Regulation of Immunoglobulin E Antibody Synthesis in Man by Antiidiotypic Antibodies

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ABSTRACT The effect of rabbit antiidiotypic antibody raised against human IgG F(ab')₂ anti-tetanus toxoid (TT) antibodies on the in vitro synthesis of TT-specific IgE antibody by peripheral blood lymphocytes (PBL) was examined in three subjects. Two of these subjects were allergic twins whose sera persistently contained IgE anti-TT antibodies. The third subject was a nonallergic individual who had a slightly elevated serum IgE (250 IU/ml) and who exhibited a transient serum IgE anti-TT response after booster immunization with TT. After appropriate absorptions rabbit anti-idiotypic (Id) IgG reacted with anti-TT antibodies of both IgG and IgE isotypes in an idiotype- and antigen-specific fashion. PBL and B cells from the three subjects studied spontaneously synthesized TT-specific IgE in culture. In all three cases, adsorption of B cells over plastic plates coated with anti-Id before culture specifically decreased the synthesis of IgE antibodies to TT but did not affect the synthesis of IgE antibodies to ragweed antigen E by PBL from the twin allergic subjects. Addition of anti-Id to cultures of PBL from all three subjects specifically inhibited the synthesis of TT-specific IgE. This inhibition was shown to be exerted both at the level of the B cells and via the generation of antigen-specific suppressor T cells from radiosensitive precursors.

The present results indicate that the synthesis of antigen-specific IgE in man is subject to regulation by idiotype anti-idiotypic interactions that can involve both B and T lymphocytes.

INTRODUCTION

Anti-Id antibodies have been shown to regulate immunoglobulin G (IgG) synthesis in experimental animals and in man (1-5). Recently, it has been shown in experimental animals that antibody of the IgE isotype shares idiotype determinants with antibody of

the IgG isotype (6). Furthermore, Blaser et al. (7) have also shown that the in vivo IgE antibody response in mice is subject to regulation by idiotype antiidiotypic interactions.

The role of anti-idiotypic (Id)¹ antibodies in the regulation of the human IgE response is unknown. Recently, it was demonstrated in our laboratory that human IgG and IgE antibodies to tetanus toxoid (TT) share idiotype determinants (8) and that autoanti-Id antibodies arise during the course of the normal human immune response to TT antigen (9). These findings strongly raised the possibility that idiotype antiidiotypic interactions may regulate the synthesis of human IgE. In the present communication we demonstrate that rabbit antibodies raised against Id determinants of human anti-TT antibodies can regulate the in vitro synthesis of anti-TT antibodies of the IgE isotype.

METHODS

Subjects. Three subjects were studied. Two of the subjects were 21-yr-old male identical twins. They had allergic rhinitis caused by multiple allergens, positive immediate hypersensitivity skin tests to multiple allergens, including ragweed, and elevated serum IgE levels (850 and 890 IU/ml, respectively). Their sera studied serially over 1 yr before the present study were found persistently to contain IgE antibodies to TT as determined by the radioallergosorbent test (mean binding of ¹²⁵I anti-IgE to TT-coupled disks in the radio-allergo-sorbent test (RAST) assay, 9.3 and 8.5%, respectively). Both subjects were studied in the fall; October through mid-November of 1980. The third subject studied was a 29-yr-old female subject who showed no positive immediate hypersensitivity skin tests to a battery of 36 common allergens and whose serum contained 250 IU/ml of IgE. 1.5 yr before the present study this subject was given a booster immunization with TT. At that time it was noticed that IgE

¹ Abbreviations used in this paper: AgE, ragweed antigen E; Anti-Id, antiidiotypic; FCS, fetal calf serum; HBSS, Hanks' Balanced Salt Solution; HSA, human serum albumin; Id, idiotype; RAST, radio-allergo-sorbent test; TT, tetanus toxoid.

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anti-TT antibodies became detectable in her serum starting the 6th d postimmunization until the end of the 2nd mo postimmunization (maximal percent binding in the RAST assay, 5.8%).

Immediately before the present study all three subjects were boosted with TT (5Lf units). IgE antibodies reappeared in the serum of the third subject for a period extending from the 5th d postimmunization to the end of the 3rd mo postimmunization (maximum percent binding in the RAST assay 6.4%).

The purposes of the study were explained to the subjects and their written consent was obtained.

