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Administration of an anti-IgE antibody inhibits CD23 expression and IgE production *in vivo*

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SUMMARY

High IgE responder BDF1 mice were immunized intraperitoneally (i.p.) with dinitrophenol$_4$ (DNP_4)–ovalbumin (OVA) in alum concomitant with intravenous (i.v.) administration of an anti-IgE monoclonal antibody (mAb). IgE levels were undetectable in mice treated with the anti-IgE antibody, whereas mice treated with isotype-matched irrelevant mAb had IgE levels comparable to that of untreated, immunized mice. Subsequent antigen challenges with DNP_4–OVA, either at weekly or monthly intervals, failed to evoke an IgE response for greater than 2 months in mice treated with anti-IgE during the primary sensitization, even though the terminal half-life of the anti-IgE antibody was 7 days. This inhibition was specific for DNP_4–OVA since the DNP_4–OVA-suppressed mice were able to respond to keyhole limpet haemocyanin (KLH). To investigate the effects of antibody treatment at the cellular level, passive transfer experiments were performed. The primary DNP-specific IgE response of adoptive transfer recipient mice was the same whether the donor cells were from mice treated with IgG or anti-IgE. Transfer of enriched T- or B-cell populations indicated that T-cell help was not compromised by administration of the anti-IgE mAb. However, splenocytes from the anti-IgE-treated mice failed to synthesize IgE *in vitro*, and flow cytometric analysis of B cells from anti-IgE-treated mice showed a dose-dependent decrease in $CD23^+$ cells following antibody treatment, which correlated with decreased serum IgE levels. Taken together, the results of these studies suggest that anti-IgE treatment suppresses IgE responses via effects on B cells rather than T cells, possibly through effects on CD23-dependent pathways.

INTRODUCTION

Since the discovery of IgE and its central role in the pathogenesis of allergic diseases,[1] many studies have focused on ways of regulating IgE synthesis. Over the past 20 years numerous investigators have demonstrated inhibition of antigen-specific IgE (as well as IgM and IgG) synthesis by treatment of mice with chemically or physically modified allergen.[2–10] Their findings suggest that differential presentation of antigens can result in suppression, rather than stimulation, of immunoglobulin (Ig) synthesis. Other investigators have shown that neonatal administration of heterologous anti-μ and anti-γ antibodies leads to suppression of serum IgE levels.[11–14] However, treatment with anti-μ or anti-γ also resulted in inhibition of other Ig isotypes[11,12,14] and produced profound changes in the composition of the peripheral T-cell pool.[13] Long-term selective inhibition of IgE isotype synthesis has been achieved in mice by administration of polyclonal anti-IgE[15,16] or syngeneic IgE.[17] Haba and Nisonoff[18] also demonstrated a relationship between the capacity of neonatal mice to produce antibodies against syngeneic IgE and long-term inhibition of IgE synthesis. In contrast, there is a report of a single injection of a polyclonal anti-IgE antiserum into rats actually increasing the differentiation of IgE secretory cells.[19]

Building on this body of work, our studies were aimed at examining the effect of treatment with a monoclonal anti-IgE antibody on antigen-specific IgE synthesis in adult, rather than neonatal, mice. Young adult BDF1 mice were treated with an anti-IgE antibody concomitant with antigen immunization. This regimen resulted in significant decreases in antigen-specific IgE synthesis without substantially altering IgG levels and without detectably altering T-cell help. Splenocytes taken from the anti-IgE-treated mice also failed to synthesize IgE *in vitro*. Furthermore, there were significantly fewer splenic B cells expressing CD23 following anti-IgE treatment compared to immunized isotype-treated controls. The effect on CD23 expression was dose-dependent and correlated with the decrease in serum IgE levels in the antibody-treated mice. A recent report indicates that IgE responses can be inhibited by *in vivo* administration of an anti-CD23.[20] The mode of action of

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the anti-CD23 antibody is proposed to be interference with binding to CD21, a newly described ligand for CD23[21] whose interaction with CD23 has been shown to be involved in IgE synthesis. Thus, the decrease in CD23 expression observed following anti-IgE treatment would lead to decreases in CD21 interactions and may contribute to the diminished IgE response.

MATERIALS AND METHODS

Animals

High IgE responder BDF1 mice were purchased from Charles River (Portage, MI) at 6–8 weeks of age. The mice, stated by the supplier to be free of adventitious agents, were allowed to acclimatize to our animal care facility for 1 week prior to use in experiments.

