In Vivo Role of Interleukin 4 in T Cell Tolerance Induced by Aqueous Protein Antigen

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Summary

High doses of aqueous protein antigens induce a form of immunological tolerance in which interleukin 2 (IL-2)- and interferon γ (IFN-γ)-secreting T helper type 1 (Th1) cells are inhibited, but IL-4-secreting (Th2) cells are not. This is manifested by reduced proliferation of antigen-specific T cells upon in vitro restimulation, and marked suppression of specific antibody responses of the immunoglobulin (Ig)G2a, IgG2b, and IgG3 isotypes, but not of IgG1 and IgE. The role of the immunomodulatory cytokine IL-4 in this model of unresponsiveness to protein antigens has been examined. Administration of tolerizing antigen itself primes splenic CD4$^+$ T cells for secretion of lymphokines, both IL-2 and IL-4. Neutralization of IL-4 in vivo with the anti-IL-4 antibody 11B11 during tolerance induction augments IFN-γ production by T cells of tolerant mice, and reverses the suppression of IgG2a, IgG2b, and IgG3. This blockade of IL-4 function does not, however, restore the proliferative responses of T cells, suggesting that reduced T cell proliferation is due to direct T cell inactivation or anergy. Inhibiting the activity of IL-4 in vivo also inhibits the expansion of antigen-specific Th2-like cells, which are resistant to the induction of unresponsiveness. Thus, the immunologic consequences of high-dose tolerance are due to a combination of clonal T cell anergy and IL-4-mediated immune regulation.

The induction of peripheral T cell tolerance to specific antigens is a well-known phenomenon (for reviews, see references 1–3). Tolerance in an individual may be due to several mechanisms, including deletion or anergy of antigen-specific lymphocytes and suppression by other cells or factors, all of which have been implicated in tolerance to self and foreign antigens. In vitro studies have shown that cloned lines of CD4$^+$ T lymphocytes can be rendered anergic by antigen stimulation in the absence of costimulatory signals (4). This form of anergy is associated with a block in IL-2 gene transcription, and, as a result, a reduction in proliferative responses. Anergy is readily induced in IL-2- and IFN-γ-secreting (Th1-type) helper clones, but not in IL-4-, IL-5-, and IL-10-secreting (Th2-type) clones (5, 6).

Recently, inhibition of specific and nonspecific immune responses has been recognized as a regulatory action of many cytokines. For instance, IL-4 and IFN-γ reciprocally antagonize the actions of each other on B cells (7), and each inhibits the differentiation of naive T cells into secretors of the other cytokine (8–10). TGF-β also antagonizes the development of IL-4-secreting T cells (11). IL-10 suppresses the activation of T cells, notably Th1 cells, by inhibiting the function of macrophages as APC (12, 13). Thus, in many experimental systems, cytokines serve as potent downmodulators of lymphocyte differentiation and activation, with IL-4 exerting an inhibitory effect on Th1-like responses, and IFN-γ doing the same

to Th2-like responses. Selective expression of cytokines is also known to be important in the hyporesponsive state associated with many chronic microbial diseases, particularly leprosy (14) and various parasitic infections (15). In these diseases, IL-4 and IL-10 expression are associated with suppressed cell-mediated immunity, whereas IFN-γ expression is found in more reactive lesions.

These observations raise the possibility that cytokine regulation plays a role in antigen-specific tolerance in vivo. Such a possibility has not been formally explored to date. Several regimens for inducing tolerance to foreign antigens in vivo have been described, including the administration of large quantities of aqueous antigens (high-dose tolerance), providing antigens in ways that selectively target costimulator-deficient APC, and antigen feeding (oral tolerance). Previously, we and others have shown that in a model of high-dose tolerance, parenteral administration of soluble protein antigens to adult mice induces unresponsiveness in Th1- but not Th2-type lymphocytes (16, 17). In this model, immunization and in vitro restimulation show that antigen-specific T cell proliferation and production of IL-2 and IFN-γ are inhibited, but secretion of IL-4 is unaffected. The antigen-specific antibody repertoire shows changes consistent with a selective inhibition of Th1 cells. Serum levels of antigen-specific IgM, IgG1, and IgE, isotypes unaffected or sustained by IL-4, are normal or even increased, while specific IgG2a, IgG2b, and IgG3,

isotypes that are enhanced by IFN-γ and suppressed by IL-4 (7, 18, 19), are greatly reduced. Direct tolerization of antigen-specific B cells does not occur in this system.

