

LYMPHOKINE CONTROL OF IN VIVO IMMUNOGLOBULIN ISOTYPE SELECTION¹

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INTRODUCTION

The discovery by Isakson and Vitetta that a T lymphocyte-produced substance could induce lipopolysaccharide (LPS)-activated murine B lymphocytes to secrete IgG1 (1) demonstrated that lymphokines could play a role in selecting the isotypes of antibody that are produced in an immune response. This observation became better defined when Vitetta, in collaboration with Ohara and Paul, traced this IgG1-inducing activity to a purified, recombinant lymphokine, now called interleukin-4 (IL-4) (2). Studies by Coffman and collaborators demonstrated that IL-4 could induce LPS-activated B cells to secrete IgE as well as IgG1, and that the lymphokine interferon- γ (IFN- γ) could inhibit this activity (3, 4). These observations were confirmed and extended by Snapper et al, who demonstrated that (a) larger doses of IL-4 were required to induce LPS-activated B cells to secrete IgE than to secrete IgG1 (5), (b) IFN- γ , in addition to inhibiting IgG1 and IgE production by B cells stimulated with LPS plus IL-4, could enhance LPS-induced IgG2a secretion, and (c) IL-4 could inhibit IgG2a secretion (6). Thus, differential secretion of IL-4 or IFN- γ could regulate the relative quantities of IgG1, IgE, and IgG2a that were made following T cell-independent induction of a humoral immune response. IL-4 is also required for the stimulation of IgE, but not necessarily IgG1, production in a T cell-dependent *in vitro* system in which B lymphocytes are cultured with antigen-specific T cells in the presence of the appropriate antigen (7, 8). Since single resting B cells can be induced in this system to generate, with high frequency, clones that include IgG1 and IgE-secreting cells, it seems likely that membrane IgM⁺ B cells are being induced to switch to the production of IgG1 and IgE. The results of this experiment do not differentiate, however, whether IL-4 promotes the actual Ig isotype switching process or selectively stimulates the growth of B cells that have switched to the secretion of IgG1 or IgE by an IL-4-independent process. The hypothesis that IL-4 acts as an isotype switch factor, which may promote translocation of the Ig heavy-chain VDJ gene segment to a site close to the γ 1 or ϵ C_H gene, is favored by observations that IL-4 can induce production of a germ-line γ 1 mRNA transcript, and, in the presence of LPS, a germ-line ϵ -chain mRNA transcript (9–12). The observation that exposure of resting, membrane IgG[−] B cells to IL-4 will allow these cells to make an IgG1 response if they are subsequently stimulated with LPS in the absence of IL-4 is also more compatible with the hypothesis that IL-4 promotes a C _{μ} to C _{γ 1} switch, rather than a selective proliferation of B cells that have undergone such a switch through an IL-4-independent process (13).

As with IL-4, IFN- γ has been shown to affect Ig isotype selection in T

cell-dependent as well as T cell-independent systems, enhancing IgG2a production and inhibiting IgG1 and IgE production in both (3, 6, 14). However, while IFN- γ can induce resting, membrane IgG⁻ B cells, in the presence of additional stimuli, to secrete IgG2a, this cytokine is not an absolute requirement for the induction of an IgG2a response (14). It is not yet clear whether IFN- γ can promote isotype switching to IgG2a at the DNA level, or whether this cytokine enhances IgG2a responses only by selectively promoting the growth or differentiation to Ig secretion of B cells that are already committed to the expression of IgG2a.

Two cytokines, IL-5 and transforming growth factor- β (TGF- β), have been implicated in the generation of in vitro murine IgA responses. Previously shown to promote the proliferation of already activated B lymphocytes and the generation of both IgM and IgG antibody responses (15–18), IL-5 was found to enhance IgA production by LPS-activated B lymphocytes to a greater extent than it enhanced the production of other isotypes (19, 20). Although IL-5 may cause a small increase in the switching of B cells to the expression of IgA, other studies have shown that this cytokine promotes IgA secretion predominantly by further activating cells that already express surface IgA (21, 22). Thus, while IL-5 may selectively promote the growth and differentiation of B cells already committed to the secretion of IgA, it does not appear to play a major role in inducing such commitment. More recent studies implicate TGF- β as an IgA switch factor, since this cytokine, in the presence of LPS, can induce surface IgA⁻ B cells to synthesize mRNA that encodes α chain, to express IgA on their plasma membranes, and to secrete this isotype (22a). The possibility that TGF- β functions physiologically as an important IgA switch factor may appear difficult to reconcile with the observation that IgA-secreting cells are found predominantly in lymphoid organs associated with the gut and the respiratory tract, while TGF- β is produced throughout the body. It has been hypothesized that TGF- β activity may reach high levels in the gut because this cytokine is secreted in an inactive form that requires proteolytic cleavage to become activated. The presence of large quantities of proteolytic enzymes of bacterial origin in the gut could promote TGF- β activation, which could, in turn, promote IgA production. This hypothesis remains to be tested by in vivo studies.

In vitro studies with human lymphoid cells have provided results that have several similarities to those observed with mouse cells. Culture of human peripheral blood mononuclear cells (PBMC) with IL-4 stimulates the development of an IgE response that is inhibitable by either IFN- γ or IFN- α (23, 24). The induction of an IgE response in this system is dependent upon the presence of both T cells and monocytes (23, 24). It requires, in addition to IL-4, the production of IL-6 as well as the estab-

lishment of direct, MHC class II-dependent, B cell-T cell interactions (25, 26). Culture of purified human B lymphocytes with helper-T-cell lines can also stimulate IgE production, which is directly proportional to the quantity of IL-4 produced by the T cells and inversely proportional to their production of IFN- γ (27). Determination of cytokine effects on Ig isotype selection has been difficult to accomplish with human B cells in a T-cell-independent system, since no known mitogen or polyclonal activator has a stimulatory effect on human B cells similar to that of LPS in the mouse. This omission has made it difficult to establish whether the stimulatory effect of IL-4 on IgE secretion is a direct one, since IL-4, which has T-cell as well as B-cell stimulatory activity, might induce T cells to produce a second mediator that could activate B cells to secrete IgE. A recent observation, that IL-4 stimulates highly purified Epstein-Barr virus (EBV)-infected human B cells to secrete IgE, however, provides evidence for a direct B-cell stimulatory role for IL-4 in the induction of a human IgE response (28). This stimulatory role does not necessarily involve a switch event at the DNA level that deletes Ig C_H genes located between VDJ and C ϵ . MacKenzie & Dosch have reported that EBV-infected human B cells, cultured at low density in the presence of activated, irradiated T cells, will secrete IgE without deleting upstream C_H genes (29). It remains to be ascertained whether the induction of IgE secretion in the absence of deletion of upstream C_H genes is a general characteristic of the IgE response, a selective characteristic of EBV-stimulated B cells, or a characteristic only of the system used by MacKenzie & Dosch.

The sole in vitro evidence that IL-4 may not always be required for the induction of an human B cells, cultured with interleukin-2 (IL-2) plus fixed, protein A-bearing *Staphylococcus aureus*, secrete IgE as well as IgG (30). Addition of exogenous IL-4 to these cultures actually suppresses both the IgG and IgE responses. It has been postulated that the IgE response in this system is derived from memory B cells that have already switched to IgE at the DNA level. However, the possibility that endogenous production of IL-4 occurs in this culture system and is responsible for the IgE response has not been eliminated.

While many similarities exist in cytokine control of IgE secretion in murine and human systems, no clear parallel has been found in the human to IL-4 and IFN- γ control of IgG1 and IgG2a production in the mouse. Although IL-4 has been reported to enhance IgG4 production selectively in one human in vitro system (31), and this IgG subclass resembles murine IgG1 in its inability to fix complement, human IgG4, unlike murine IgG1, makes only a very small contribution to T-dependent immune responses to soluble proteins. No evidence of IFN- γ control of human IgG subclass

selection, or of IL-5 or TGF- β control of human IgA production, has yet been published.

While the roles of IL-4 and IFN- γ in IgG subclass selection may differ in mouse and human, the interspecies similarity in regulation of IgE secretion by these two cytokines indicated that their relative production would play a major role in determining the extent to which an immune response included IgE production. Studies by Mosmann and by Bottomly provide a mechanism for such differential secretion of these cytokines. Murine CD4⁺ T lymphocyte clones were found to secrete either IFN- γ (and IL-2), or IL-4 (and IL-5), but not both IFN- γ and IL-4 (32–35). CD4⁺ T-cell clones that secreted IFN- γ and IL-2 were termed Th1 cells; those that secreted IL-4 and IL-5, Th2 cells (32). Thus, in the mouse, an immune stimulus that predominantly activated precursors of the Th2 cells, or induced a multipotent precursor to differentiate predominantly in the Th2 direction, might stimulate an antibody response that was characterized by production of mostly IgG1 and IgE, while a stimulus that resulted in the generation of activated Th1 cells might generate a predominantly IgG2a response. Since IgG1, IgG2a, and IgE differ in their abilities to fix complement, to bind to IgGFC receptors, and to participate in the induction of mast cell degranulation, this differential lymphokine secretion and the resultant isotype selection could provide a means for the host to respond to challenge with a variety of different microbes and toxins in a way that might most effectively eliminate the pathogen yet minimize damage to itself. More recent studies, however, suggest that CD4⁺ T-cell clones do not all clearly fit into the Th1 or Th2 categories. These studies demonstrate that a considerable percentage of mouse CD4⁺ T cells, and the majority of human CD4⁺ T cells, produce both IL-4 and IFN- γ (27, 35–41). Some of these CD4⁺ T cells, which combine characteristics of Th1 and Th2 cells and have been termed Th0 cells (35, 41), may represent precursors of the Th1 or Th2 CD4⁺ subpopulations. Although it is unclear whether CD4⁺ T cells with pure Th1 or Th2 secretory characteristics exist *in vivo*, it is clear that T-cell responses to different stimuli (or in different mouse strains, to the same stimulus) can have a predominantly Th1 or Th2 character (see below). This has proven to be particularly important in murine leishmaniasis, in which a host response that has Th1 characteristics is associated with a self-limited infection, while a response that has Th2 characteristics is associated with an overwhelming, generalized infection that eventually kills the host (42–44).

Elegant and exciting as these observations and concepts were, they did not prove that lymphokines had the same effects on the generation of an *in vivo* antibody response as had been observed *in vitro*. *In vitro* experiments had been purposefully set up to demonstrate an effect. Cell numbers

and types and lymphokine and LPS concentrations had been chosen arbitrarily. Possibly, a lymphokine that had an effect in vitro might not be present in the necessary quantity, at the required place, at the proper time to have the same effect in an in vivo response. In addition, in vitro experiments may have omitted stimuli that are important in an in vivo response. Such stimuli might, in vivo, supersede the in vitro-demonstrated effects of IL-4 or IFN- γ in the regulation of Ig isotype selection. Finally, inhibitors of some IL-4 or IFN- γ effects might be present in vivo, so that effects of these lymphokines demonstrable in vivo might be blocked in intact animals.