Reagents

Purified rat anti-mouse IgE (clone R35–92) and purified rat IgG1 isotype control antibody were purchased from Pharmingen (San Diego, CA). The rat anti-mouse IgE antibody binds both soluble IgE and IgE on mast cells and B cells; it is capable of causing histamine release *in vitro* (data not shown). Dinitrophenol (DNP) was conjugated to ovalbumin (OVA) or human serum albumin (HSA) (Sigma, St Louis, MO) as previously described.[22] Alum adjuvant was purchased from Intergen (Purchase, NY).

Immunization protocols

Groups of six mice per treatment regimen were immunized intraperitoneally (i.p.) with 1 μg DNP_4–OVA in 1 mg alum. Some mice were also immunized i.p. with 5 μg keyhole limpet haemocyanin (KLH) in 1 mg alum. Unless otherwise indicated, immunized mice were untreated, treated intravenously (i.v.) with 100 μg anti-IgE or treated i.v. with 100 μg IgG isotype-matched irrelevant antibody, either in 20-μg doses for five consecutive days or as a single bolus concomitant with immunization. Mice were then boosted i.p. every 4 weeks with 1 μg DNP_4–OVA in 1 mg alum.

In vitro *immunoglobulin synthesis*

Splenocytes (2×10^5 cells/ml) from anti-IgE or isotype control-treated mice were incubated in RPMI-1640 medium containing 10% fetal bovine serum (Gibco, Grand Island, NY) and supplemented with glutamine, penicillin–streptomycin, and 5×10^{-5} M 2-mercaptoethanol. Cultures were incubated in 5% CO_2 at 37°, harvested at 96 hr, and the total IgE and IgG concentrations in the supernatants were measured using the ELISA format described.

Immunoglobulin determinations

Sera from weekly bleeds were assessed for total IgE and antigen-specific IgG levels by ELISA. Total IgE was measured by coating flat-bottomed microtitre plates with 2 μg/ml of polyclonal rabbit anti-mouse IgE (Genentech, S. San Francisco, CA) in 0·05 M Na_2CO_3 buffer, pH 9·6, overnight at 4°. Monoclonal IgE (Pharmingen) was used as the reference standard. Serial dilutions of serum samples from the treatment groups were then added to the coated wells and the amount of murine IgE which bound to the plate was detected using biotinylated FcεRI IgG immunoadhesion[22] followed by streptavidin–horseradish peroxidase (Zymed, S. San Francisco, CA). The presence of the anti-IgE antibody in the samples had no effect on the ability to detect serum IgE using this assay format. IgG anti-DNP antibodies in the test sera were also measured by ELISA using rat anti-DNP–OVA (Zymed) as the reference standard. Each well was coated with 2·5 mg/ml DNP coupled to HSA (DNP–HSA) for 24 hr at 4°.[23] The test sera were added to the antigen-coated plates and the amount of IgG which bound to antigen was detected using a 1 : 5000 dilution of goat anti-mouse IgG horseradish peroxidase-conjugated antiserum (Boehringer Mannheim, Indianapolis, IN). Antigen-specific IgE was determined by passive cutaneous anaphylaxis (PCA) reaction in rats, as previously described, [24] or by ELISA. The assay format was identical to that described for detecting DNP-specific IgG except the detecting reagent was biotinylated FcεRI IgG immunoadhesion followed by streptavidin–horseradish peroxidase.

Adoptive transfer

Donor mice for adoptive transfer experiments were immunized and either treated with anti-IgE antibody or treated with IgG irrelevant antibody. After 4 weeks the mice were killed, their spleens removed, and single-cell suspensions were prepared by triturating the organs between frosted glass slides. Following density gradient separation to remove the red cells, 5×10^6 viable splenocytes from each of the antibody-treated groups, or enriched B- or T-cell subpopulations from the anti-IgE-treated mice, were transferred into naive recipient mice via a lateral tail vein. B- and T-cell fractions were prepared using magnetic bead separation with the MACS separator system (Becton Dickinson, Palo Alto, CA). B-cell enriched populations were prepared by depletion of T cells using 20 μl of anti-Thy-1.2 microbeads (Becton Dickinson). Likewise, T-cell enriched populations were prepared by depletion of B-cells using 20 μl of B220 microbeads (Becton Dickinson). The degree of enrichment was assessed by FACS analysis. Unfractionated splenocyte fractions contained 62% $B220^+$ and 19% Thy-1^+ cells. B-cell enriched populations were 83% $B220^+$, while T-cell enriched populations were 68% Thy-1^+. Two hours following adoptive transfer of the various cell populations, the recipients, along with naive control mice, were immunized i.p. with 1 μg DNP_4–OVA in 1 mg alum.