We have now explored the contribution of IL-4 to the establishment of high-dose tolerance, and its effect on lymphokine-producing T cells in tolerized animals. An IL-4 antagonist augments antibody responses in tolerant animals and increases IFN-γ production by T cells, but does not prevent the block in the IL-2-dependent proliferation of antigen-specific Th1-like cells. In addition, neutralization of IL-4 during tolerance induction reveals that IL-4 serves as a growth and differentiation factor for Th2 cells in vivo, and accounts for their expansion in tolerized animals. Thus, high-dose tolerance in vivo may be due to a combination of clonal anergy of Th1 cells and inhibition mediated by Th2-derived cytokines.

Materials and Methods

Antigens, Cytokines, and Antibodies. TNP-conjugates of OVA, BSA, and rabbit gamma globulin (Sigma Chemical Co., St. Louis, MO) were prepared as described previously (19). Purified protein derivative (PPD)[1] of *Mycobacterium tuberculosis* was kindly donated by Lederle Biologicals (Pearl River, NY). Murine IL-4 was generously provided by Dr. Steve Gillis (Immunex Corp., Seattle, WA); murine IL-2 was from Genzyme (Boston, MA). HPLC-purified 11B11, the neutralizing mAb for IL-4 (20), was kindly provided by Dr. Craig Reynolds (Biological Response Modifiers Program, National Cancer Institute, Frederick, MD). The neutralizing mAb for IL-2, S4B6 (21), was a gift from Dr. Tim Mosmann (University of Alberta, Edmonton, Canada).

Induction of High-Dose Tolerance and 11B11 Treatments. Female CAF₁ mice (BALB/cJ × A/J)F₁ (The Jackson Laboratory, Bar Harbor, ME), aged 6–8 wk, were used for all experiments. Animals were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and those prepared by the Committee of Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. To induce tolerance, animals were given the indicated quantity of TNP-OVA in PBS intraperitoneally, or the equivalent volume of PBS, on days 1, 3, and 5 of the experiment. On day 8, animals were immunized in the footpad with ~100 μg TNP-OVA in CFA (IFA and fixed *M. tuberculosis*; Difco Laboratories, Detroit, MI). On day 17, mice were bled for serum and killed, and draining lymph nodes were collected. In experiments analyzing splenic responses, spleens were harvested on experiment day 8 before immunization of the mice.

For anti-IL-4 mAb treatments, animals were given 2 mg of HPLC-purified 11B11 in PBS on days 0 and 4 of the experiment, and 1 mg on day 8. Control animals were given an equivalent volume of PBS. This dose of 11B11 has been shown to block 99% of the IL-4 dependent, nematode-induced primary IgE response in mice (22).

Cell Preparation, Cultures, and Lymphokine Bioassays. Lymphocyte suspensions from draining lymph nodes or spleen were prepared by grinding tissue through sterile wire mesh; for lymph node cells, lymphocytes from two mice in each treatment group were pooled. CD4+ cells were depleted from splenocytes using two rounds of anti-CD4 antibodies, GK1.5 (23) and RL172 (24), and guinea pig complement (Cedarlane Laboratories, Hamilton, On-

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[1] *Abbreviation used in this paper:* PPD, purified protein derivative.

tario, Canada). This procedure effectively depleted >98% of CD4+ cells. For proliferation assays, 4 × 10⁵ cells were cultured in 0.2 ml RMPI 1640 supplemented with 2 mM L-glutamine, penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 10 mM Hepes (all from Gibco Laboratories, Grand Island, NY), 5 × 10⁻⁵ M 2-ME, and 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT), at 37°C in 96-well flat-bottomed microculture plates in 5% CO₂. OVA or PPD was added as indicated in the figures; proliferation was measured on day 3 of culture by [³H]TdR incorporation. For lymphokine production assays, 6 × 10⁵ lymph node cells or 8 × 10⁵ spleen cells were cultured in medium alone or medium with antigen as indicated, and supernatants were collected at 48 or 72 h. IL-2 levels were measured by using the IL-2/IL-4-sensitive indicator line HT-2 in the presence of the blocking antibody 11B11 (10 μg/ml) as described (16). IL-4 levels were determined using the IL-4-dependent indicator line CT.4S (25; kindly provided by Drs. R. Seder and W. E. Paul, National Institutes of Health) in the presence of the anti-IL-2 antibody S4B6 (1:1,000 dilution of ascites) to assure specificity. In this assay, half-maximal stimulation of CT.4S cells is seen with 10–20 U/ml of rIL-4, the units having been originally defined by the suppliers on the basis of B cell costimulation assays. IFN-γ activity was measured by inhibition of proliferation of the B lymphoma line WEHI 279 as described (16). Concentrations in units/milliliter were determined using purified recombinant cytokines as standards, and were based on concentrations provided by the suppliers. Standard deviations were all <10% of sample values.