Antigens and antisera. TT and DT antigens were obtained from Massachusetts Biological Laboratories purified over Sephadex G-200 columns and radiolabeled with ^{125}I as described in reference 10. Ragweed antigen E was obtained from Calbiochem Laboratories, La Jolla, CA and extensively dialyzed against phosphate-buffered saline (PBS). Human serum albumin (HSA) was obtained from the American Red Cross. Rabbit anti-human IgE raised in rabbits against IgE myeloma P.S. (a kind gift of Dr. Ishizaka) was absorbed extensively with pooled human IgG, IgM, and IgA myelomas and human kappa lambda Bence Jones proteins. Rabbit anti-human IgE used to coat the plastic plates in the solid-phase radioimmunoassay was immunosorbent purified over Sepharose 4B cross-linked to P.S. myeloma IgE. By radioimmunoassays this immunosorbent purified rabbit anti-human IgE bound ^{125}I IgE (P.S.) and ^{125}I IgE (N.D.) myeloma proteins, but not human IgG, IgM, and IgA myelomas. The ^{125}I rabbit antihuman IgE used in the radioimmunoassay was purchased from Pharmacia Fine Chemicals, Piscataway, NJ, as the RAST reagent and had a specific activity of 12 $\mu\text{Ci}/\mu\text{g}$.

Determination of IgE and of antigen-specific IgE antibodies in the serum. Total IgE and antigen-specific IgE were determined in the serum using the commercially available PRIST and RAST assays (Pharmacia Fine Chemicals). TT and AgE were cross-linked to cyanogen bromide-activated cellulose disks as described in reference 11. The specificity of the RAST was ascertained by showing that purified IgE but not IgG (10 μg each) completely blocked the binding of ^{125}I -anti-IgE to the antigen-coated disks preincubated with a known positive serum.

Production, absorption, and characterization of anti-idiotypic antisera. Idiotypic antisera were raised in rabbits against IgG (Fab) $_2$ anti-TT antibodies isolated from the serum of each of the three subjects studied. The IgG (Fab) $_2$ anti-TT was obtained 7 and 10 d after a booster immunization with TT that was given 1 yr or more before the present study. The antisera were prepared as described in reference 10 except that the IgG-enriched fraction of human serum was absorbed extensively against the insolubilized IgG fraction of rabbit anti-human IgE (8). IgG F(ab) $_2$ fragments obtained by pepsin digestion followed by Sephadex G-150 chromatography were passed over a TT-immunosorbent column pretreated with NaSCN. The material that did not bind to the column was designated as TT nonreactive IgG F(ab) $_2$. The material that was bound to the TT column (4–7 mg) was eluted with 3 M NaSCN and designated IgG F(ab) $_2$ anti-TT or “idiotype”. This material contained <50 pg/ml of IgE as determined by a solid-phase radioimmunoassay described below. It was used to immunize a rabbit as described in reference 10. IgG was isolated from the rabbit serum by DEAE column chromatography and suspended at 10 mg/ml in PBS. 10 ml of rabbit anti-Id IgG was absorbed twice, respectively, with (a) 2 g of Sepharose-bound TT nonreactive IgG F(ab) $_2$ derived from the idiotype donor. (b) 2 g of Se-

pharose-bound pooled human IgG (Cohn fraction II), (c) 1 ml of packed erythrocytes obtained from the idiotype donor, (d) 1 ml of packed nylon wool purified T cells derived from leukopheresis units, and (e) 1 ml of packed Epstein-Barr virus (EBV) transformed human B cell line.