Immunofluorescence and flow cytometry

At the indicated times after antigen challenge and anti-IgE antibody treatment, mice were killed, their spleens removed and cells dispersed using frosted glass slides. The single-cell suspension was subjected to an LSM (Organon Teknika Corporation, Durham, NC) density gradient, the cells at the interface washed twice, then resuspended at 5×10^6 cells/ml in phosphate-buffered saline (PBS) containing 0·1% bovine serum albumin (BSA) and 0·01% sodium azide. Splenocytes were sequentially stained for B220/CD45R (B-cell marker) expression and CD23 (low affinity IgE receptor) expression by incubation with appropriate concentrations of antibody specific for murine B220 conjugated to Red613 (Gibco), followed by fluorescein-conjugated anti-CD23 antibody (Pharmingen). Cells were analysed by gating on the lymphocyte population and examining $B220^+$ $CD23^+$ cells by two-colour fluorescence using a FACScan flow cytometer (Becton Dickinson, San Jose, CA).

Pharmacokinetic studies

The pharmacokinetic properties of anti-IgE monoclonal antibodies mAb were investigated in female BDF1 mice following an i.v. (n = 15) injection of 40 μg/mouse. Mice were killed at the specified times over 30 days (1 mouse/time-point) and blood was collected. The serum concentration of mAb was determined by a solid-phase assay using anti-rat IgG to capture anti-IgE mAb, and biotinylated mouse IgE at a saturating concentration for detection.

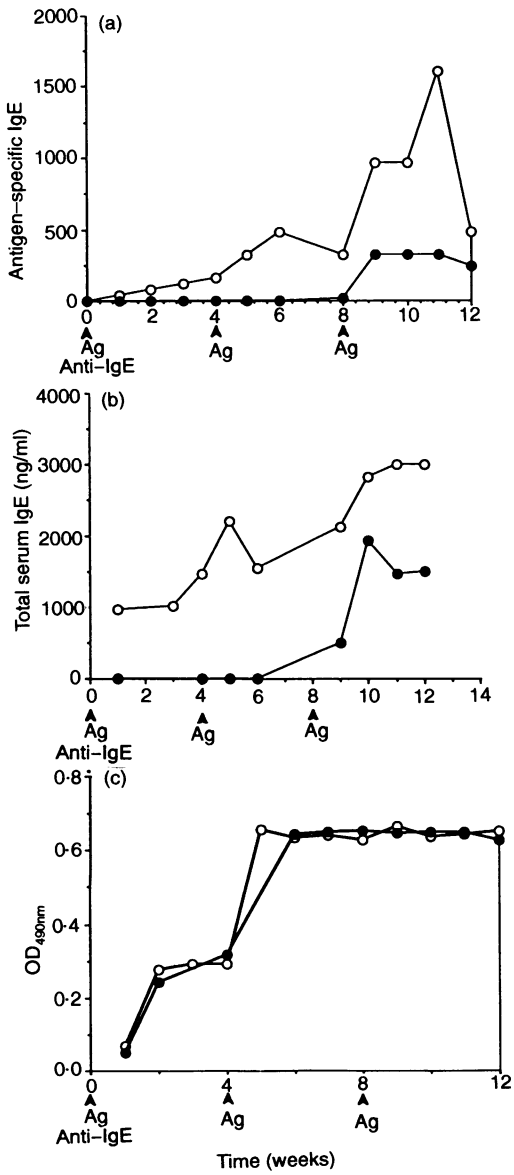

Figure 1. Anti-IgE antibody-mediated inhibition to IgE synthesis, but not IgG synthesis, in response to antigen (Ag) immunization. Mice were immunized i.p. (arrows) with 1 μg DNP_4–OVA in 1 mg alum and treated i.v. with either 20 μg IgG isotype-matched irrelevant antibody (○) or 20 μg anti-IgE mAb (●) on days 0, 1, 2, 3 and 4. The serum DNP-specific IgE titres (a) were determined by passive cutaneous anaphylaxis reaction in rats. Total serum IgE (b) and DNP specific IgG titres (c) were assessed by ELISA (n = 6 mice/group).

Statistical analysis

Statistically significant differences between treatment groups were established by analysis of variance; follow-up comparisons between individual groups were made using Duncan's multiple range test. Differences were judged to be statistically significant if $P < 0{\cdot}05$. In the text and figures all data shown are means and standard deviations.