USE OF IN VIVO SYSTEMS TO STUDY LYMPHOKINE EFFECTS ON Ig ISOTYPE SELECTION

Three in vivo systems that induce polyclonal Ig secretion were used in the initial investigations of whether IL-4 and IFN- γ have the same effects on Ig isotype selection in vivo as in vitro. First, a system was studied in which mice are injected intravenously with 800 μ g of an affinity purified goat antibody to mouse IgD (GaM δ). GaM δ injection directly activates B lymphocytes by cross-linking their IgD (45). The activated B lymphocytes process the goat IgG that has bound to their surface and present it to T lymphocytes, which in turn become activated and secrete lymphokines, including IL-4 (45-48). The T-cell help that is generated induces B cells to proliferate and differentiate into Ig-secreting cells (49). This proliferation and differentiation is on a massive level; by 8 days after GaM δ injection an approximately five-fold increase has occurred in spleen cell number, and as many as one third of spleen cells secrete IgG1, with the result that serum IgG1 levels can increase over 100-fold during a three-day period (49, 50). Serum IgE levels increase by a similar factor; however, IgE levels are usually only 0.1% of IgG1 levels in these mice (51). A smaller, two- to five-fold increase in serum IgG2a levels is also usually observed (51).

In a second system, mice have been inoculated with third stage larvae of the parasitic nematode *Nippostrongylus brasiliensis* (Nb). This induces an IgE response similar in magnitude to that observed in the GaM δ -injected mice, but a relatively small IgG1 response that can be observed only in mice that initially have low serum IgG1 levels (52-54). Serum IgG2a levels actually decrease in these mice (54). The difference in IgG1:IgE ratios between the GaM δ and the Nb systems suggested already that secretion of these two Ig isotypes is regulated differently.

A third system, in which mice are injected intravenously with killed, fixed *Brucella abortus* (BA), induces a very different Ig isotype pattern;

considerable quantities of IgG2a are secreted while IgG1 and IgE responses are small or absent (55).

IL-4 IS REQUIRED FOR THE INDUCTION OF PRIMARY, POLYCLONAL IgE, BUT NOT IgG1 RESPONSES IN VIVO

The GaM δ and Nb systems were used first to study the requirement for IL-4 for the induction of primary, polyclonal IgG1 and IgE antibody responses (Figure 1). In these experiments BALB/c mice were injected with either GaM δ or Nb, and they simultaneously received either 20 mg of monoclonal rat IgG1 anti-mouse IL-4 antibody (produced by the hybridoma 11B11) (56), 20 mg of a control rat IgG1 antibody (anti-NP, produced by the hybridoma J4-1), or no additional antibody. Mice were bled

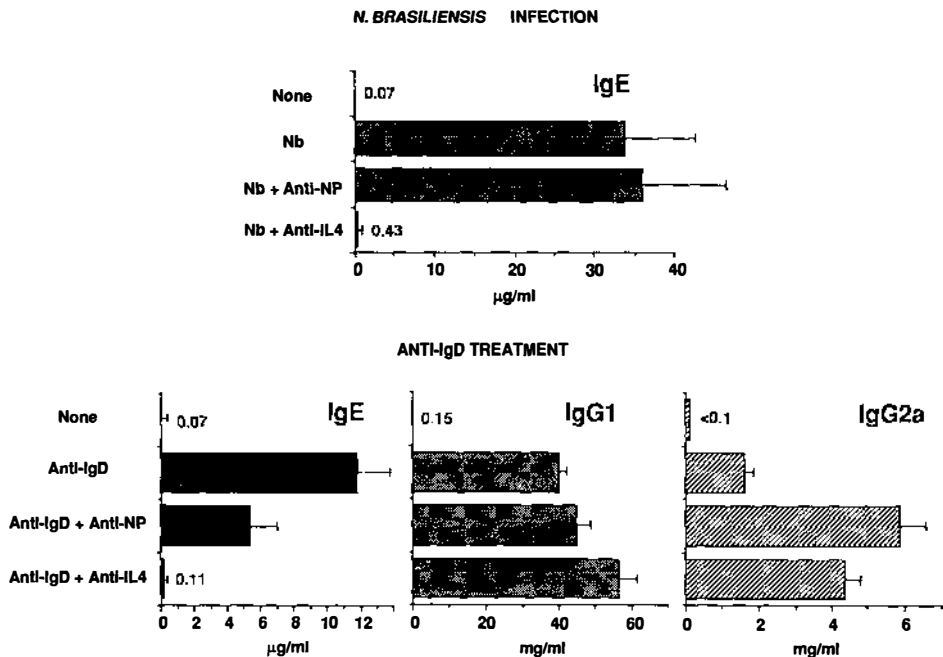


Figure 1 Anti-IL-4 antibody blocks in vivo polyclonal IgE responses. BALB/c mice (5/group) were left untreated, inoculated with Nb larvae, or injected with GaM δ antibody $\pm$ monoclonal anti-IL-4 antibody or a control antibody (anti-NP). Mice were bled 14 days after Nb inoculation or 9 days after GaM δ injection. Serum IgG1 and IgG2a levels were determined by radial immunodiffusion; IgE levels by ELISA. Geometric means and standard errors of serum Ig concentrations are shown.

at the time of the peak antibody response, and serum IgG1, IgG2a, and IgE levels were determined. It was initially hypothesized, because of the *in vitro* data, that anti-IL-4 antibody would block both the IgG1 and IgE responses. Surprisingly, while anti-IL-4 antibody inhibited IgE production in both the GaM δ and Nb systems by greater than 99%, it had no consistent, specific effect on IgG1 production or IgG2a production (57).

Since less IL-4 is required *in vitro* for the generation of an IgG1 response than for the generation of an IgE response (5), it was possible that the doses of anti-IL-4 antibody used neutralized IL-4 sufficiently to block IgE production almost totally, but not sufficiently to limit an IgG1 response. This possibility was investigated by studying whether anti-IL-4 antibody could decrease B-cell class-II MHC (Ia) expression in Nb-inoculated mice, since IL-4 induces murine B cells to express increased quantities of cell surface Ia (58), and because this effect requires considerably less IL-4 *in vitro* than does the induction of an IgG1 response (C. M. Snapper, F. D. Finkelman, unpublished data). Anti-IL-4 antibody was found to inhibit both the IgE response and the increase in B-cell Ia expression in Nb-inoculated mice, but again the antibody failed to inhibit the IgG1 response (57). This result demonstrated that the failure of anti-IL-4 antibody to inhibit IgG1 production could not result simply from incomplete neutralization of IL-4. It left open, however, the possibility that IL-4 is delivered to precursors of IgG1, but not IgE-secreting cells, in a manner that may not be susceptible to antibody neutralization, such as direct cell-to-cell contact.

INHIBITION OF A PRIMARY, GAM δ -INDUCED IGE RESPONSE BY A MONOCLONAL ANTI-IL-4 RECEPTOR (IL-4R) ANTIBODY

The production of a rat IgG2a monoclonal antibody that binds with high affinity to the mouse IL-4R and blocks IL-4 binding (M. P. Beckmann, K. A. Schooley, B. Gallis, T. V. Bos, D. Friend, A. R. Alpert, R. Raunio, S. K. Dower, K. S. Prickett, P. E. Baker, L. S. Park, "Monoclonal antibodies recognizing the murine interleukin-4 receptor. Characterization of two antibodies which immunoprecipitate and inhibit receptor function," submitted) has allowed *in vivo* studies to be performed in which even delivery of IL-4 to a B cell by direct, cell-to-cell contact can be blocked. This is because anti-IL-4R antibody can inhibit an IL-4 effect in advance of IL-4 secretion, by binding to B-cell IL-4 receptors and making them refractory to stimulation by IL-4 secreted later. Anti-IL-4 antibody, in contrast, can

have no effect until the actual time that IL-4 is secreted, and thus, it must be continuously present at concentrations sufficient to neutralize secreted IL-4 before it can bind to the IL-4R.

To study the *in vivo* effects of anti-IL-4R antibody on Ig isotype selection during an immune response, some BALB/c mice were left untreated; others were injected with GaM δ , but no additional antibody, or with GaM δ plus a range of doses of anti-IL-4R antibody, anti-IL-4R antibody, or both anti-IL-4 and anti-IL-4R antibodies. Still others were injected with GaM δ plus control rat IgG1 and/or IgG2a antibodies. Sera obtained 8 days later were analyzed for IgG1, IgG2a, and IgE concentrations (Figure 2). Anti-IL-4R antibody was approximately five-fold more potent than anti-IL-4 antibody at inhibiting GaM δ -induced IgE production by 95%, although the differences in the concentrations of these antibodies required to inhibit IgE production to a smaller extent were less marked. Neither anti-IL-4 antibody nor anti-IL-4R antibody significantly inhibited the IgG1 responses made eight days after GaM δ injection, although both antibodies somewhat inhibited the IgG1 responses made by these mice ten days after

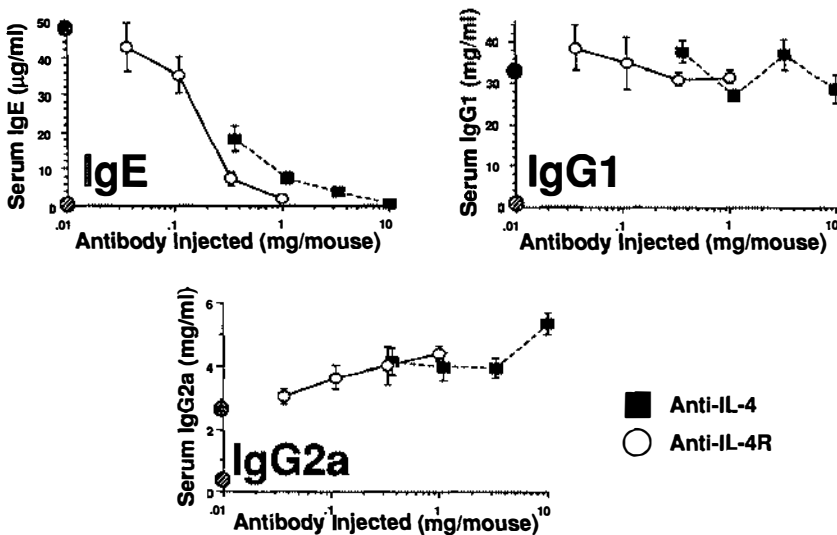


Figure 2 Effects of anti-IL-4 and anti-IL-4 receptor antibodies on the generation of polyclonal Ig responses in GaM δ antibody-injected mice. BALB/c mice (5/group) were injected with GaM δ antibody plus the doses shown or either monoclonal anti-IL-4 antibody or anti-IL-4 receptor antibody and were bled 8 days later. Serum IgE levels were determined by ELISA; IgG1 and IgG2a levels by radial immunodiffusion. Geometric means and standard errors are shown. Diagonal-filled and black circles on ordinates indicate serum Ig levels of untreated mice and mice treated only with GaM δ antibody, respectively.

injection (data not shown). At neither time, however, did the combination of anti-IL-4 and anti-IL-4R antibodies inhibit IgG1 production more completely than did either antibody alone (data not shown). This observation indicates that IgG1 production in GaM δ -stimulated mice, unlike IgG1 production in some *in vitro* systems, must be largely independent of IL-4. Both anti-IL-4 and anti-IL-4R antibodies modestly enhanced IgG2a responses in this study, but no synergistic or additive enhancement was observed when these antibodies were administered together.