Assays for Anti-TNP Antibodies. Serial dilutions of sera were analyzed for TNP-specific antibodies by ELISA. Titers of anti-TNP IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes were determined using plates coated with TNP-BSA, and isotype-specific alkaline phosphatase–conjugated developing reagents as described previously (19). TNP-specific IgE was measured using a pan-IgE capture reagent, followed by a sandwich ELISA of serum, TNP-rabbit gamma globulin, and anti–rabbit Ig reagents as described previously (16).

Results and Discussion

Tolerogenic Antigen Stimulates Lymphokine-producing T Cells. If IL-4 plays a role in the induction or maintenance of high-dose tolerance, then tolerogenic antigens should themselves stimulate IL-4 production. To test this, aqueous TNP-OVA was injected into mice intraperitoneally at doses previously shown to induce tolerance (16). Splenocytes were isolated and examined for lymphokine production upon restimulation with OVA in culture. As shown in Table 1, at high cell numbers splenocytes from TNP-OVA-pretreated mice secreted low but detectable, antigen-inducible levels of IL-4 and IL-2 upon restimulation with OVA. Proliferative responses of these cells were minimal (not shown), and mice given aqueous TNP-OVA did not contain detectable anti-TNP IgG1 or IgG2a antibodies in their sera (16).

It has recently been demonstrated that an important source of IL-4 is a non-B, non-T population of cells that belong to the mast cell/basophil lineage and release lymphokine in response to Fc receptor–mediated signaling (26, 27). It was possible that aqueous antigens induced Igs capable of sensitizing Fc receptors on these cells, facilitating IL-4 release on subsequent exposure to antigen. Two approaches were used to establish the cellular source of IL-4. First, depletion of CD4+ cells from splenocytes was found to completely abolish antigen-

induced secretion of both IL-2 and IL-4 (Table 1). Second, TNP-BSA was shown to be incapable of stimulating IL-4 secretion by splenocytes from TNP-OVA-pretreated mice (Table 1). These results indicate that crosslinking bound anti-TNP Ig on inflammatory cells is not the mechanism of IL-4 release, and that lymphokine production is carrier specific, as expected of a T cell response. Thus, even tolerogenic aqueous protein antigens at high doses are capable of "priming" splenic IL-2- and IL-4-producing CD4+ T cells.

Anti-IL-4 Antibody Enhances Specific Antibody Production in Tolerized Mice but Does Not Prevent the Block in T Cell Proliferation. The above results raised the possibility that high-dose tolerance may, at least in part, be due to the effects of IL-4, which is stimulated by tolerogenic antigen. Were this the case, specifically antagonizing the effects of IL-4 ought to reduce or prevent tolerance. To test this, mice were treated with aqueous TNP-OVA at tolerogenic doses on days 1, 3, and 5 of the experiment, given 2 mg of the anti-IL-4 mAb 11B11 on days 0 and 4 and 1 mg on day 8, and were immunized with antigen in adjuvant on day 8. Serum antibody levels