RESULTS

Anti-IgE antibody treatment suppresses IgE synthesis in mice

BDF1 high IgE responder mice were immunized i.p. with 1 μg DNP_4–OVA in 1 mg alum on day 0. On days 0–4, mice were treated with 20-μg doses of anti-IgE mAb or an isotype-matched control antibody (Fig. 1). Mice were rechallenged with antigen again at 4 and 8 weeks. There was no effect of the irrelevant IgG antibody treatment compared to untreated mice on any parameters tested. However, treatment with anti-IgE antibody resulted in long-term inhibition of both antigen-specific (Fig. 1a) and total IgE synthesis (Fig. 1b). The observed suppression lasted throughout the primary and secondary antigen challenge. Serum IgE was completely undetectable until after the third antigen injection, at which time levels of IgE were measurable in the serum of mice treated with anti-IgE antibody. There was no detectable effect on the level of antigen-specific serum IgG as a result of anti-IgE antibody treatment (Fig. 1c).

Subsequent studies revealed that anti-IgE antibody treatment was equally effective in suppressing IgE synthesis whether administered over a 5-day period or in a single injection concomitant with i.p. antigen immunization. Therefore, in subsequent studies a single i.v. injection of antibody was administered. The observed inhibitory effect was dose dependent. As shown in Fig. 2, 50 or 100 μg of anti-IgE antibody, when given at the time of antigen priming, resulted in undetectable primary or secondary IgE responses, while a 20-μg dose inhibited the primary response but failed to affect the secondary IgE response.

A single i.v. injection of 100 μg anti-IgE antibody, along with antigen challenge, suppressed IgE synthesis even in the

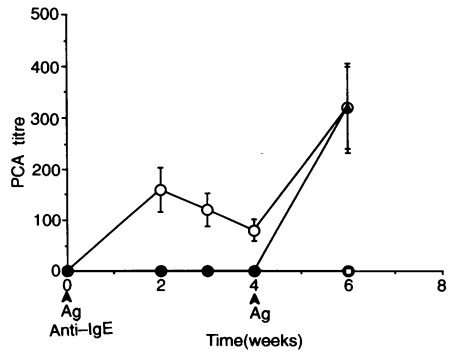

Figure 2. Dose–response of anti-IgE antibody-mediated inhibition of IgE synthesis. Mice were treated i.v. with either 100 μg IgG (○), 100 μg anti-IgE (●), 50 μg anti-IgE (□) or 20 μg anti-IgE (▲) concomitant with i.p. immunization on day 0. Mice were challenged with DNP_4–OVA antigen on week 4. Antigen-specific IgE was determined by passive cutaneous anaphylaxis (n = 6 mice/group).

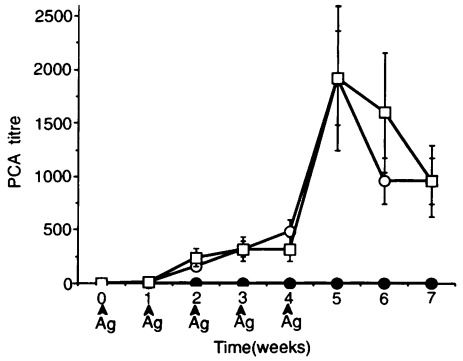

Figure 3. Effect of weekly antigen challenge on anti-IgE antibody-mediated IgE inhibition. Mice were treated with a single i.v. injection (○) of 100 μg IgG isotype-matched control antibody or anti-IgE mAb (●) on day 0, then immunized five times at weekly intervals with 1 μg DNP_4–OVA in 1 mg alum. Control mice were immunized on days 0 and 28 to assess the effects of multiple antigen challenges on the IgE response (□). Serum levels of antigen-specific IgE was assessed using passive cutaneous anaphylaxis (n = 6 mice/group).

face of chronic antigen challenge (Fig. 3). In this study, mice were treated with antibody at the time of primary immunization and challenged for 5 consecutive weeks with DNP_4–OVA in alum. Antigen-specific IgE titres were decreased throughout the course of the experiment, even though the mice were treated only once with the anti-IgE mAb. The chronic immunization regimen itself had no apparent suppressive effects on the level of IgE produced in the sham-treated mice.

