administered during sensitization. The greater effect of TGF-β_1 during the latter part than during the early part of the CIA induction phase in the present studies also suggests an influence on the effector rather than the immunization process. In view of the lack of correlation between the effect of TGF-β_1 on anti-CII serum levels and its effect on arthritis, the inhibitory effect on antibody production, seen when TGF-β_1 is injected close to the time of primary (days 0–4) and/or secondary (days 28–32) CII injections, appears relatively unimportant for its antiarthritic effect. It seems more likely that TGF-β_1 counteracts a T-cell-mediated event that is particularly sensitive to TGF-β_1 during the third week after immunization with CII. Administration of antibodies to TGF-β during this time caused an accelerated appearance of CIA, suggesting that endogenously produced TGF-β can affect CIA development. In view of the protective effect of anti-TNF administered during that same period, it seems that TNF production during the third week after immunization is important for CIA development.

TNF release by sensitized T cells and/or macrophages in the joint may cause upregulation of Ia expression on the synovial cells. Ia^+ dendritic cells are prominent in the synovia and synovial exudates from arthritic joints (43, 44), and, thus, downmodulation of Ia expression by TGF-β_1, similar to its reported effect on murine Langerhans cells (24), human glioma cells (45), and human macrophages (46), could play a role in its protective effect against arthritis induction. As shown by Nathan and coworkers (47), TGF-β suppresses nitric oxide production by macrophages and has a general deactivating effect on these cells, probably also affecting their ability to produce TNF-α in inflammatory lesions. It seems more likely that TGF-β inhibits the production rather than the effects of TNF-α, since simultaneous treatment with TGF-β_1 and TNF-α resulted in a similar enhancement of CIA as treatment with TNF-α alone. An important role for TNF-α in the *in vivo* chain of events leading to arthritis is also indicated by the finding that TNF-α transgenic mice spontaneously develop arthritis (48).

It is of interest that TGF-β_1 protects against CIA and EAE when injected systemically (31, 49–51) but causes arthritis when injected into a joint (28). Most likely the chemotactic and fibrogenic effects (52) of TGF-β_1 are important in this local response, although it has also been shown that, in synergy with other growth factors, TGF-β_1 is capable of inducing chondrocyte proliferation (53).

The augmenting effect of TNF-α and IL-1α on CIA agrees with earlier observations (54). The known antagonistic effects of TGF-β_1 and TNF-α (15, 16, 24), the inhibitory effect of TGF-β_1 on IL-1 receptor expression (55), and the ability of TGF-β to enhance production of IL-1 receptor antagonist (56) suggest that an *in vivo* balance between the effects of these cytokines may determine the outcome of an autoimmune response. The downmodulation effects of the IL-1 receptor antagonist on prostaglandin E_2 and collagenase production in human rheumatoid synovial cells (57) further suggest the importance of IL-1 in the rheumatoid disease process.

In previous studies, TGF-β_1 appeared to maintain and/or cause the development of suppressor cell function in chicken spleen cells *in vitro* (19). Further studies are needed to evaluate the possible role of the promotion of suppressor cell induction by TGF-β_1 in its protective effect on experimental autoimmune diseases. In the present studies a single simultaneous injection of TGF-β_1 did not increase the effectiveness of i.v. injected CII in inducing resistance to induction of CIA. The lack of a counteracting effect of either IL-1α or

Table 2. Effect of TNF-α on tolerance induction by i.v. injected CII

Additional treatment(s)	Response on day 40		Response on day 60		
	CIA incidence, %	Serum Ab, μg/ml	CIA incidence, %	Serum Ab, μg/ml	*n*
None	53	1426 ± 200	63	238 ± 37	19
CII (day −7)	0	1910 ± 609[a,b]	11	429 ± 107[c,d]	9
CII (day −7), TNF-α (days −7, −3)	0	450 ± 127[a]	0	153 ± 45[c,e]	9
CII (day −7), TNF-α (days −7, −3, 0–4, 7–11, 14–18, 28–32)	13	846 ± 212[b]	0	423 ± 71[d,e]	8

All groups (*n* mice per group) were immunized with 100 μg of CII in CFA on day 0 and received booster injections of 100 μg i.p. on day 28. Days of CII (25 μg, i.v.) and TNF-α (0.5 μg, i.p.) injections are in relation to day of immunization with CII and CFA. Serum antibody (Ab) values are means ± SE. Student's *t* test: $P = 0.03$, a and c; not significant, b and d; $P < 0.005$, e.