and T cell responses to in vitro restimulation were determined 9 d later. Titers of serum anti-TNP antibody of different isotypes are shown in Fig. 1. As previously reported (16), pretreatment with tolerogenic antigen markedly suppressed the production of IgG2a, IgG2b, and IgG3, all of which are inhibitable by IL-4 (18, 19), but did not inhibit IgG1 and IgE, which are enhanced by or dependent on IL-4 (Fig. 1, *left*). When tolerance was induced in a parallel set of mice treated with 11B11, the production of IgG2a, IgG2b, and IgG3 was greatly increased (Fig. 1, *right*). Mice given the lower tolerogenic doses and 11B11 had titers two- to fivefold higher for these isotypes than even control mice that were immunized without prior administration of tolerogen. At the highest dose of tolerogen, the 11B11-treated mice showed reduced production of these isotypes compared with mice not pretreated with tolerogenic antigen. This is probably due to the induction of anergy in T cells, which results in reduced expansion of helper T cells in vivo. Anti-IL-4 antibody treatment abolished specific IgE production, demonstrating that IL-4 was effectively neutralized by 11B11 in vivo. 11B11 treat-

Table 1. *Splenocytes from Tolerogen-treated Mice Secrete IL-2 and IL-4*

				Lymphokine responses to restimulation with:			
Tolerogen	Dose	Cells	Medium	OVA (300 μg/ml)	OVA (1,000 μg/ml)	OVA (2,000 μg/ml)	TNP-BSA (1,000 μg/ml)
	μg				*U/ml*		
IL-2							
PBS		SPL	1.10	0.48	1.83	1.51	NT*
		CD4 dep.	<0.2	<0.2	<0.2	<0.2	NT
TNP-OVA	50	SPL	0.40	2.44	3.52	6.16	NT
		CD4 dep.	<0.2	<0.2	<0.2	<0.2	NT
TNP-OVA	200	SPL	2.11	3.04	3.79	4.07	NT
		CD4 dep.	<0.2	<0.2	<0.2	<0.2	NT
TNP-OVA	1,000	SPL	1.25	0.86	3.06	4.30	NT
		CD4 dep.	<0.2	<0.2	<0.2	<0.2	NT
IL-4							
PBS		SPL	0.14	0.14	0.20	0.17	<0.1
		CD4 dep.	<0.1	<0.1	<0.1	<0.1	<0.1
TNP-OVA	SPL	50	<0.1	0.22	0.73	0.57	<0.1
		CD4 dep.	<0.1	<0.1	<0.1	<0.1	<0.1
TNP-OVA	200	SPL	0.14	0.25	0.56	0.96	0.12
		CD4 dep.	<0.1	<0.1	<0.1	<0.1	<0.1
TNP-OVA	1,000	SPL	<0.1	0.20	0.40	0.58	0.12
		CD4 dep.	<0.1	<0.1	<0.1	<0.1	<0.1

Mice were pretreated with PBS or aqueous TNP-OVA at the indicated doses on days 1, 3, and 5. Splenocytes were collected on day 8, and cultured at 8 × 10⁵ per well in medium alone, or with antigen at the indicated concentrations (μg/ml). CD4+ T cells were depleted from splenocytes by treatment with anti-CD4 antibodies and complement, and also cultured at 8 × 10⁵ per well (CD4 dep.). Lymphokine levels were determined from supernatants taken from cultures on day 2 (IL-2) or day 3 (IL-4). Data are means of concentrations of lymphokines from two mice per group. SE were <10% of sample values in all cases. Results are from one representative experiment out of three.
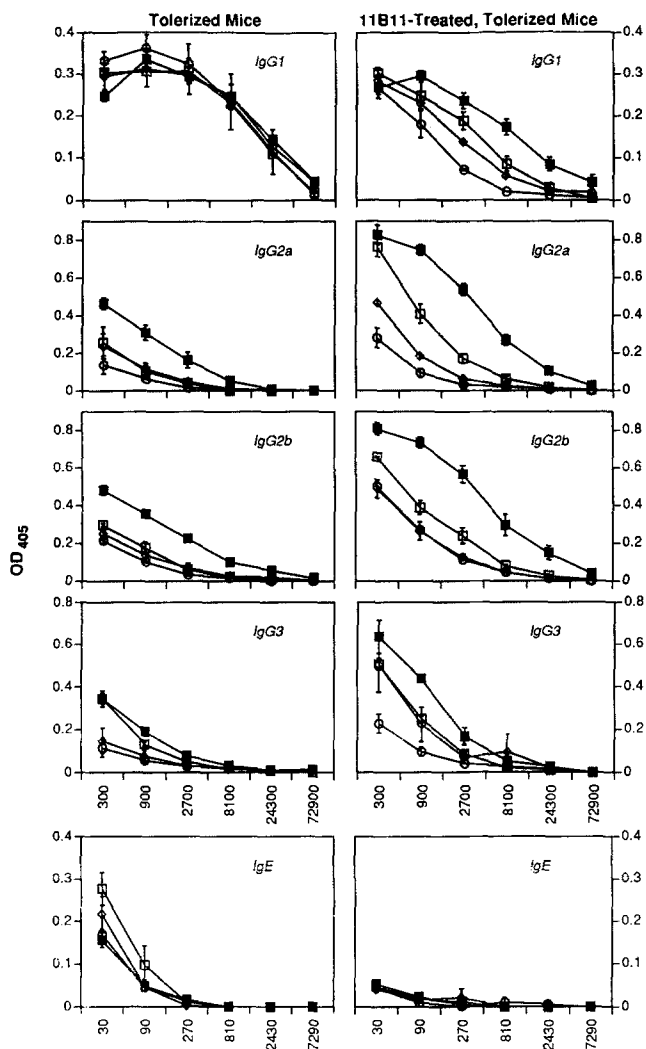* NT, not tested.