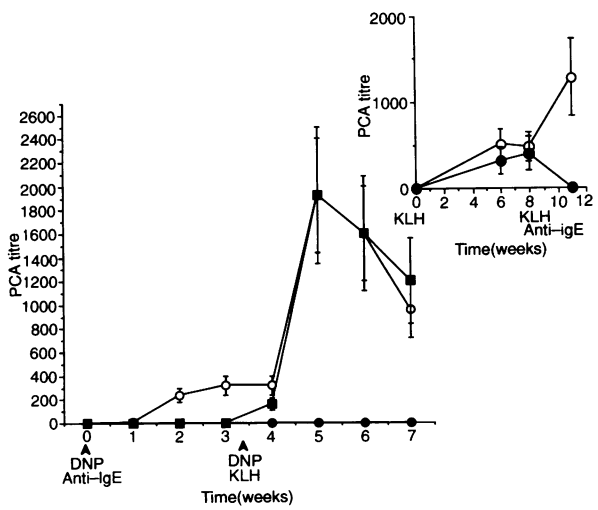

Figure 4. Anti-IgE antibody-treated mice respond following alternative antigen challenge. Mice were immunized with DNP_4–OVA and treated with either 100 μg IgG control antibody (○) or 100 μg anti-IgE antibody (●). At week 3, one group of anti-IgE-treated mice was immunized i.p. with 5 μg KLH in 1 mg alum (■). At week 4, all treatment groups were challenged with DNP_4–OVA. Inset: mice were immunized with 2 μg KLH in alum. At week 6, mice were rechallenged with KLH and treated with 100 μg IgG (○) or anti-IgE (●). Serum antigen-specific IgE levels were determined weekly by passive cutaneous anaphylaxis (n = 6 mice/group).

Immunization with an alternative antigen, KLH, results in IgE synthesis in mice suppressed to DNP_4–OVA

Studies were undertaken to determine if treatment with anti-IgE antibody would affect the response to a second antigen given during the time the anti-DNP IgE responses were undetectable. Immunization with KLH 4 weeks after anti-IgE treatment produced normal KLH-specific IgE responses in these mice (Fig. 4). In contrast, when mice with suppressed DNP-specific IgE responses were re-immunized with DNP_4–OVA at this same 4-week interval, these mice failed to mount a detectable DNP-specific IgE response. These results cannot be explained by the need for higher concentrations of anti-IgE antibody since comparable antibody concentrations (100 μg) inhibited both DNP and KLH responses (Fig. 4, inset).

Anti-IgE treatment *in vivo* also suppresses *in vitro* IgE synthesis

In a separate set of experiments, DNP–OVA-immunized mice were treated with the anti-IgE or isotype-control antibody. Spleens were harvested from these mice 1 week after antibody administration and cultured to determine if the effects on IgE synthesis persisted *in vitro*. After 96 hr, IgE levels of 60 ng/ml were measured in the splenocyte cultures from the IgG-treated mice. In contrast, no IgE was detectable in the cultures prepared from the anti-IgE-treated mice (Fig. 5). As was seen *in vivo*, *in vitro* addition of the anti-IgE antibody to splenocytes from IgG-treated mice depressed IgE synthesis to 50% of the control value. There was no difference in IgG levels between the treated or untreated mice.

Anti-IgE antibody treatment does not alter the capacity of splenocytes to adoptively transfer T-cell help

To determine whether anti-IgE antibody treatment affected the functionality of the T-cell and/or B-cell compartment, adoptive

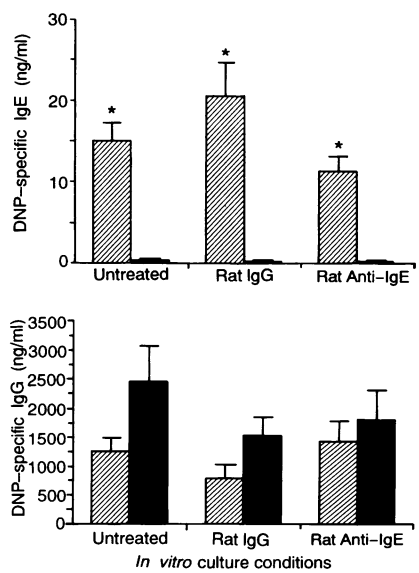

Figure 5. Anti-IgE treatment *in vivo* suppresses synthesis by splenocytes *in vitro*. Spleen cells from IgG- (□) or anti-IgE- (■) treated mice were cultured for 96 hr and antigen-specific IgE and IgG levels were assayed by ELISA. Results are means ± SD.* Significance at $P < 0.05$.