TNF-α on the tolerizing influence of i.v. injected CII is perhaps surprising. In studies with i.v. injected human immunoglobulin in mice, Weigle *et al.* (41) have shown that simultaneously injected IL-1 prevents the induction of unresponsiveness to human immunoglobulin, mimicking the well-established effect of lipopolysaccharide (58). The findings suggest that the nature of the antigen may influence the ease with which the induction of unresponsiveness can be abrogated. It has been reported by others that the protection afforded by prior i.v. injection of CII is due to the activation of $CD4^+$ $pgp1^+$ T cells with suppressor cell activity (59), whereas that induced by human immunoglobulin is primarily due to deletion (or inactivation) of responsive T and B cells (60).

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1. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, G. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7443–7446.
2. Cremer, M. A., Hernandez, A. D., Townes, A. S., Stuart, J. M. & Kang, A. H. (1983) *J. Immunol.* **131,** 2995–3000.
3. Nagler-Anderson, C., van Vollenhoven, R. F., Gurish, M. F., Bober, L. A., Siskind, G. W. & Thorbecke, G. J. (1988) *Cell. Immunol.* **113,** 447–461.
4. van Vollenhoven, R. F., Nagler-Anderson, C., Soriano, A., Siskind, G. W. & Thorbecke, G. J. (1988) *Cell. Immunol.* **115,** 146–155.
5. Arita, C., Kaibara, N., Hotokebuchi, T., Takagishi, K. & Arai, K. (1987) *Clin. Immunol. Immunopathol.* **43,** 354–361.
6. Takagishi, K., Kaibara, N., Hotokebuchi, T., Arita, C., Morinaga, M. & Arai, K. (1986) *Ann. Rheum. Dis.* **45,** 339–344.
7. Ranges, G. E., Sriram, S. & Cooper, S. M. (1985) *J. Exp. Med.* **162,** 1105–1110.
8. Banerjee, S., Wei, B. Y., Hillman, K., Luthra, H. S. & David, C. S. (1988) *J. Immunol.* **141,** 1150–1154.
9. Cooper, S. M., Sriram, S. & Ranges, G. E. (1988) *J. Immunol.* **141,** 1958–1962.
10. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowiew, S., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163,** 1037–1050.
11. Ristow, H. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5531–5533.
12. Kehrl, J. H., Roberts, A. B., Wakefield, L. M., Jakowlew, S., Sporn, M. B. & Fauci, A. S. (1986) *J. Immunol.* **137,** 3855–3860.
13. Kehrl, J. H., Taylor, A. S., Delsing, G. A., Roberts, A. B., Sporn, M. B. & Fauci, A. S. (1989) *J. Immunol.* **143,** 1868–1874.
14. 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.
15. 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.
16. Chantry, D., Turner, M., Abney, E. & Feldmann, M. (1989) *J. Immunol.* **142,** 4295–4300.
17. Straub, C. & Zubler, R. H. (1989) *J. Immunol.* **142,** 87–93.
18. Shalaby, M. R. & Ammann, A. J. (1988) *Cell. Immunol.* **112,** 343–350.
19. Quere, P. & Thorbecke, G. J. (1990) *Cell. Immunol.* **129,** 468–477.
20. 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.
21. Ranges, G. E., Figari, I. S., Espevik, T. & Palladino, M. A., Jr. (1987) *J. Exp. Med.* **166,** 991–998.
22. Espevik, T., Figari, I. S., Ranges, G. E. & Palladino, M. A., Jr. (1988) *J. Immunol.* **140,** 2312–2316.
23. Belsito, D. V., Epstein, S. P., Schultz, J. M., Baer, R. L. & Thorbecke, G. J. (1989) *J. Immunol.* **143,** 1530–1536.
24. Epstein, S. P., Baer, R. L., Thorbecke, G. J. & Belsito, D. V. (1991) *J. Invest. Dermatol.* **96,** 832–837.
25. Lotz, M., Kekow, J. & Carson, D. A. (1990) *J. Immunol.* **144,** 4189–4194.
26. Saxne, T., Palladino, M. A., Heinegard, D., Talal, N. & Wollheim, F. A. (1988) *Arthritis Rheum.* **31,** 1044–1049.
27. Lafyatis, R., Thompson, N. L., Remmers, E. F., Flanders, K. C., Roche, N. S., Kim, S.-J., Case, J. P., Sporn, M. B., Roberts, A. B. & Wilder, R. L. (1989) *J. Immunol.* **143,** 1142–1148.