The capacity of rabbit anti-Id IgG to bind ^{125}I -idiotype and to inhibit the binding of ^{125}I -labeled TT to IgG and to IgE from the idiotype donor were assessed as described in reference 10. 10 μg of F(ab) $_2$ anti-TT were radiolabeled with ^{125}I to a specific activity of 2–4,000 cpm/ng. Each of a set of conical plastic Beckman microfuge tubes (Beckman Instruments, Inc., Wakefield, MA) received 10–20 ng of ^{125}I F(ab) $_2$ anti-TT followed by 100 μl of different dilutions of preimmune or immune rabbit IgG in PBS. After an incubation period of 1 h at 37°C, normal rabbit serum and goat anti-rabbit IgG were added at equivalence (taking into consideration the contribution of preimmune or immune rabbit IgG). The tubes were incubated again at 37°C for 1 h, then at 4°C overnight. The precipitates that formed were washed in a Beckman microcentrifuge for 5 min at 12,000 rpm with ice-cold PBS containing 0.5% human serum albumin and 0.5% Tween 20 (washing buffer). After three washes the precipitates were counted for radioactivity in a Tri-Carb gamma scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). To determine whether anti-Id recognized antigen binding sites on IgG, the dilution of human IgG necessary to precipitate 70% of the total amount of precipitable radiolabeled TT was determined. Each of a set of Beckman microfuge tubes received in succession 10 ng of radiolabeled antigen, different dilutions of preimmune or postimmune rabbit IgG (preabsorbed with Sepharose 4-B cross-linked to TT and DT), and 100 μl of the appropriate dilution of human IgG determined above. After an incubation period of 1 h at 37°C, myeloma IgG and rabbit anti-human IgG were added at equivalence and the precipitates were processed as described above. To determine whether anti-Id inhibited antigen binding to IgE, cellulose disks cross-linked to rabbit anti-human IgE were obtained from Pharmacia Diagnostics and the disks were incubated for 6 h at room temperature with 0.1 ml of a dilution of human serum, which resulted in the binding of 70% of the radioactivity in 10 ng of ^{125}I -TT or ^{125}I -AgE. The disks were then washed extensively with washing buffer, incubated overnight at room temperature with 10 ng of ^{125}I -radiolabeled antigen in the presence or absence of antiidiotypic antiserum. After extensive washing the disks were counted for radioactivity.

Cell isolation and fractionation. Heparinized blood was obtained from the subjects between the 8th and 35th d postimmunization. Mononuclear cells were isolated from peripheral blood over Ficoll-hypaque and washed five times with Hanks' Balanced Salt Solution (HBSS) prior to further fractionation.

T cells were obtained by rosetting mononuclear cells with neuraminidase-treated sheep erythrocytes (E) and recovering the E rosette-forming cells from the bottom of a Ficoll-hypaque gradient after lysis of erythrocytes with Tris-buffered 0.87% NH_4Cl . Cells that did not form rosettes with E were recovered from the top of the Ficoll gradient, and used as a B cell-rich suspension. The details of these procedures and the composition of the cell fractions are found in reference 12.

Cell cultures for the measurement of IgE synthesis in vitro. Cells were suspended at a concentration of $1 \times 10^6/\text{ml}$ in RPMI 1640 medium containing 10% fetal calf serum (FCS), penicillin (100 $\mu\text{g}/\text{ml}$) and streptomycin (50 $\mu\text{g}/\text{ml}$) and cultured in triplicate in 1-ml aliquots in $12 \times 75\text{-mm}$ sterile plastic tubes. After 7 d of incubation in 5% CO_2 in

air at 37°C the culture fluid supernatants were collected and assayed for their content of total IgE and of antigen-specific IgE. At the initiation of the cultures 1-ml aliquots of cell suspension were frozen and thawed three times, allowed to incubate for 7 d at 37°C, then spun at 200 g for 10 min and their supernatants were collected. Parallel 6-d cultures were carried in the presence of cycloheximide (Sigma Chemical Co., St. Louis, MO, 100 µg/ml) to ascertain that the IgE measured at the end of the 6-d culture period represented *de novo* synthesized IgE.

Radioimmunoassay of IgE in culture supernatants. IgE present in culture supernatants were determined by a solid-phase radioimmunoassay as described (13). Triplicate wells of a plastic polyvinyl 96-well microtiter plate (Dynatech Laboratories, Inc., Alexandria, VA) were coated with a 0.4-mg/ml solution of immunosorbent purified rabbit anti-human IgE (P.S.) by placing 200 µl of the reagent in the V-shaped wells. This reagent was derived from the IgG fraction of the serum of a rabbit immunized with purified myeloma P.S. (a kind gift of Dr. Ishizaka). This rabbit IgG was absorbed with insolubilized human myelomas IgG, IgM, IgA, and with K and L Bence Jones proteins, then immunosorbent-purified over an IgE P.S. column. It reacted solely with ¹²⁵I-IgE (P.S. and N.D. the latter a gift of Dr. Johansson, Uppsala, Sweden), but not with ¹²⁵I radiolabeled IgG, IgA, IgM, K, or L chains. The rabbit anti-IgE-containing plates were incubated for 16 h in a humidified chamber at room temperature. The wells were aspirated, washed twice with PBS, filled for 2 h at room temperature with a 10% solution of HSA then washed thrice with PBS-1% bovine serum albumin (BSA). Duplicate wells then received 200 µl of sample or standard and after an overnight incubation at room temperature were aspirated, washed three times in PBS-1%, HSA-0.05%, Tween 20, twice in PBS (washing buffer), and then received 200 µl of ¹²⁵I rabbit anti-human IgE obtained from Pharmacia, Inc. (RAST reagent, sp act, 12 µCi/µg). After a 6-h incubation the wells were aspirated, washed three times with washing buffer, three times with PBS, and five times with distilled water. The wells were then cut and their content of radioactivity was measured in a gamma spectrometer (model 1185, Tracor Analytic, Stamford, CT) by counting over a 10-min interval.