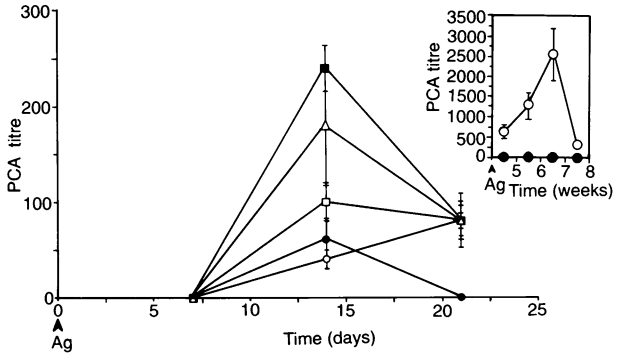

Figure 6. Anti-IgE antibody treatment has no effect on the capacity of splenocytes to passively transfer T-cell help. Donor mice were immunized i.p. with 1 μg DNP_4–OVA in 1 mg alum and treated with 100 μg IgG control antibody (○) or 100 μg anti-IgE antibody (●). At 4 weeks, half of the mice were challenged with antigen (inset) and the remainder were killed to obtain spleen cells for adoptive transfer. Naive recipient mice received no cells (○), 5×10^6 viable splenocytes i.v. from either IgG antibody control-treated donors (□) or from anti-IgE antibody-treated donor mice (■), or purified T cells (△) or B-cells (●) from anti-IgE-treated donors. Two hours after adoptive transfer, recipient mice were immunized with DNP_4–OVA. Antigen-specific IgE titres were assessed weekly (n = 6 mice/group).

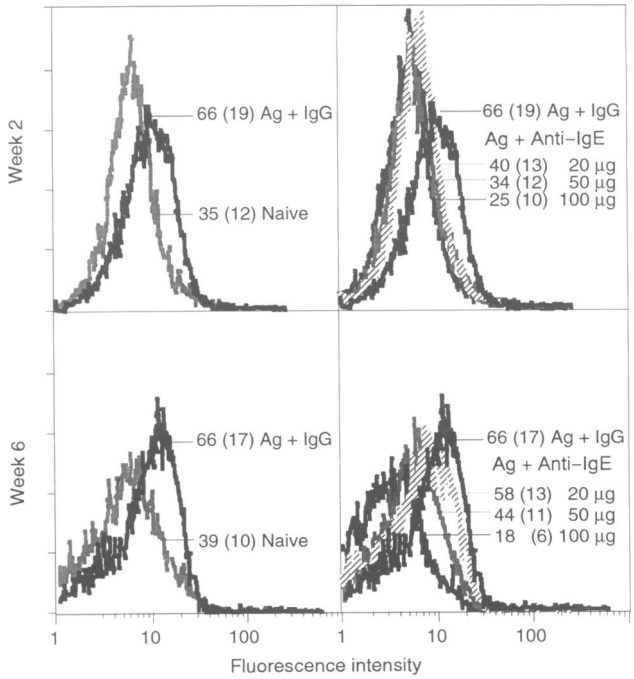

Figure 7. CD23 expression on splenic B cells. Spleen cells from control mice and mice treated with various concentrations of anti-IgE antibody were examined 2 weeks after a primary (week 2) or 2 weeks after a secondary (week 6) antigen challenge for CD23 expression on $B220^+$ B cells. Numbers represent percentage $B220^+$ $CD23^+$ cells in the spleens of mice treated with the various doses of anti-IgE antibody. Numbers in parentheses represent mean fluorescence intensity.

transfer experiments were performed. Mice were treated with either IgG isotype-matched control antibody or with anti-IgE mAb at the time of immunization. Four weeks later, half the mice in each of the treatment groups were rechallenged with antigen to confirm suppression of IgE synthesis (Fig. 6, inset). The remaining mice were killed and splenocytes, enriched B-cell or T-cell subpopulations were passively transferred into naive recipients, and the recipients were then immunized with DNP_4–OVA (Fig. 6). A shift in the kinetics of the IgE response was detectable in the sera of mice that received unfractionated splenocytes or the T-cell enriched fraction from immunized donors compared to sham reconstituted mice. This suggests that T-cell priming had occurred in the antigen-IgE-treated mice. This is further supported by the observation that, in mice adoptively transferred with unfractionated splenocytes or the enriched T-cell fraction, the peak IgE titres were similar to the levels normally attained at a secondary antigen response. In contrast, there was no difference in the magnitude or kinetics of the antigen-specific IgE response in mice that received only B cells from IgE-suppressed donors compared to sham adoptive-transfer controls. If anything, the IgE response of mice given the B-cell enriched fraction was more transient than the sham controls.