The observation that an anti-IL-4R antibody is as selective and at least as effective as an anti-IL-4 antibody at blocking an *in vivo* IgE response enhances the possibility that approaches that block the effects of IL-4 on the B lymphocyte may be useful for the therapy of IgE-mediated disorders. Repeated injection of the tremendous quantities of a foreign anti-IL-4 antibody that would be required to achieve a clinically significant effect in a human would be impractical and would most likely stimulate the production of human antibodies that would neutralize the anti-IL-4 antibody. The successful and selective *in vivo* inhibition of IgE production by anti-IL-4R antibody suggests that it may be possible to use a smaller IL-4R antagonist pharmacologically with similar effects. The recent cloning of the IL-4R gene (B. Mosley, M. P. Beckmann, C. J. March, R. L. Idzerda, S. D. Gimpel, T. V. Bos, D. Friend, A. Alpert, D. Anderson, J. Jackson, J. M. Wignall, C. Smith, B. Gallis, J. E. Sims, D. Urdal, M. B. Widmer, D. Cosman, L. S. Park, "The murine interleukin-4 receptor: Molecular cloning and characterization of secreted and membrane bound forms," submitted) should facilitate the production and screening of such IL-4R antagonists.

A LATE, NON-IL-4-DEPENDENT EFFECT OF CD4⁺ T CELLS IS REQUIRED FOR THE GENERATION OF AN *IN VIVO* IgE RESPONSE

Studies have been performed in GaM δ -injected mice to determine the period of time during which T-cell help, in general, and IL-4, in particular, are required for the generation of an IgE response (60). To do this, mice were first injected with GaM δ . These mice were also injected, up to six days later, with anti-IL-4 antibody, to block IL-4-mediated effects, with anti-CD4 antibody, to block all help mediated by CD4⁺ T cells (61), or with a control antibody (anti-NP). Mice were all bled 8 days after injection with GaM δ , and serum IgG1 and IgE levels were determined. As in previous experiments, anti-IL-4 antibody had no consistent effect on IgG1 production, but almost totally blocked the IgE response if the antibody

was given up to four days after GaM δ . Anti-CD4 antibody almost totally blocked the IgG1 response if given up to four days after GaM δ , and still almost totally blocked the IgE response if given as late as five days after GaM δ . Since the production of IL-4 in GaM δ -injected mice cannot be detected until three days after GaM δ injection, this experiment localizes the time during which the presence of IL-4 is important for the generation of an IgE response to the two-day period that extends from three to five days after GaM δ injection. In addition, these studies suggest that from five to six days after GaM δ injection some form of T-cell help other than IL-4 is important in the generation of an IgE response, while the IgG1 response appears to be independent of T help five days after GaM δ injection. This suggestion was supported by experiments which ruled out more trivial explanations for these results by demonstrating that: (a) IgE and IgG1 responses appear at the same time in GaM δ antibody-injected mice. (b) IgE and IgG1 responses in these mice are equally inhibited by limiting doses of anti-CD4 antibody. (c) Even low doses of anti-CD4 antibody, which inhibit IgE responses less than do optimal doses of anti-IL-4 antibody if given two days after GaM δ , inhibit IgE responses more than do optimal doses of anti-IL-4 antibody if both are given five days after GaM δ (60). No progress has yet been made, however, in the identification of this additional, late acting form of T cell help that may contribute specifically to an IgE response.

IL-4 IS REQUIRED IN VIVO TO SUSTAIN ESTABLISHED IgE RESPONSES

The unexpected isotype specificity of IL-4 in the induction of IgE secretion *in vivo* suggested that an approach aimed at blocking IL-4 production or IL-4 effects might be of use in the treatment of IgE-mediated allergic disorders. However, for an IL-4 based therapeutic approach to such disorders to have any chance for success, it would have to be able to decrease an already established IgE response and to prevent the establishment of a new response. To investigate this, a study was made of the ability of anti-IL-4 antibody to accelerate the decline in an Nb-induced IgE response, when given at the time of the peak response. Anti-IL-4 antibody clearly accelerated the decline in this response, as did anti-CD4 antibody (57).

Since serum IgE levels in Nb-inoculated mice spontaneously decline a few days after Nb are expelled, it was possible that the IL-4 requirement for maintenance of IgE production in the Nb system might not be relevant to human allergy patients, in whom serum IgE levels normally stay elevated for long periods of time. For this reason, the IL-4-dependence of an

established, chronic murine IgE response was investigated. Such a response is made by mice inoculated with the nematode *Heligmosomoides polygyrus* (Hp). Unlike Nb, Hp is not spontaneously expelled by infected mice; neither do Hp-induced IgE responses spontaneously fall (62). Despite these considerations, when anti-IL-4 antibody was given to Hp-infected mice after large IgE responses had already occurred, the antibody still induced serum IgE levels to decline substantially within one week of administration (Figure 3). After three weeks of anti-IL-4 antibody treatment, serum IgE levels were reduced by greater than 95% compared to those levels observed in untreated or control (anti-NP) antibody-treated, Hp-infected mice. All mice continued to excrete large numbers of Hp ova, however. Anti-IL-4 antibody did not suppress all Hp-induced immune responses non-specific-

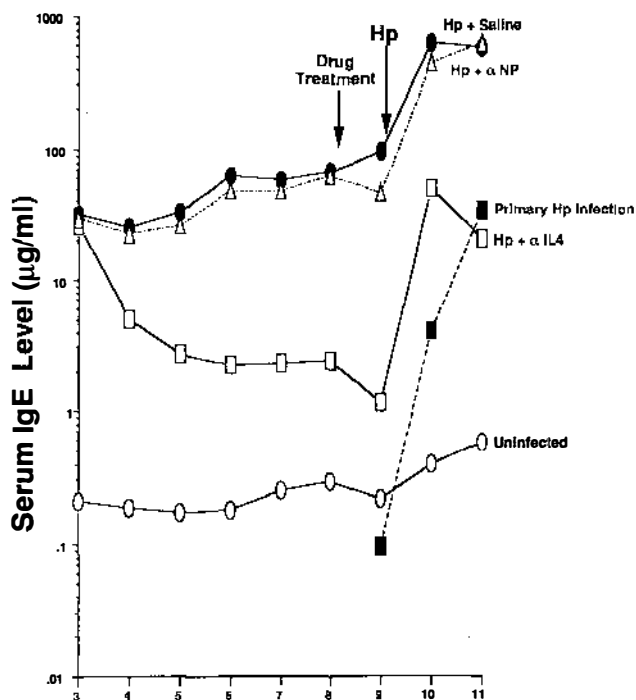


Figure 3 Effect of chronic anti-IL-4 antibody treatment on an established, ongoing IgE response to *Heligmosomoides polygyrus* (Hp). BALB/c mice (5/group) were left untreated or were infected with Hp larvae. Starting three weeks later, mice were bled weekly, and Hp-inoculated mice were injected weekly with saline, anti-IL-4 antibody, or anti-NP antibody (control) for the duration of the experiment. At 58 days after Hp inoculation mice were cured by drug treatment, and 65 days after injection mice were reinoculated with Hp. An additional group of previously untreated mice was inoculated with Hp at this time. Serum IgE levels were determined by ELISA. Geometric mean values are shown.

cally; substantial increases in polyclonal serum IgG1 levels continued to be seen after anti-IL-4 antibody treatment. Thus, the maintenance of even an established, ongoing IgE response is IL-4 dependent.

IL-4 IS REQUIRED TO GENERATE AND SUSTAIN A SECONDARY, ANTIGEN-SPECIFIC, IN VIVO IgE RESPONSE

To determine whether secondary, antigen-specific IgE responses are as dependent upon IL-4 as are primary, polyclonal IgE responses, mice were first primed with an intraperitoneal injection of 1 μ g of TNP-KLH on alum, rested for three weeks, and boosted in the same way with the same dose of the same antigen. At the time of the boost, mice were intravenously injected with anti-IL-4 antibody, anti-CD4 antibody, anti-NP antibody, or saline. Serum was obtained seven days later and IgE, IgG1, and IgG2a anti-TNP levels, as well as total IgE levels, were determined (Figure 4). Both the secondary IgE anti-TNP and the polyclonal responses appeared to be completely blocked by anti-IL-4 antibody, while the IgG1 and IgG2a anti-TNP responses were not significantly or specifically altered. In contrast, anti-CD4 antibody inhibited the production of all three isotypes of anti-TNP antibody. Thus, IL-4 appears to be required for the production of both primary polyclonal and secondary antigen-specific IgE, but not IgG responses.