Figure 1. Effects of 11B11 treatment during tolerance induction on antigen-specific antibody isotypes. Mice were treated with PBS (■) or rendered tolerant with aqueous TNP-OVA (□, three 50-μg doses; ◇, three 200-μg doses; ○, three 1,000-μg doses) before subcutaneous immunization with TNP-OVA in CFA. In each group, half the animals were given 11B11 during tolerance induction (*right*) and the other half PBS (*left*). Sera from immunized mice were analyzed for TNP-specific Ig by ELISA. The ordinate axes are fold dilutions of serum; antibody titers are means from two mice, shown as OD_{405}. Error bars are SD.
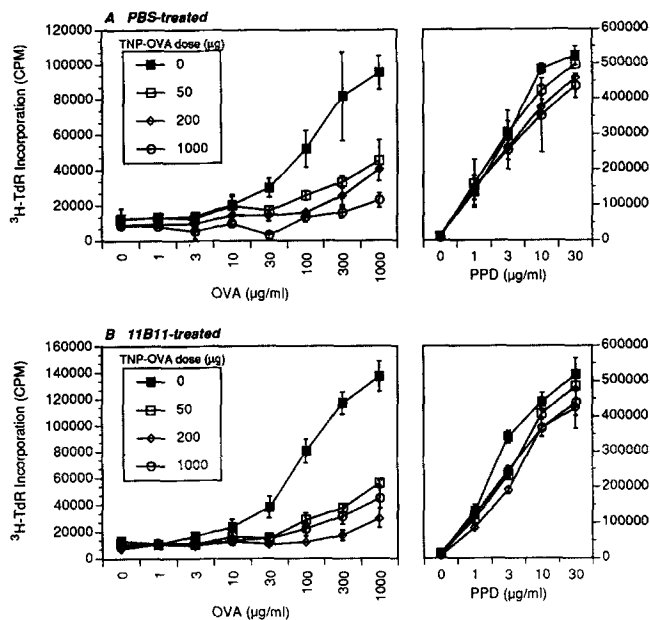

Figure 2. 11B11 treatment does not prevent the block of T cell proliferation induced by aqueous antigen. Mice were pretreated with PBS or aqueous TNP-OVA at the indicated doses, and immunized with TNP-OVA in CFA. Mice in *B* were also treated with 11B11, while those in *A* received an equivalent volume of PBS. Immune lymph node cells pooled from two mice per group were cultured at 4×10^5 per well with either OVA or PPD as indicated. Data are proliferation on day 3 expressed as cpm; error bars denote SD.

ment also had a modest effect on IgG1 production in the tolerized mice, consistent with the demonstration that IL-4 potentiates but is not required for switching to IgG1 (28). In summary, the levels of antibodies that are reduced in tolerized mice were increased by neutralization of IL-4.