Anti-IgE mAb treatment alters CD23 expression concomitant with suppression of IgE synthesis

To further investigate the effects of anti-IgE mAb treatment on B-cell populations, mice were immunized and treated with various doses of the anti-IgE antibody, killed at the specific times and their splenocytes removed and stained for surface marker expression (Fig. 7). Expression of CD23, the low-affinity receptor for IgE, was up-regulated as a result of immunization. When examined 2 weeks after antibody administration, it was apparent that treatment with 20, 50 or 100 μg of the anti-IgE antibody prevented this increase in CD23-expressing cells. Indeed, the percentage of $CD23^+$ cells in these mice was similar to the levels found for splenic B cells from unimmunized mice. Over time, the number of $CD23^+$ splenic B cells increased in the anti-IgE-treated mice. However, only the mice treated with the 20-μg dose of anti-IgE attained levels comparable to that of control immunized mice by week 6. Interestingly, the enhanced CD23 expression correlated with loss of inhibition of IgE levels in this group of anti-IgE-treated mice.

Pharmacokinetics of anti-IgE mAb in mice

To determine the pharmacokinetic profile of anti-IgE mAb, mice were injected i.v. with 40 μg mAb, whereafter serum

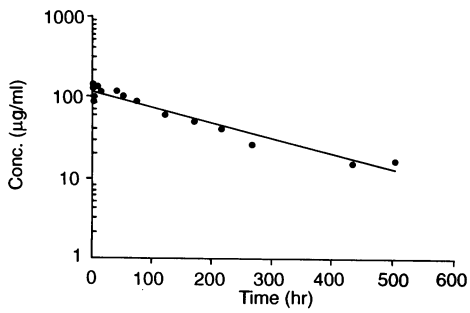

Figure 8. Pharmacokinetic profile of anti-IgE mAb in mice. The i.v. serum concentration versus time data were described by a biexponential equation ($C_t = 4{\cdot}53e^{-50{\cdot}84t} + 1{\cdot}55e^{-0{\cdot}45t}$).

concentrations of the antibody were determined (Fig. 8). The terminal half-life of anti-IgE mAb was approximately 7 days.

DISCUSSION

Other investigators have demonstrated potent immunosuppressive effects on humoral immunity by administration of anti-immunoglobulin antibodies.[11,12] Indeed, class-specific Ig suppression has been achieved when the appropriate anti-isotype antisera were used for the treatments .[16,25,26] We have extended these previous findings to demonstrate that administration of an anti-IgE antibody in the context of antigen can inhibit antigen-specific IgE synthesis in adult mice.

The duration of inhibition of the IgE response was dose-dependent, since administration of 20 μg of the anti-IgE antibody maintained suppression for 4 weeks while the injection of 100 μg of the antibody suppressed the IgE response for over 2 months. This suggests that a critical concentration of the antibody was required to maintain suppression. This decrease in IgE levels cannot be explained by an inability to detect IgE bound to the anti-IgE antibody, since the ELISA format was developed to allow quantification of both free and antibody-complexed IgE. Likewise, since the anti-IgE antibody does not block binding of IgE to the high-affinity receptor on mast cells, it has no ability to interfere with detection of antigen-specific IgE by PCA (data not shown). It is also unlikely that the observed suppression of the IgE response to DNP–OVA can be explained by the anti-IgE antibody merely removing serum IgE through normal immune complex clearance, since the duration of the inhibition was longer lasting than would be expected for an antibody with a serum half-life of 7 days. This is further supported by the values obtained for serum IgE in these mice. When total blood volume is taken into consideration, the total serum IgE was greater than the amount of anti-IgE which was administered. Also, immunization with a second antigen, KLH, 3 weeks after treatment with the anti-IgE antibody, elicited a primary response to KLH while the IgE response to a secondary challenge with DNP–OVA remained suppressed. The response to KLH could be explained by a decline in the serum concentrations of the anti-IgE antibody to levels insufficient to induce suppression. However, these animals remained unresponsive to challenge with DNP–OVA at the 4-week time-point. Taken together, these data suggest that a portion of the B-cell population was anergic toward DNP–OVA. This is further supported by the ability of these animals to respond to tertiary challenge with DNP–OVA at week 10 with an IgE response similar in magnitude and kinetics to the tertiary response in the control mice. Thus it appears that the DNP–OVA-responsive B-cell population in the anti-IgE-treated animals was primed but anergic during the primary and secondary antigen challenges.

The adoptive transfer experiments indicate that transfer of a B-cell enriched fraction from antigen-primed and anti-IgE-treated mice had no positive effect on the response of the naive recipients to a primary immunization. This is in contrast to the transfer of unfractionated or T-cell-enriched splenocyte fractions from antigen-primed and anti-IgE-treated mice. Upon primary antigen exposure, recipients receiving unfractionated splenocytes from anti-IgE-treated mice produced an IgE response equivalent in magnitude to a secondary response and roughly equivalent to the response of mice given unfractionated cells from isotype-control mice. Mice receiving the fraction enriched for T cells mounted an ever higher response, which probably reflects an increase in the proportion of T-helper cells due to fractionation. Thus it appears that T-cell help was unaffected by antibody treatment.