Experiments were also performed to study whether IL-4 is required to maintain the antigen-specific IgE response induced by TNP-KLH on alum. When given at the time of the peak IgE anti-TNP response to a second

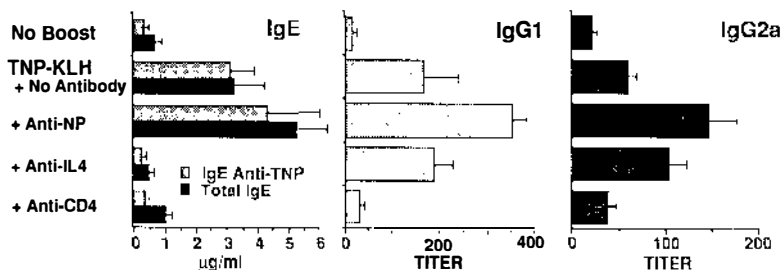


Figure 4 Isotype-specific inhibition of secondary anti-TNP response. BALB/c mice (5/group) were all primed with TNP-KLH on alum. Three weeks later mice were injected with saline (no boost) or with TNP-KLH on alum plus saline, a control antibody (anti-NP), anti-IL-4 antibody, or anti-CD-4 antibody. Mice were bled 7 days after the boost. Serum IgE, IgG1, and IgG2a anti-TNP antibody levels and total IgE concentration were determined by ELISA and are shown as geometric means and standard errors.

immunization with TNP-KLH on alum, anti-IL-4 antibody again accelerated the decline in serum IgE levels, although the decline was considerably slower than that observed in the Nb system (Figure 5). The slowness of the anti-IL-4 antibody-induced decline in serum IgE anti-TNP antibody levels in this system is compatible with an observation that anti-IL-4 antibody failed to decrease, over a 7–10-day culture period, *in vitro* IgE production by human B cells that had differentiated to the stage of IgE secretion *in vivo* (G. Delespesse, personal communication; D. Vercelli, H. H. Jabara, C. Cunningham-Rundles, J. S. Abrams, D. B. Lewis, J. Meyer, L. C. Schneider, D. Y. M. Leung, R. Geha, "Regulation of IgE synthesis in the hyper-IgE syndrome." Submitted). When coupled with evidence from the GaM δ system that the last phase of the induction of an IgE response is IL-4-independent, both observations suggest that the role of IL-4 in the maintenance of an IgE response is to recruit new cells to secrete IgE, rather than to sustain IgE secretion by already fully differentiated plasma cells. The possibility cannot, however, be ruled out that IL-4 both stimulates B cells to become committed to the synthesis of IgE

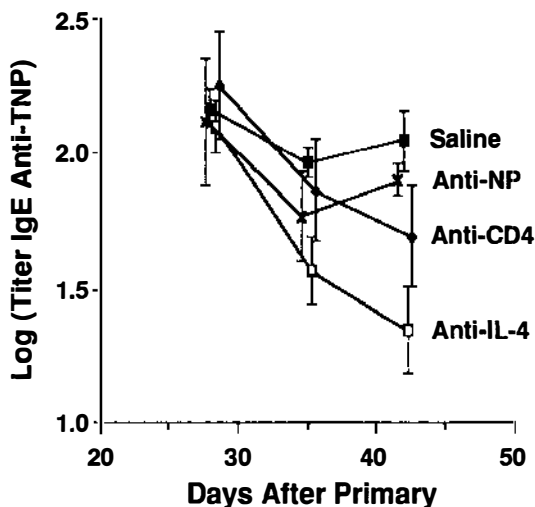


Figure 5 IL-4 maintains antigen-specific IgE responses. BALB/c mice (5/group) were immunized with TNP-KLH on alum and were boosted with the same antigen three weeks later. One and two weeks after the boost, mice were injected with saline, anti-NP antibody (control), anti-IL-4 antibody, or anti-CD4 antibody. Mice were bled two and three weeks after the boost, and serum IgE anti-TNP titers were determined by ELISA. Geometric means and standard errors are shown.

and extends the life of IgE secreting cells after they have begun to secrete IgE, but that IL-4 is not required during the period that IgE-committed cells differentiate to IgE secretion.

An additional, somewhat surprising result of studies in the TNP-KLH system is that anti-CD4 antibody, unlike anti-IL-4 antibody, did not significantly accelerate the decline in serum IgE anti-TNP levels. This raises the possibility that IL-4 made by a cell other than a CD4⁺ T cell is important in sustaining IgE responses in this system. In this regard it is noteworthy that mast cell lines secrete IL-4 in response to cross-linking of their high affinity IgE receptors (63), and that the spleens of TNP-KLH/alum-immunized mice contain non-B, non-T cells that secrete this cytokine when stimulated with IgE-containing immune complexes (S. Z. Ben Sasson, G. Le Gros, personal communication).

SOME IgE PRODUCTION DURING AN IN VIVO SECONDARY, POLYCLONAL RESPONSE APPEARS TO BE IL-4-INDEPENDENT

Not all IgE responses appear to be totally dependent upon the presence of IL-4. Mice that are inoculated for a first time with Nb, allowed to recover for about one month, then reinoculated with the same parasite, make a secondary polyclonal IgE response that is approximately three- to four-fold greater in magnitude than the primary response and that appears much more rapidly than the primary response. Injection of anti-IL-4 antibody at the time of the second Nb inoculation inhibits IgE production by at least 65%, and, in some experiments, by as much as 90%; however, the nearly 100% inhibition of IgE production that occurs when anti-IL-4 antibody is given at the time of primary Nb inoculation is never observed. Experiments in which various doses of anti-IL-4 antibody were administered to try to inhibit IgE responses to primary or secondary Nb inoculation (Figure 6) indicate that while even relatively small doses of anti-IL-4 antibody inhibit most of the secondary IgE response, some of this response cannot be inhibited even by large doses of this antibody. This failure suggests that this response has both IL-4-dependent and IL-4-independent components. We have hypothesized that the IL-4-independent part of this response may be generated by memory B cells that have already switched to IgE expression. This hypothesis, if correct, would indicate that IL-4 is important *in vivo* only for isotype switching, and not for a further differentiative process by which cells that have already switched to IgE become IgE-secreting cells. Given our results, this hypothesis would

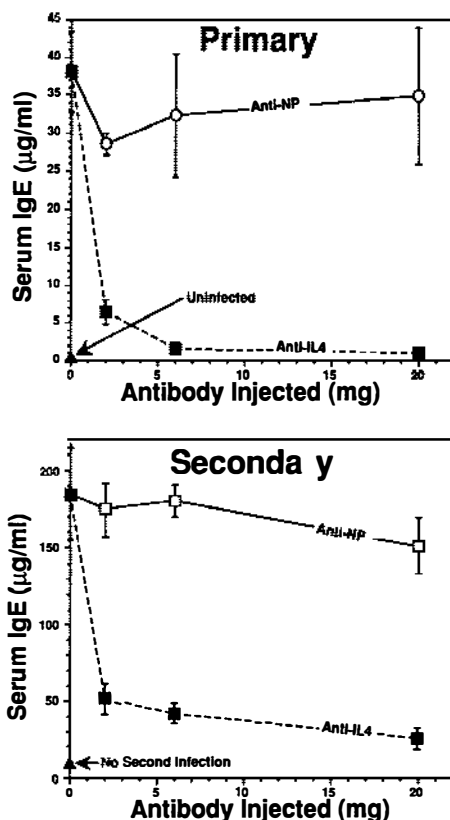


Figure 6 Inhibition of primary and secondary polyclonal IgE responses to Nb infection by anti-IL-4 antibody. Previously untreated BALB/c mice were left untreated or were inoculated with Nb larvae. Mice additionally received two injections of either anti-IL-4 or anti-NP (control) antibody at the doses shown. At the same time, mice that had received an initial Nb inoculation approximately six weeks earlier were re-inoculated with Nb and were injected twice with anti-IL-4 or anti-NP antibody at the indicated doses. Mice were bled 13 days after the primary inoculation or 7 days after the second inoculation, and IgE levels, shown as geometric means and standard errors, were determined by ELISA.

also require that only a small percentage of the IgE response generated in mice given a second Nb inoculation is derived from cells that have switched to IgE during the initial Nb inoculation. An alternate explanation for the apparent partial IL-4-independence of the secondary Nb-induced polyclonal IgE response is that so much IL-4 may be generated during this response that complete neutralization is impossible even with large doses of anti-IL-4 antibody. To investigate this possibility an experiment tested

the effects of anti-IL-4 antibody on both IgE production and B-cell Ia expression in mice given primary or secondary Nb infections. While anti-IL-4 antibody again failed to completely inhibit the secondary IgE response, it did completely block the Nb-induced increase in B-cell Ia expression (64). Since *in vitro* studies have demonstrated that induction of an IgE response requires much more IL-4 than does the induction of increased B-cell Ia expression, this result makes it unlikely that incomplete IL-4 neutralization could explain incomplete inhibition of the secondary Nb-induced IgE response.

Partial IL-4-independence of a secondary IgE response is also seen in mice treated with anti-IL-4 antibody after an IgE response has been established by Hp infection (Figure 3). When these mice are cured of Hp by drug administration, then reinfected with Hp while continuing to receive an anti-IL-4 antibody, serum IgE levels increase by greater than 30-fold. The serum levels attained, however, are still reduced by 95% compared to those that develop in similarly treated mice that have not received anti-IL-4 antibody. Thus, while part of the secondary IgE response in both the Nb and Hp systems appears to be IL-4 independent, in both systems this response accounts for only a small percentage of the total response.

IL-4 IS NOT REQUIRED DURING A PRIMARY IN VIVO IMMUNE RESPONSE TO ALLOW THE GENERATION OF A SECONDARY IgE RESPONSE

To determine if inhibition of IL-4 effects during a primary Nb infection would alter the IgE response made during a second infection with this parasite, the magnitudes and kinetics of these responses were compared in mice that received no anti-IL-4 antibody treatment with those that developed in mice treated with anti-IL-4 antibody (*a*) only during the first Nb inoculation, (*b*) only during the second Nb inoculation, or (*c*) during both inoculations (Figure 7). As before, anti-IL-4 antibody inhibited IgE production during a primary Nb response by over 98%. However, when inoculated with third-stage larvae of Nb six weeks after the initial inoculation, these mice generated an IgE response both larger and more rapid than that made by mice not previously infected with Nb, although not as large as that made by mice initially infected with Nb in the absence of anti-IL-4 antibody. In contrast, administration of anti-IL-4 antibody at the time of both primary and secondary Nb inoculation suppressed the secondary IgE response by over 99%, compared to that made by mice infected

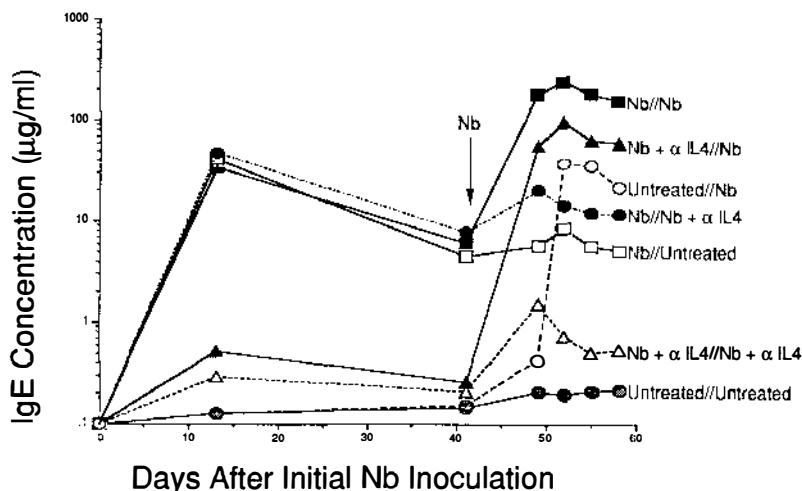


Figure 7 Effect of anti-IL-4 antibody on IgE responses to primary and secondary Nb inoculation. BALB/c mice (5/group) were left untreated or inoculated with Nb larvae and bled 13 days after inoculation. Mice were again bled 42 days after the initial inoculation and were left untreated or were re-inoculated with Nb one day after that. Mice were injected with anti-IL-4 antibody or saline at the time of and 7 days after the initial Nb inoculation, and at the time of and 4 days after the second Nb inoculation. Mice were bled 6, 9, 12, and 15 days after the second inoculation. Serum IgE levels, determined by ELISA, are shown as geometric means. The legend at the right of the figure indicates the treatment received during the period of initial inoculation//period of second inoculation.