The induction of tolerance is also characterized by reduced T cell proliferation (anergy) after immunization and in vitro restimulation with antigen. In such experimental systems, T cell proliferation induced by restimulation with antigen in vitro is directly proportional to the priming and clonal expansion of antigen-specific T cells as a result of immunization in vivo. As shown in Fig. 2 *A*, in mice pretreated with tolerogenic doses of aqueous TNP-OVA and immunized with

TNP-OVA in CFA, the subsequent proliferation of T cells in response to restimulation with OVA was inhibited. Proliferative responses to PPD, a component of the adjuvant, were not affected, demonstrating the antigenic specificity of the T cell unresponsiveness. Treating mice with 11B11 at the time of the induction of unresponsiveness to soluble antigen did not prevent the subsequent block in T cell proliferation (Fig. 2 *B*). Thus, the unresponsiveness of T cells in this model is likely due to deletion or functional inactivation (anergy) of antigen-specific T cells in vivo, and is not caused by IL-4-mediated suppression.

Anti-IL-4 Antibody Alters the Profile of Lymphokine Production by T Cells in Tolerant Animals. The observation that anti-IL-4 antibody significantly reverses the suppression of some antibody isotypes in tolerant animals may be explained by several potential actions of IL-4. This cytokine may be responsible for the expansion of Th2 cells, the inhibition of IFN-γ production by viable Th1 cells, and the negation of the effects of IFN-γ on isotype switching by B cells. We therefore examined the profile of cytokines produced by T cells from animals that were pretreated with aqueous antigen with or without 11B11 and then immunized. In accordance with previous results (16), T cells from tolerant mice (without 11B11 treatment) showed reduced IL-2 and IFN-γ secretion upon restimulation in vitro, but no suppression, and even a modest increase, in IL-4 production. In contrast, T cells from mice given tolerogen and 11B11 produced markedly less IL-4 and more IFN-γ than those from animals treated with tolerogen

Table 2. *Effect of Anti-IL-4 on Lymphokine Production by T Cells from Tolerant Animals*

Tolerogen	Dose	Medium	Lymphokine responses to restimulation with:		
			OVA (300 μg/ml)	OVA (1,000 μg/ml)	PPD (30 μg/ml)
	μg			*U/ml*	
IL-2 without 11B11 treatment					
PBS		<0.2	3.33	5.60	3.93
TNP-OVA	50	<0.2	0.66	1.39	2.44
TNP-OVA	200	<0.2	0.76	0.84	4.03
TNP-OVA	1,000	<0.2	0.36	0.97	9.32
IL-2 with 11B11 treatment					
PBS		<0.2	3.76	7.76	2.99
TNP-OVA	50	<0.2	0.88	1.46	8.76
TNP-OVA	200	<0.2	0.27	0.99	7.32
TNP-OVA	1,000	<0.2	<0.2	0.76	6.12
IL-4 without 11B11 treatment					
PBS		<0.1 (199)	<0.1 (723)	0.21 (2,491)	NT*
TNP-OVA	50	<0.1 (591)	0.26 (3,273)	0.36 (5,559)	NT
TNP-OVA	200	<0.1 (474)	0.25 (3,128)	0.48 (7,249)	NT
TNP-OVA	1,000	<0.1 (696)	<0.1 (895)	0.18 (1,987)	NT
IL-4 with 11B11 treatment					
PBS		<0.1 (863)	0.23 (2,686)	0.20 (2,240)	NT
TNP-OVA	50	<0.1 (854)	**<0.1 (948)**	**0.11 (1,577)**	NT
TNP-OVA	200	<0.1 (26)	**<0.1 (132)**	**<0.1 (577)**	NT
TNP-OVA	1,000	<0.1 (523)	<0.1 (723)	**<0.1 (686)**	NT
IFN-γ without 11B11 treatment					
PBS		0.58	8.98	27.64	33.33
TNP-OVA	50	0.53	4.74	12.32	20.23
TNP-OVA	200	0.59	3.25	6.47	20.56
TNP-OVA	1,000	0.52	0.94	0.91	28.05
IFN-γ with 11B11 treatment					
PBS		0.32	9.32	30.49	33.57
TNP-OVA	50	0.50	**11.86**	**19.86**	23.32
TNP-OVA	1,000	0.30	**6.59**	**12.98**	31.05