28. Allen, J. B., Manthey, C. L., Hand, A. R., Ohura, K., Ellingsworth, L. & Wahl, S. M. (1990) *J. Exp. Med.* **171,** 231–246.
29. Ruddle, N. H., Bergman, C. M., McGrath, M. L., Lingenheld, E. G., Grunnet, M. L., Padula, S. J. & Clark, R. B. (1990) *J. Exp. Med.* **172,** 1193–1200.
30. Selmaj, K., Raine, C. S. & Cross, A. H. (1991) *Ann. Neurol.* **30,** 694–700.
31. Kuruvilla, A. P., Shah, R., Hochwald, G. M., Liggitt, H. D., Palladino, M. A. & Thorbecke, G. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2918–2921.
32. Pennica, D., Hayflick, J. S., Bringman, T., Palladino, M. A. & Goeddel, D. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6060–6064.
33. Kramer, S. M. & Carver, M. E. (1986) *J. Immunol. Methods* **93,** 201–206.
34. Derynck, R., Jarrett, 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.
35. Lucas, C., Bald, L. N., Fendly, B. M., Mora-Worms, M., Figari, I. S., Patzer, E. J. & Palladino, M. A. (1990) *J. Immunol.* **145,** 1415–1422.
36. Sheehan, K. C. F., Ruddle, N. H. & Schreiber, R. D. (1989) *J. Immunol.* **142,** 3884–3893.
37. Trentham, D. E., Townes, A. S. & Kang, A. H. (1977) *J. Exp. Med.* **146,** 857–868.
38. Wood, F. D., Pearson, C. M. & Tanaka, A. (1969) *Int. Arch. Allergy Appl. Immunol.* **35,** 456–467.
39. Van Vollenhoven, R. F., Nagler-Anderson, C., Stecher, V. J., Soriano, A., Connolly, K. M., Nguyen, H. T., Siskind, G. W. & Thorbecke, G. J. (1988) *Aging Immunol. Infect. Dis.* **1,** 159–176.
40. Havell, E. A., Fiers, W. & North, R. J. (1988) *J. Exp. Med.* **167,** 1067–1085.
41. Weigle, W. O., Scheuer, W. V., Hobbs, M. V., Morgan, E. L. & Parks, D. E. (1987) *J. Immunol.* **138,** 2069–2074.
42. Hom, J. T., Butler, L. D., Riedl, P. E. & Bendele, A. M. (1988) *Eur. J. Immunol.* **18,** 881–888.
43. Lindblad, S., Klareskog, L., Hedfors, E., Forsum, U. & Sundstrom, C. (1983) *Arthritis Rheum.* **26,** 1321–1332.
44. Zvaifler, N. J., Steinman, R. M., Kaplan, G., Lau, L. L. & Rivelis, M. (1985) *J. Clin. Invest.* **76,** 789–800.
45. Zuber, P., Kuppner, M. C. & De Tribolet, N. (1988) *Eur. J. Immunol.* **18,** 1623–1626.
46. Czarniecki, C. W., Chiu, H. H., Wong, G. H. W., McCabe, S. M. & Palladino, M. A. (1988) *J. Immunol.* **140,** 4217–4223.
47. Ding, A., Nathan, C. F., Graycar, J., Derynck, R., Stuehr, D. J. & Srimal, S. (1990) *J. Immunol.* **145,** 940–944.
48. Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D. & Kollias, G. (1991) *EMBO J.* **10,** 4025–4031.
49. Kuruvilla, A. P., Shah, R., Hochwald, G. M., Palladino, M. A. & Thorbecke, G. J. (1990) *FASEB J.* **4,** A2258 (abstr.).
50. Allen, J. B., Brandes, M. E., Costa, G. L., Ogawa, Y. & Wahl, S. M. (1990) *Lymphokine Res.* **5,** 15 (abstr.).
51. Karpus, W. J. & Swanborg, R. H. (1991) *J. Immunol.* **146,** 1163–1168.
52. Sporn, M. B., Roberts, A. B., Wakefield, L. M. & Assoian, R. K. (1986) *Science* **233,** 532–534.
53. Hiraki, Y., Inoue, H., Hirai, R., Kato, Y. & Suzuki, F. (1988) *Biochim. Biophys. Acta* **969,** 91–99.
54. Hom, J. T., Bendele, A. M. & Carlson, D. G. (1988) *J. Immunol.* **141,** 834–841.
55. 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.
56. Turner, M., Chantry, D., Katsikis, P., Berger, A., Brennan, F. M. & Feldmann, M. (1991) *Eur. J. Immunol.* **21,** 1635–1639.
57. Arend, W. P., Welgus, H. G., Thompson, R. C. & Eisenberg, S. P. (1990) *J. Clin. Invest.* **85,** 1694–1697.
58. Louis, J. A., Chiller, J. M. & Weigle, W. O. (1973) *J. Exp. Med.* **138,** 1481–1495.
59. Meyers, L. K., Stuart, J. M. & Kang, A. H. (1989) *J. Immunol.* **143,** 3976–3980.
60. Parks, D. E., Doyle, M. V. & Weigle, W. O. (1978) *J. Exp. Med.* **148,** 625–638.