twice in the absence of anti-IL-4 antibody, and by over 80% as compared to that made by mice given anti-IL-4 antibody only at the time of the secondary infection. These observations indicate that: (a) “priming” for a secondary IgE response can occur even when the primary response is largely blocked by neutralization of IL-4; and (b) when an IgE response to an initial immunization is blocked by neutralization of IL-4, continued IL-4 neutralization is both necessary and able to block the development of an IgE response to a second immunization. Thus, the IL-4-independent component of a secondary IgE response develops in Nb-infected mice only when IL-4 is present during the primary response. These observations are compatible with the hypotheses that: (a) the increased speed and magnitude of the secondary polyclonal IgE response in Nb-primed mice results primarily from the IL-4-independent generation of memory T cells; (b) the IL-4-independent component of the IgE response to a second Nb infection is derived from B cells that have become committed to IgE production in response to IL-4 during a primary Nb infection; and (c) a relatively small percentage of the IgE produced during a second Nb infection is derived from these B cells.

MODIFICATION OF HUMORAL, IN VIVO IMMUNE RESPONSES BY INTERFERONS

As noted earlier, in addition to establishing that IL-4 can contribute to the generation of IgG1 and IgE responses, *in vitro* murine studies have demonstrated that the lymphokine IFN- γ can inhibit these responses and contribute to the generation of IgG2a responses. To determine whether IFN- γ can have these same effects *in vivo*, the abilities of recombinant, purified IFN- γ and of a purified, monoclonal anti-IFN- γ antibody to alter the isotypes of Ig produced in mice immunized with GaM δ or BA were investigated. In an initial series of experiments, BALB/c mice injected with GaM δ also received twice daily intraperitoneal injections of 782 to 100,000 units of IFN- γ two, three, and four days after GaM δ . Mice were bled eight days after GaM δ injection, and their serum IgE, IgG1 and IgG2a levels were determined. Doses of IFN- γ as low as 1500 units induced increased IgG2a production; this effect was greatest at IFN- γ doses of 3000 to 6000 units. Significant inhibition of IgG1 and IgE responses was first noted when a dose of 12,500 units of IFN- γ was used, and the degree of inhibition increased as the dose of IFN- γ was further raised. At higher IFN- γ doses, enhancement of IgG2a responses was no longer observed; however, inhibition of production of this isotype did not occur until doses were reached that made mice severely ill (100,000 units) (55). Thus, in this system, less IFN- γ was required to enhance IgG2a production than to inhibit IgG1 and IgE production, and inhibition of the IgG1 and IgE responses required an identical IFN- γ dose.

Injection of anti-IFN- γ antibody together with GaM δ had an effect opposite to that of IFN- γ (Figure 8). Anti-IFN- γ antibody not only blocked

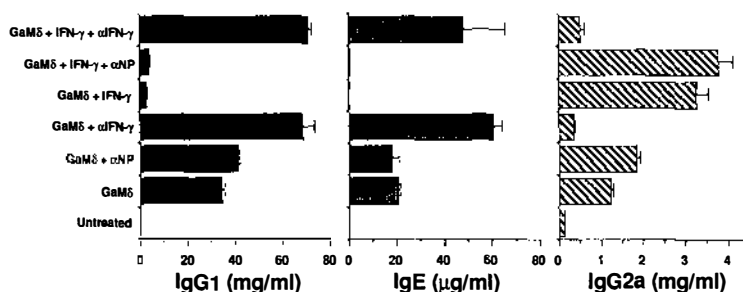


Figure 8 Effects of IFN- γ and anti-IFN- γ antibody on Ig secretion in GaM δ -treated mice. BALB/c mice (4/group) were left untreated or were injected with GaM δ antibody $\pm$ IFN- γ and/or anti-IFN- γ antibody or anti-NP antibody (control). Mice were bled 8 and 10 days after GaM δ antibody injection. Day 8 serum IgG1 and IgE levels, and day 10 serum IgG2a levels are shown as geometric means and standard errors.

the ability of IFN- γ to inhibit GaM δ -induced IgG1 and IgE responses and enhance the GaM δ -induced IgG2a response; but also enhanced GaM δ -induced IgG1 and IgE responses and blocked the GaM δ -induced IgG2a response (55). Thus, sufficient IFN- γ can be made during an in vivo immune response to inhibit IgG1 and IgE production, and to stimulate IgG2a production. This issue was further studied in a system in which mice were injected with killed, fixed BA, which stimulates a large polyclonal IgG2a response, but relatively little or no IgG1 or IgE production (55). Injection of mice with anti-IFN- γ antibody considerably, but incompletely, inhibited the BA-induced IgG2a response, and considerably amplified the BA-induced IgG1 response, but did not lead to the generation of an IgE response. When given to mice that had also been injected with GaM δ , BA acted very much like IFN- γ , in that it inhibited GaM δ -induction of IgG1 and IgE production and allowed a large IgG2a response to be produced. Anti-IFN- γ antibody blocked the ability of BA to inhibit GaM δ induction of an IgG1 response but did not block the ability of BA to inhibit GaM δ induction of IgE production (55). Thus, while BA induction of IFN- γ production can explain the ability of BA to block IgG1 production, inhibition of IgE production by BA must involve an additional mechanism.

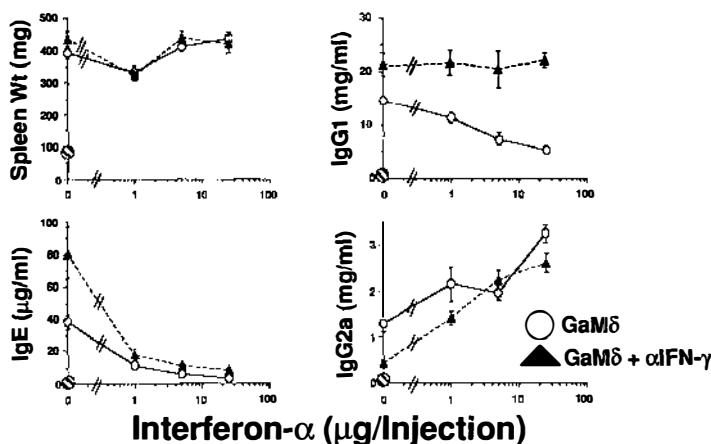


Figure 9 Effect of interferon- α on GaM δ -induced polyclonal antibody responses. BALB/c mice (5/group) were injected with GaM δ antibody $\pm$ monoclonal anti-IFN- γ antibody, and 2, 3, and 4 days later, received two injections/day of recombinant mouse IFN- α at the doses shown. Mice were bled and their spleens were removed and weighed 8 days after GaM δ injection. Serum IgG1, IgG2a, and IgE levels were determined and are shown as geometric means and standard errors. Ig and spleen weights for mice that were not treated with GaM δ are shown as diagonal-filled circles.

Since BA has been shown to induce IFN- α production in mice (65, 66), and IFN- α blocks IgE production in both murine and human in vitro systems (23; and C. M. Snapper, F. D. Finkelman, unpublished data), in vivo murine studies with this cytokine were performed. When injected into GaM δ -treated mice (Figure 9), IFN- α produced effects that resembled those of IFN- γ (suppression of IgG1 and IgE production and stimulation of IgG2a production), but, unlike IFN- γ , did not have obvious toxic effects at doses that caused substantial alterations in Ig isotype selection. In the presence of monoclonal anti-IFN- γ antibody, IFN- α continued to suppress the GaM δ -induced IgE response and enhance IgG2a production, but no longer inhibited the IgG1 response. These observations suggest that IFN- α may act directly at the B-cell level to stimulate IgG2a production and suppress IgE production, while its inhibitory effects on IgG1 production result either from induction of IFN- γ production or from synergistic enhancement of the inhibitory effect of IFN- γ . While studies to determine the physiological roles of IFN- α and IFN- β in the control of Ig isotype selection await the availability of large quantities of antibodies that specifically neutralize these cytokines, the relative lack of toxicity of IFN- α , as compared to IFN- γ , may make the former cytokine a better candidate than the latter for the treatment of human IgE-mediated disorders.

TREATMENT OF HUMAN HYPER-IgE SYNDROME PATIENTS WITH IFNs

Both IFN- α and IFN- γ have been used for the treatment of small numbers of patients with the hyper-IgE syndrome. IFN- α_{2b} , given to one hyper-IgE syndrome patient at a dose of 3×10^6 U subcutaneously twice a week for a period of four weeks, resulted in an approximately 50% reduction in serum IgE levels without affecting serum levels of other Ig isotypes. The severe atopic dermatitis that was present in this patient cleared completely after three weeks of treatment, but reappeared in a milder form eight days after the last IFN- α dose (67). Of five patients treated with 50–100 μ g of IFN- γ three times a week for two weeks, the serum IgE levels of two decreased by approximately 50%, and all exhibited considerable decreases in the “spontaneous” production of IgE by their peripheral blood mononuclear cells when cultured in vitro (C. L. King, J. I. Gallin, H. L. Malech, S. L. Abramson, T. B. Nutman, “Regulation of immunoglobulin production in hyper-immunoglobulin-E recurrent-infection syndrome by interferon- γ .” Submitted). In a separate study (67a), treatment of 15 patients with atopic dermatitis and high serum IgE levels with 10 μ g/M² of IFN- γ five times a week for one week, then sequentially with 50 and 100 μ g/M²

of IFN- γ five times a week during the two subsequent weeks, failed to consistently decrease serum IgE levels. However, the in vivo IFN- γ treatment was associated with a significant decrease in the "spontaneous" in vitro production of IgE by peripheral blood mononuclear cells from most of these patients. These studies suggest that cytokines, measures that alter cytokine production, or measures that alter cytokine effects, may be useful in the therapy of IgE-mediated disorders. Further investigation is required to confirm these preliminary observations, to determine optimal doses of these cytokines, and to compare their effectiveness and toxicity with each other, with agents that block IL-4 production or effects, and with combinations of these cytokines and agents, in the treatment of IgE-mediated disorders.