Mice were tolerized with TNP-OVA at the indicated doses and half were treated with 11B11, before subcutaneous immunization with TNP-OVA in CFA. Immune lymph node cells (6 × 10⁵) were cultured with medium, OVA, or PPD at the concentrations indicated. Supernatants from day 2 cultures were analyzed for IL-2; supernatants taken on day 3 were analyzed for IL-4 and IFN-γ. Data are means of lymphokine concentrations. SE were <10% of sample values in all cases. For IL-4, the actual values of CT4S proliferation minus the background (Δcpm) induced by culture supernatants at 50% (vol/vol) are also shown. In this experiment, CT4S proliferation in medium (background) was 2,120 ± 247 cpm; with 1 U/ml IL-4, 12,328 ± 915 cpm; 12.5 U/ml IL-4, 41,015 ± 2,996 cpm; and 25 U/ml IL-4, 79,243 ± 1,824 cpm. Groups showing significant effects of 11B11 are indicated in bold. Results are from one representative experiment out of three.
* NT, not tested.

alone, but showed no change in IL-2 secretion (Table 2). In three experiments, administration of tolerogenic antigen (200–1,000 μg) reduced subsequent in vitro IL-2 production by immune T cells by an average of 86.5%, and reduced IFN-γ production by 89.4%, compared with T cells from immunized (and not tolerized) mice. In mice given tolerogen and 11B11, IL-2 production remained reduced by 91%, whereas IFN-γ levels were restored to 43% of controls. In all three experi-

ments, IL-4 secretion by immune T cells was almost completely suppressed by prior administration of 11B11. Thus, neutralizing the effects of IL-4 at the time of induction of tolerance in Th1 cells has two consequences on lymphokine-producing T cells: it prevents the concomitant expansion of IL-4-secreting Th2 cells, and it enhances IFN-γ production. Anti-IL-4 does not prevent the inhibition of IL-2 secretion by T cells from tolerized mice, suggesting that the block in IL-2 is a direct effect of the tolerogen, presumably from a form of T cell anergy. Surviving, anergized Th1-like cells are nonetheless secreting IFN-γ, reminiscent of findings from in vitro experiments where T cell anergy causes near complete inhibition of IL-2 secretion and only partial inhibition of IFN-γ secretion (4).

Based on the results described above, the following sequence of events in tolerance induced by high doses of aqueous antigens can be envisioned. Administration of aqueous antigen induces clonal anergy in tolerance-sensitive, IL-2-producing cells of the Th1 subset, a direct consequence of antigen recognition. Tolerance in these cells may be due to antigen presentation by APC such as resting B cells or macrophages, which lack necessary costimulators (29). Anergy of Th1 cells is manifested by a block in clonal expansion in vivo, which is assayed by reduced IL-2 production and proliferation upon restimulation with antigen. At the same time, aqueous antigen stimulates IL-4 production by CD4⁺ T cells, enhancing the differentiation of Th2-like cells that are resistant to tolerization. Subsequent immunization with antigen and adjuvant induces further expansion of Th2-like cells, which serve as a source of continuing IL-4 production. This IL-4 may mediate several of the immunologic changes seen in tolerant mice. First, IL-4 drives the production of IgG1 and IgE, and inhibits the antigen-induced switching to and secretion of IgG2a, IgG2b, and IgG3 isotypes. Second, IL-4 inhibits IFN-γ production by any residual anergic Th1 cells that have retained the ability to secrete IFN-γ. IL-4 has been shown to inhibit IFN-γ production by T cells in vitro (30, 31). Finally, the Th2 cells that are expanded by IL-4 may secrete other cytokines, such as IL-10, that further suppress Th1-dependent responses (12, 13).

These findings indicate that there are multiple mechanisms of tolerance operating at the organismal and the cellular level. The phenomenon of tolerance in the intact animal, as measured by antibody responses, is attributable in part to the suppressive actions of IL-4, whereas the tolerogen-induced inhibition of Th1 cells is a form of cellular unresponsiveness or anergy that is largely independent of exogenous cytokines. The roles of cytokines in other models of tolerance remain to be explored. Identifying the mechanisms of tolerance has important implications for the design of therapeutic strategies to inhibit pathologic immune responses. High-dose tolerance may be especially effective for disorders caused by Th1-mediated delayed-type hypersensitivity reactions. Additionally, in high-dose tolerance, selective activation of Th2-type T cells may account for skewing immune reactions towards hyporesponsive states, as has been found in chronic microbial infections with high antigenic loads. Antagonizing the cytokine activities augmented in such diseases may be one way of ameliorating these conditions.

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