Given the effects seen in these experiments on B-cell responsiveness and the extensive body of literature on the role of CD23-bearing B cells in IgE regulation, we examined the $CD23^+$ B-cell population in the anti-IgE-treated mice. Many of the reports of down-regulation of Ig synthesis by anti-Ig treatment show an integral role for binding to CD23 and inhibition of IgE responses.[27,28]

Following antibody treatment, there appeared to be a dose-dependent inhibition of the up-regulation of CD23 expression which occurs upon immunization. This decrease in CD23 expression paralleled the decreased response to DNP–OVA. Furthermore, the recovery of the IgE response to DNP–OVA was accompanied by increased expression of CD23. In light of the report that an anti-CD23 mAb inhibited *in vivo* IgE synthesis via blocking CD21/23 interactions,[20] one might speculate that the B-cell anergy and inhibition of antigen-specific IgE production following anti-IgE resulted from the failure to up-regulate CD23.

FCεRII can act quite efficiently in the capture and presentation of antigens associated with IgE[29] and may preferentially present antigen for IgE T-helper type 2 (Th2) responses. Since the presentation of certain antigens has been associated with either Th1 or Th2 cell development, one of the IgE feedback mechanisms may be linked to the presentation of IgE-bound antigen(s) which stimulates Th2 cell development. If the anti-IgE antibody is interfering with IgE synthesis by blocking interactions with CD23-bearing B cells, this could explain the ability of relatively low doses of the anti-IgE antibody to eliminate the very high IgE levels stimulated by this immunization protocol.

Since interleukin-4 (IL-4) is known to up-regulate CD23 expression, it is possible that antibody treatment interfered with IL-4 production. While serum IL-4 levels were undetectable in these mice, IL-4 levels were measurable in a murine model of *Schistosoma mansoni* which leads to exaggerated IgE production (Amiri *et al.*, manuscript in preparation). In those experiments, serum IL-4 levels rose to detectable levels by week 7 of the infection. Interestingly, anti-IgE treatment of *Schistosoma*-infected mice completely prevented this rise in IL-4 levels. When examined *in vitro*, concanavalin A-stimulated splenocytes from antibody-treated mice produced less IL-4 than splenocytes from control mice. Thus the decrease in serum IL-4 may result from the decrease in IL-4 secretion by T cells. Alternatively the decrease in IgE levels produced by antibody treatment could lead to a decrease in mast cell degranulation and result in decreased release of IL-4 from the granules. Mast cells granules have been shown to be an important source of IL-4.[30] In the studies described herein, inhibition of release of IL-4 locally by either of these mechanisms may have contributed to the decrease in CD23 expression.

It is also possible that the decreased serum concentrations of IgE contributed to the lower CD23 levels, since binding of IgE to CD23 has been shown to stabilize FcεRII expression at the cell surface;[31] or treatment with the anti-IgE antibody may have cross-linked cell FcεRII (sCD23) via bound IgE, resulting in loss of CD23 from the cell surface, which has been shown to

increase receptor internalization and degradation.[32] This could also lead to a reduction in the amount of soluble FCεRII released from the B-cell surface.[33] Production of sCD23 is one of the putative roles for CD23$^+$ B cells in the regulation of the IgE response. However, since Fab fragments of the antibody produced results similar to that of the intact antibody this is an unlikely explanation (data not shown).

Alternatively, the anti-IgE antibody may be acting via binding to membrane IgE. Yuan[34] showed that anti-Ig inhibited lipopolysaccharide (LPS)-induced Ig synthesis by specific inhibition of transcriptional factors required for enhanced initiation of sIg mRNA. Chen *et al.*[35] extended these findings to show that down-regulation of endogenous μ-mRNA following anti-μ treatment was a result of decreased OTF2 DNA binding. Experiments are under way to determine the effect of anti-IgE treatment on the transcriptional regulation of IgE synthesis. Alternatively, anti-IgE treatment may lead to clonal deletion of specific IgE-bearing B cells. This seems unlikely, however, since in other experiments we have seen no decrease in IgE splenic B cells following antibody treatment.

Regardless of the mechanism, treatment of mice with an anti-IgE produces potent inhibition of IgE responses. There is increasing evidence that a complex interaction of cytokines, such as IL-4 and IL-13, and regulatory factors, such as the soluble FcεRII or IgE 'binding' factor, play a role in regulation of IgE responses. It will be important to determine the relative contributions of each of the events outlined in the overall scheme of IgE synthesis.

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