IFN- γ CAN CONTRIBUTE TO, BUT IS NOT REQUIRED FOR, THE INDUCTION OF ALL ANTIGEN-SPECIFIC IgG2a RESPONSES

The ability of IFN- γ to enhance an IgG2a response is not limited to polyclonal IgG2a responses, since anti-IFN- γ antibody selectively inhibits both the IgG2a anti-DNP response in mice immunized with DNP-*Bordetella pertussis* (68) and the IgG2a anti-FITC response in mice immunized with FITC-BA (Table 1). IFN- γ does not seem to be an absolute requirement for the generation of an IgG2a response, however, since these responses are only partially inhibited by anti-IFN- γ antibody. The lack of complete inhibition is not likely a result of incomplete neutralization of IFN- γ , since a ten-fold increase in the dose of anti-IFN- γ antibody did not further inhibit the generation of an IgG2a anti-FITC antibody response to FITC-BA (data not shown). Furthermore, if IFN- γ were an absolute requirement for the generation of an IgG2a response, it might be anticipated that the small IgG2a responses made to antigens that induce primarily an IgG1 response would be at least as easy to inhibit with anti-IFN- γ antibody as the large IgG2a responses made to antigens that induce primarily that IgG isotype. This is not the case. While the relatively large IgG2a anti-FITC response to FITC-BA is strongly inhibited by anti-IFN- γ antibody, the small IgG2a anti-FITC responses to FITC-sheep erythrocytes, FITC-Ficoll, or FITC-KLH are only slightly inhibited by the same antibody (Table 1). In some experiments no significant inhibition of FITC-sheep erythrocyte-induced IgG2a anti-FITC antibody production was observed (data not shown). Thus, it seems likely that IFN- γ can enhance some, but is not required for the induction of all, murine IgG2a antibody responses.

Table 1 Effects of anti-IFN- γ antibody on IgG1 and IgG2a anti-FITC antibody responses to different FITC-substituted antigens. Mice were immunized with the antigens shown and were injected with 1 mg of either monoclonal anti-IFN- γ antibody or an isotype-matched rat IgG control antibody (anti-NP) at the same time. Mice were bled 7 to 10 days later and serum IgG1 and IgG2a anti-FITC antibody titers were determined by ELISA. Numbers shown are geometric means for groups of five mice, $\times / \div$ (geometric standard errors).

Immunogen	Anti-FITC titer	
	IgG1	IgG2a
...	< 10	< 10
FITC-BA + anti-NP	120 (1.38)	3,820 (1.20)
FITC-BA + anti-IFN- γ	112 (1.19)	475 (1.07)
FITC-SRBC + anti-NP	318 (1.06)	28 (1.06)
FITC-SRBC + anti-IFN- γ	333 (1.13)	17 (1.08)
FITC-GaM δ + anti-NP	27,000 (1.09)	303 (1.05)
FITC-GaM δ + anti-IFN- γ	29,800 (1.08)	165 (1.18)
FITC-KLH + anti-NP	252 (1.21)	54 (1.29)
FITC-KLH + anti-IFN- γ	308 (1.21)	33 (1.20)
FITC-Ficoll + anti-NP	387 (1.08)	165 (1.22)
FITC-Ficoll + anti-IFN- γ	354 (1.10)	96 (1.17)

IFN- γ INHIBITS POLYCLONAL IgG1 RESPONSES MORE THAN ANTIGEN-SPECIFIC IgG1 RESPONSES

While IFN- γ appears to have a similar stimulatory effect on the polyclonal and antigen-specific IgG2a responses to FITC-BA or to GaM δ , it inhibits polyclonal IgG1 responses to a considerably greater extent than antigen-specific IgG1 responses. This is seen both in studies in which exogenous IFN- γ inhibits the GaM δ -induced goat IgG-specific IgG1 response less than the polyclonal IgG1 response to the same immunogen (Figure 10) and in studies in which anti-IFN- γ antibody fails to enhance the FITC-specific IgG1 response to several antigens, including FITC-BA (Table 1), even though it considerably enhances the polyclonal IgG1 response to BA (55). While the mechanism for differentially affecting polyclonal and antigen-specific responses is not immediately apparent, the consequence of this effect is to conserve the immune system by focusing a humoral immune response more precisely toward the stimulating antigen. It is not currently known whether polyclonal IgE responses are also inhibited by IFN- γ to a greater extent than are antigen-specific responses. This is an

important question to resolve, since an agent that inhibited all IgE responses could ameliorate IgE-mediated disorders, while an agent that selectively inhibited polyclonal IgE responses could exacerbate these disorders by increasing the percentages of mast cell IgE receptors occupied by IgE molecules specific for pathogenic antigens. Unfortunately, this is a difficult issue to resolve, because it is hard to quantitate accurately antigen-specific IgE in the presence of much larger amounts of polyclonal IgE and antigen-specific IgG, and because IFN- γ stimulation of immune responses that results from enhancement of macrophage and dendritic cell antigen presentation (69–71) can be difficult to separate from more specific effects on Ig isotype selection.

ENDOGENOUSLY PRODUCED IFN- γ CAN LIMIT “SPONTANEOUS” IN VIVO PRODUCTION OF IgE

To determine if IFN- γ has a role in limiting IgE production in animals that are not purposefully immunized, BALB/c mice were treated from birth with weekly injections of saline or with either a monoclonal anti-IFN- γ antibody or an isotype-matched control antibody. When bled at twelve weeks of age, the anti-IFN- γ antibody-treated mice had serum IgE levels that averaged nearly four-fold more than those of the control mice (4.28 μ g/ml vs 1.15 μ g/ml). Serum IgG1 and IgG2a levels were similar in anti-IFN- γ antibody-treated and control antibody-treated mice (P. Cohen, F. D. Finkelman, manuscript in preparation). This observation suggests that endogenously produced IFN- γ may have a role in limiting environmental antigen stimulation of IgE production.

IL-5 PROMOTES EOSINOPHILIC RESPONSES, BUT DOES NOT CONTRIBUTE TO IgE OR IgG1 RESPONSES IN SOME MURINE IN VIVO SYSTEMS

In in vitro mouse studies IL-5 has been found to stimulate antibody production by enhancing both proliferation and differentiation of activated B cells (15–18), to be required for the induction of Ig secretion by B cells cultured with antigen-specific Th2 cell clones in the presence of the appropriate antigen (72, 73), especially to promote the synthesis and secretion of IgA (19–22), and to stimulate eosinophil development and activation (74). In vivo studies provide support only for the last of these effects. Studies in which the effects of a monoclonal anti-IL-5 antibody have been studied in mice infected with *Schistosoma mansoni* (75), Nb (76), or Hp (Figure 11), have all shown that this antibody blocks the

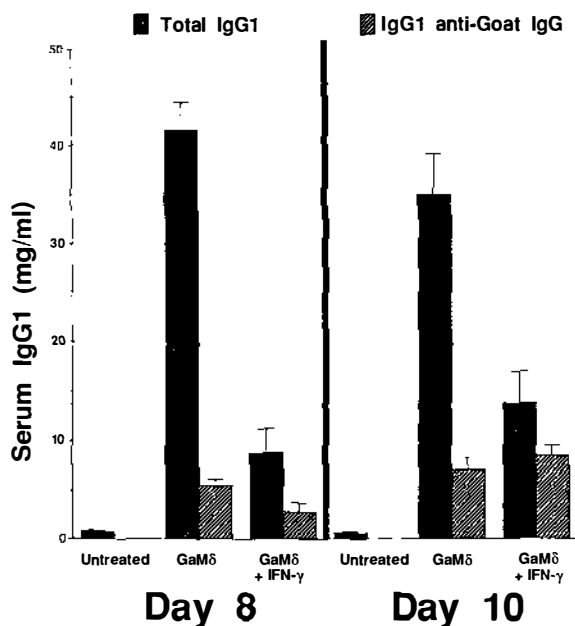


Figure 10 Inhibition of polyclonal and goat IgG-specific antibody responses by IFN- γ . BALB/c mice (5/group) were left untreated or were injected with GaM δ antibody. One group of GaM δ -injected mice also received two daily injections of 25,000 units of IFN- γ twice daily 2, 3, and 4 days after GaM δ injection. Mice were bled eight and ten days after GaM δ injection. Total serum IgG1 levels were determined by radial immunodiffusion; serum IgG1 anti-goat IgG levels were determined by ELISA. Serum Ig levels are shown as geometric means and standard errors.

development of both blood and tissue eosinophil responses but fails to affect IgG1 or IgE responses, even though similar IL-5 concentrations are required to stimulate lymphocyte and eosinophil responses *in vitro*. Anti-IL-5 antibody, at a dose 20-fold greater than that required to completely block eosinophilia in the parasite models, also fails to block IgG1, IgG2a, or IgE responses in GaM δ -treated mice, which do not develop eosinophilia (J. F. Urban, I. M. Katona, F. D. Finkelman, unpublished data). In both the GaM δ and Hp systems, treatment with a combination of anti-IL-4 and anti-IL-5 antibodies had no effect on the generation of IgG responses and was no more effective at blocking IgE responses than anti-IL-4 antibody alone (Figure 11, F. D. Finkelman, J. F. Urban, I. M. Katona, R. L. Coffman, T. R. Mosmann, unpublished data). These observations suggest that IL-5, while crucial to the development of eosinophilia, may have little *in vivo* role in the generation of antibody responses. It is

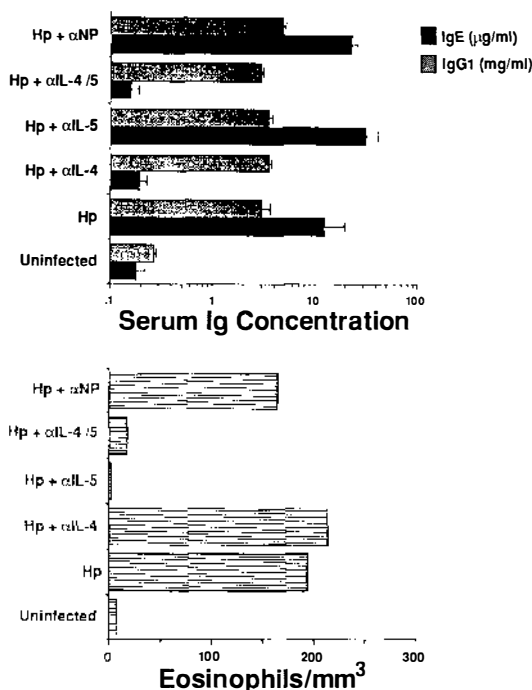


Figure 11 IL-5 contributes to the development of eosinophilia, but not to IgE or IgG1 responses in Hp-infected mice. BALB/c mice (5/group) were left untreated or were inoculated with Hp. Some groups received 2 mg of monoclonal anti-IL-5 antibody (TRFK-5), 10 mg of monoclonal anti-IL-4 antibody (11B11), both antibodies, or 12 mg of a control (J4-1 anti-NP) antibody, once a week, starting at the time of Hp inoculation. Blood eosinophil levels, serum IgE levels (shown as $\mu\text{g/ml}$), and serum IgG1 levels (shown as mg/ml) were all determined 13 days after Hp inoculation.

possible, however, that IL-5 acts at a considerable distance from its site of production in the stimulation of eosinophil responses, but stimulates B lymphocytes that are in direct contact with the T lymphocytes that produce it. If this is the case, it might be more difficult for an anti-IL-5 antibody to inhibit IL-5 induction of B cell, than eosinophil responses. It is also noteworthy that no studies of anti-IL-5 antibody effects on the generation of in vivo IgA responses have yet been reported.

SUMMARY AND CONCLUSIONS

Several specific conclusions can be drawn from these studies:

1. IL-4 is required for the generation of both primary polyclonal and secondary antigen-specific IgE responses in vivo.

2. IL-4 is required to maintain established, ongoing, antigen-specific and polyclonal IgE responses.

3. Most, but not all, polyclonal IgE production during a secondary immune response is IL-4-dependent. Memory B cells that have already switched to IgE at the DNA level may no longer require stimulation with IL-4 to be induced to secrete IgE.

4. The generation of a secondary IgE response is not dependent upon the presence of IL-4 during primary immunization. However, if IL-4 is not present during primary immunization, it is required during secondary immunization for the generation of an IgE response.

5. IL-4 does not appear to be required for the generation of *in vivo* IgG1 responses, and in at least some instances, does not contribute significantly to the generation of IgG1 responses *in vivo*.

6. A late-acting form of T-cell help other than IL-4 appears to be required for the generation of an IgE, but not an IgG1 response.

7. An antibody that inhibits IL-4 binding to IL-4 receptors affects Ig isotype selection in the same way as an antibody that neutralizes IL-4.

8. IFN- γ can act in both spontaneous and induced immune responses to suppress IgE production.

9. IFN- γ can also suppress IgG1 production and stimulate IgG2a production. However, IFN- γ appears to suppress polyclonal IgG1 responses more than antigen-specific IgG1 responses, and it enhances, but is not required for, the generation of IgG2a responses.

10. IFN- α appears to resemble IFN- γ in its ability to inhibit IgE and enhance IgG2a responses in GaM δ -injected mice, but it requires the presence of IFN- γ to suppress IgG1 production in these mice.

11. Both IFN- α and IFN- γ appear to be able to decrease IgE production in some human patients.

12. There is no direct evidence that IL-5 contributes to the generation of *in vivo* antibody responses.

Two general conclusions may also be drawn. First, sufficient evidence has now accumulated to support the view that approaches aimed at the control of IL-4 and IFN production or effects may be useful in the control of IgE-mediated disorders. Secondly, *in vivo* studies sometimes support hypotheses that have been generated in simpler, more controllable *in vitro* systems, but sometimes indicate that *in vitro* observations have little *in vivo* applicability. Despite their complexity and expense, *in vivo* studies will continue to be required to further elucidate the mechanisms by which cytokines contribute to the generation of immune responses and to the control of Ig isotype selection in intact animals.

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Literature Cited

1. Isakson, P. C., Pure, E., Vitetta, E. S., Krammer, P. H. 1982. T cell derived B cell differentiation factor(s). Effect on the isotype switch of murine cells. *J. Exp. Med.* 155: 734-48
2. Vitetta, E. S., Ohara, J., Myers, C., Layton, J., Krammer, P. H., Paul, W. E. 1985. Serological, biochemical, and functional identity of B cell-stimulatory factor I and B cell differentiation factor for IgG1. *J. Exp. Med.* 161: 1726-31
3. Coffman, R. L., Carty, J. 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J. Immunol.* 136: 949-54
4. Coffman, R. L., Ohara, J., Bond, M. W., Carty, J., Zlotnik, A., Paul, W. E. 1986. B cell stimulatory factor-I enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136: 4538-41
5. Snapper, C. M., Finkelman, F. D., Paul, W. E. 1988. Differential regulation of IgG1 and IgE synthesis by interleukin 4. *J. Exp. Med.* 167: 183-96
6. Snapper, C. M., Paul, W. E. 1987. Interferon- γ and B cell stimulatory factor-I reciprocally regulate Ig isotype production. *Science* 236: 944-47
7. Coffman, R. L., Seymour, B. W., Lebman, D. A., Hiraki, D. D., Christiansen, J. A., Shrader, B., Cherwinski, H. M., Savelkoul, H. F. J., Finkelman, F. D., Bond, M. W., Mosmann, T. R. 1988. The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol. Rev.* 102: 5-28
8. Lebman, D. A., Coffman, R. L., 1988. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. *J. Exp. Med.* 168: 853-62
9. Stavnezer, J., Radcliffe, G., Lin Y.-C., Nietupski, J., Berggren, L., Sitia, R., Severinson, E. 1988. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA* 85: 7704-8
10. Rothman, P., Lutzker, S., Cook, W., Coffman, R., Alt, F. W. 1988. Mitogen plus interleukin 4 induction of C ϵ transcription in B lymphoid cells. *J. Exp. Med.* 168: 2385-89
11. Berton, M. T., Uhr, J. W., Vitetta, E. S. 1989. Induction of germline S gamma 1 transcripts in normal resting B cells by interleukin-4 prior to switch recombination to IgG1 and inhibition by interferon-gamma. *Proc. Natl. Acad. Sci. USA* 86: 2829-33
12. Esser, C., Radbruch, A. 1989. Rapid induction of transcription of unrearranged S-gamma-1 switch regions in activated murine B cells by interleukin-4. *EMBO J.* 8: 483-88
13. Snapper, C. M., Paul, W. E. 1987. B cell stimulatory factor-I (interleukin 4) prepares resting murine B cells to secrete IgG1 upon subsequent stimulation with bacterial lipopolysaccharide. *J. Immunol.* 139: 10-17
14. Stevens, T. L., Bossie, A., Sanders, V. M., Fernandez-Botran, R., Coffman, R. L., Mosmann, T. R., Vitetta, E. S. 1988. Regulation of antibody isotype secretion

- by subsets of antigen-specific helper T cells. *Nature* 334: 255-58
15. Swain, S. L., Howard, M., Kappler, J., Marrack, P., Watson, J., Booth, R., Wetzel, G. D., Dutton, R. W. 1983. Evidence for two distinct classes of B cell growth factor which have activities in different functional assays. *J. Exp. Med.* 158: 822-35
 16. Swain, S. L. 1985. Role of BCGF II in the differentiation to antibody secretion of normal and tumor B cells. *J. Immunol.* 134: 3934-43
 17. Takatsu, K., Tanaka, K., Tominaga, A., Hamaoka, T. 1980. Antigen-induced T cell-replacing factor [TRF]. III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.* 125: 2646-53
 18. Kinashi, T., Harada, N., Severinson, E., Tanabe, T., Sideras, P., Konishi, M., Azuma, C., Tominaga, A., Bergstedt-Lindqvist, S., Takahashi, M., Matsuda, F., Yaoita, Y., Takatsu, K., Honjo, T. 1986. Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. *Nature* 324: 70-73
 19. Coffman, R. L., Shrader, B., Carty, J., Mosmann, T. R., Bond, M. W. 1987. A mouse T cell product that preferentially enhances IgA production. I. Biologic characterization. *J. Immunol.* 139: 3685-90
 20. Murray, P. D., McKenzie, D. T., Swain, S. L., Kagnoff, M. F. 1987. IL5 and IL4 produced by Peyer's patch T cells selectively enhance IgA expression. *J. Immunol.* 139: 2669-74
 21. Harriman, G. R., Kunimoto, D. Y., Elliot, J. F., Paetkau, V., Strober, W. 1988. The role of IL5 in IgA B cell differentiation. *J. Immunol.* 140: 3033-39
 22. Beagley, K. W., Eldridge, J. H., Kiyono, H., Everson, M. P., Koopman, W. J., Honjo, T., McGhee, J. R. 1988. Recombinant murine IL-5 induces high rate IgA synthesis in cycling IgA-positive Peyer's patch B cells. *J. Immunol.* 141: 2035-42
 - 22a. Coffman, R. L., Lebman, D. A., Shrader, B. 1989. Transforming growth factor- β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J. Exp. Med.* 170: 1039-44
 23. Pene, J., Rousset, F., Briere, F., Chretien, I., Bonnefoy, J. Y., Spits, H., Yokota, T., Arai, K., Banchereau, J., de Vries, J. 1988. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E₂. *Proc. Natl. Acad. Sci. USA* 85: 6880-84
 24. Sarfati M., Delespesse G. 1988. Possible role of human lymphocyte receptor for IgE (CD23) or its soluble fragments in the in vitro synthesis of human IgE. *J. Immunol.* 141: 2195-99
 25. Vercelli, D., Jabara, H. H., Arai, K.-I., Geha, R. S. 1989. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. *J. Exp. Med.* 169: 1295-1308
 26. Vercelli, D. H., Jabara, H., Arai, K., Yokota, T., Geha, R. S. Endogenous IL-6 plays an obligatory role in IL-4 induced human IgE synthesis. *Eur. J. Immunol.* In press
 27. Del Prete, G. F., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Ricci, M., Banchereau, J., de Vries, J., Romagnani, S. 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J. Immunol.* 140: 4193-98
 28. Thyphronitis, G., Tsokos, G. C., June, C. H., Levine, A. D., Finkelman, F. D. 1989. IgE secretion by Epstein-Barr virus-infected purified human B lymphocytes is stimulated by interleukin-4 and suppressed by interferon- γ . *Proc. Natl. Acad. Sci. USA* 86: 5580-84
 29. MacKenzie, T., Dosch, H.-M., 1989. Clonal and molecular characteristics of the human IgE-committed B cell subset. *J. Exp. Med.* 169: 407-30
 30. Splawski, J. B., Jelinek, D. F., Lipsky P. E. 1989. Immunomodulatory role of IL-4 on the secretion of Ig by human B cells. *J. Immunol.* 142: 1569-75
 31. Spiegelberg, H. L., Nusslein, H. G. 1989. Interleukin-4 induces human B cells to secrete both IgE and IgG4. *FASEB J.* 3: A967 (Abstr.)
 32. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., Coffman, R. L. 1986. Two types of murine helper T cell clone: I. Definition according to the profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136: 2348-57
 33. Killar, L., MacDonald, G., West, J., Woods, A., Bottomly, K. 1987. Cloned, Ia-restricted T cells that do not produce interleukin 4 (IL-4)/B cell stimulatory factor 1 (BSF-1) fail to help antigen-specific B cells. *J. Immunol.* 138: 1674-79
 34. Bottomly, K. 1988. A functional dichotomy in CD4 T lymphocytes. *Immunol. Today* 9: 274-77
 35. Mosmann, T. R., Coffman, R. L. 1989. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different

- functional properties. *Annu. Rev. Immunol.* 7: 145–73
36. Palaird, X., de Waal Malefijt, R., Yssel, H., Blanchard, D., Chretien, I., Abrams, J., de Vries, J., Spits, H. 1988. Simultaneous production of IL2, IL4, and IFN γ by activated human CD4 $^{+}$ and CD8 $^{+}$ T cell clones. *J. Immunol.* 141: 849–55
 37. Maggi, E., Del Prete, G., Macchia, D., Parronchi, P., Tiri, A., Chretien, I., Ricci, M., Romagnani, S. 1988. Profiles of lymphokine activities and helper function for IgE in human T cell clones. *Eur. J. Immunol.* 18: 1045–50
 38. Gajewski, T. F., Fitch, F. W. 1988. Antiproliferative effect of IFN- γ in immune regulation. I. IFN- γ inhibits the proliferation of Th2 but not Th1 murine helper T cell clones. *J. Immunol.* 140: 4245–52
 39. Wong, R. L., Ruddle, N. H., Padula, S. J., Lingenheld, E. G., Gergman, C. M., Rugen, R. V., Epstein, D. I., Clark, R. B. 1988. Subtypes of helper cells: non-inflammatory type 1 helper T cells. *J. Immunol.* 141: 3329–34
 40. Kelso, A., Gough, N. M. 1988. Coexpression of granulocyte-macrophage colony-stimulating factor, γ -interferon, and interleukins 3 and 4 is random in murine alloreactive T-lymphocyte clones. *Proc. Natl. Acad. Sci. USA* 85: 9189–93
 41. Firestein, G. S., Roeder, W. D., Laxer, J. A., Townsend, K. S., Weaver, C. T., Hom, J. T., Linton, J., Torbett, B. E., Glasebrook, A. L. 1989. A new murine CD4 $^{+}$ T cell subset with an unrestricted cytokine profile. *J. Immunol.* 143: 518–25
 42. Muller, I., Pedrazzini, T., Farrell, J. P., Louis, J. 1989. T-cell responses and immunity to experimental infection with *Leishmania major*. *Annu. Rev. Immunol.* 7: 561–78
 43. Scott, P., Natovitz, P., Coffman, R., Pearce, E., Sher, A. 1988. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. *J. Exp. Med.* 168: 1675–84
 44. Heinzel, F. P., Sadick, M. D., Holaday, B. J., Coffman, R. L., Locksley, R. M. 1989. Reciprocal expression of interferon γ or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J. Exp. Med.* 169: 59–72
 45. Finkelman, F. D., Scher, I., Mond, J. J., Kung, J. T., Metcalf, E. S. 1982. Polyclonal activation of the murine immune system by an antibody to IgD. I. Increase in cell size and DNA synthesis. *J. Immunol.* 129: 629–37
 46. Finkelman, F. D., Smith, J., Villacreses, N., Metcalf, E. S. 1984. Polyclonal activation of the murine immune system by an antibody to IgE. VII. Demonstration of the role of non-antigen specific T help in vivo B cell activation. *J. Immunol.* 133: 550–55
 47. Finkelman, F. D., Ohara, J., Goroff, D. K., Smith, J., Villacreses, N., Mond, J. J., Paul, W. E. 1986. Production of BSF-1 during an in vivo, T-dependent immune response. *J. Immunol.* 137: 2878–85
 48. Finkelman, F. D., Malek, T. R., Shevach, E. M., Mond, J. J. 1986. In vivo and in vitro expression of an interleukin 2 receptor by murine B and T lymphocytes. *J. Immunol.* 137: 2252–59
 49. Finkelman, F. D., Scher, I., Mond, J. J., Kessler, S., Kung, J. T., Metcalf, E. S. 1982. Polyclonal activation of the murine immune system by an antibody to IgD. II. Generation of polyclonal antibody production and cells with surface IgG. *J. Immunol.* 129: 638–46
 50. Finkelman, F. D., Smith, J., Villacreses, N., Metcalf, E. S. 1985. Polyclonal activation of the murine immune system by an antibody to IgD. VI. Influence of doses of goat anti-mouse δ and normal goat IgG on B lymphocyte proliferation and differentiation. *Eur. J. Immunol.* 15: 315–20
 51. Finkelman, F. D., Snapper, C. M., Mountz, J. D., Katona, I. M. 1987. Polyclonal activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction of a polyclonal IgE response. *J. Immunol.* 138: 2826–30
 52. Katona, I. M., Urban, J. F. Jr., Scher, I., Kanellopoulos, C., Finkelman, F. D. 1983. Induction of an IgE immune response in mice infected with *Nippostrongylus brasiliensis*. Characterization of lymphoid cells with intracytoplasmic or surface IgE. *J. Immunol.* 130: 350–56
 53. Finkelman, F. D., Katona, I. M., Urban, J. F. Jr., Snapper, C. M., Ohara, J., Paul, W. E. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine BSF-1. *Proc. Natl. Acad. Sci. USA* 83: 9675–78
 54. Lebrun, P., Spiegelberg, H. L. 1987. Concomitant immunoglobulin E and immunoglobulin G1 formation in *Nippostrongylus brasiliensis*-infected mice. *J. Immunol.* 139: 1459–65

55. Finkelman, F. D., Katona, I. M., Mosmann, T. R., Coffman, R. L. 1988. Interferon- γ regulates the isotypes of immunoglobulin secreted during in vivo humoral immune responses. *J. Immunol.* 140: 1022-27
56. Ohara J., Paul, W. E. 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature* 315: 333-36
57. Finkelman, F. D., Katona, I. M., Urban, J. F. Jr., Holmes, J., Ohara, J., Tung, A. S., Sample, J. vG., Paul, W. E. 1988. Interleukin-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* 141: 2335-41
58. Noelle, R., Krammer, P. H., Ohara, J., Uhr, J. W., Vitetta, E. S. 1984. Increased expression of Ia antigens on resting B cells, an additional role for B-cell growth factor. *Proc. Natl. Acad. Sci. USA* 81: 6149-53
59. Deleted in proof
60. Finkelman, F. D., Holmes, J., Urban, J. F. Jr., Paul, W. E., Katona, I. M. 1989. T help requirements for the generation of an in vivo IgE response: a late acting form of T cell help other than interleukin 4 is required for IgE but not for IgG1 production. *J. Immunol.* 142: 403-8
61. Wilde, D. B., Marrack, P., Kappler, J., Dialsynas, D. P., Fitch, F. W. 1983. Evidence implicating L3T4 in class II MHC antigen reactivity: monoclonal antibody GK1.5 (anti-L3T4a) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T cell lines. *J. Immunol.* 131: 2178-83
62. Urban, J. F. Jr., Katona, I. M., Finkelman, F. D. 1989. The role of CD4⁺ T cells in the IgE response and immunity to *Heligmosomoides polygyrus*. *FASEB J.* 3: A1357 (Abstr.)
63. Plaut, M., Pierce, J. H., Watson, C. J., Hanley-Hyde, J., Nordan, R. P., Paul, W. E. 1989. Mast cell lines produce lymphokines in response to cross-linkage of Fc ϵ RI or to calcium ionophores. *Nature* 339: 64-67
64. Finkelman, F. D., Katona, I. M., Urban, J. F. Jr., Paul, W. E. 1989. Control of in vivo IgE production in the mouse by interleukin-4. *Ciba Foundation*, London, England. In press.
65. Youngner, J. S., Stinebring, W. R. 1965. Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pre-treated with *Escherichia coli* endotoxin or infected with *Mycobacterium tuberculosis*. *Nature (London)* 208: 456
66. Youngner, J. S. 1977. Properties of interferon induced by specific antigens. *Tex. Rept. Biol. Med.* 35: 17
67. Souillet, G., Rousset, F., de Vries, J. E. 1989. Alpha-interferon treatment of patient with hyper IgE syndrome. *Lancet* 1: 1384
- 67a. Leung, D. Y. M., Boguniewicz, M., Vercelli, D., Jabara, H., Geha, R. S. 1989. Gamma interferon inhibits IgE production in patients with atopic dermatitis. *Clin. Res.* 37: 239A (Abstr.)
68. Hagen, M., Essani, N. A., Strejan, G. H. 1989. Role of interferon-gamma in the modulation of the IgE response by 2, 4-dinitrophenyl-*Bordetella pertussis* vaccine in the mouse. *Eur. J. Immunol.* 19: 441-46
69. Steeg, P. S., Moore, R. N., Johnson, H. M., Oppenheim, J. S. 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. *J. Exp. Med.* 156: 1780-93
70. Murray, H. W., Spitalny, G. L., Nathan, C. F. 1985. Activation of mouse peritoneal macrophages in vitro and in vivo by interferon- γ . *J. Immunol.* 134: 1619-22
71. Trichieri, G., Perussia, B. 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. *Immunol. Today* 6: 131-36
72. Boom, W. H., Liano, D., Abbas, A. K. 1988. Heterogeneity of helper/inducer T lymphocytes. II. Effects of interleukin 4 and interleukin 2-producing T cell clones on resting B lymphocytes. *J. Exp. Med.* 167: 1352-63
73. Rasmussen, R., Takatsu, K., Harada, N., Takahashi, T., Bottomly, K. 1988. T cell-dependent hapten-specific and polyclonal B cell responses require release of interleukin 5. *J. Immunol.* 140: 705-12
74. Sanderson, C. J., O'Garra, C. J., Waren, D. J., Klaus, G. G. B. 1986. Eosinophil differentiation factor also has B cell growth factor activity: proposed name interleukin 4. *Proc. Natl. Acad. Sci. USA* 83: 437-40
75. Sher, A., Coffman, R. L., Hieny, S., Scott, P., Cheever, A. W. 1989. Interleukin-5 (IL-5) is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. *Proc. Natl. Acad. Sci. USA* In press
76. Coffman, R. L., Seymour, B. W. P., Hudak, S., Jackson, J., Rennick, D. 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245: 308-10