IgE values were read from a standard curve constructed using known amounts of IgE. Nonspecific binding of IgE to plates coated with HSA was negligible (~40 cpm). Also there was negligible binding (<50 cpm) to anti-IgE coated wells that received 0.1 ml of 100 µg/ml solutions of IgG, IgM, and IgA myeloma proteins. We used the commercial Pharmacia reference standards for calibrations. Essentially identical standard curves were obtained. The lower limit of the sensitivity of this assay varied between 50 and 150 pg/ml depending on the interval of time elapsed between the iodination of the rabbit ¹²⁵I-anti-human IgE and the performance of the assay. The amount of *de novo* IgE present in the supernatant of a given culture was determined to be the total amount of IgE present in that supernatant minus the mean IgE present in the supernatants of the triplicate parallel cultures to which cycloheximide has been added.

Radioimmunoassay of antigen-specific IgE in culture supernatants. This assay was performed exactly as the assay for total IgE except that the plates were coated with 1 mg/ml solution of TT antigen or ragweed antigen E.

The net counts per minute of ¹²⁵I anti-IgE bound to antigen-coated wells preincubated with culture supernatants was obtained by subtracting the counts per minute of ¹²⁵I anti-IgE bound in the presence of supernatants of cycloheximide-treated cultures (mean of triplicates usually <100

cpm) from the counts per minute of ¹²⁵I anti-IgE bound in the presence of supernatants from the untreated cultures. The specificity of this assay was established by demonstrating that addition of TT antigen (1 µg/well) to the samples tested blocked completely the binding of ¹²⁵I anti-IgE to the wells. The content of antigen-specific IgE in a given supernatant was compared with the content of antigen-specific IgE in a standard serum pool obtained by pooling of the sera of subjects 1 and 2 at a 1:1 ratio. Dilutions of this serum pool were made in RPMI 1640 medium containing 10% FCS. Nonspecific binding of ¹²⁵I anti-IgE to plates coated with antigen (TT and AgE) and preincubated with culture medium was always <45 cpm and was subtracted from the counts per minute of ¹²⁵I anti-IgE bound in the presence of the dilutions of the standard serum pool. 1 ml of a 1/100 dilution of the standard pool was arbitrarily assigned a content of 2,000 U of TT-specific IgE antibody and 2,000 U of AgE-specific IgE antibody. Fig. 1 depicts the relationship between counts per minute of ¹²⁵I anti-IgE bound to wells coated with AgE or TT and incubated with various dilutions of the standard pool. Samples in which the net binding of ¹²⁵I anti-IgE exceeded threefold the background (~45 cpm) were considered to be positive. Thus, the lower limit of sensitivity of the assay was ~11 U (55 U/ml) of TT-specific IgE and ~7 U (35 U/ml) of AgE-specific IgE. The coefficient of variation of triplicate determinants averaged 14±8%. The coefficient of variation between the means of four determinations performed at four different times on three different samples averaged 15, 21, and 13%, respectively.

Absorption of B cells on plates coated with rabbit anti-IgG. Plastic petri dishes were incubated overnight at room temperature with a 1-mg/ml solution of rabbit Id IgG

FIGURE 1 Standard curve for radioimmunoassay of antigen-specific IgE. Numbers on the abscissa represent units of antigen-specific IgE with the content of 1 ml of standard serum pool being assigned an arbitrary value of 2,000 U. Numbers on the ordinate represent net counts per minute of ¹²⁵I anti-IgE bound to wells coated with TT or AgE and incubated with 200 µl of different dilutions of the standard pool. Net counts per minute were obtained by subtracting the counts per minute bound in the absence of the standard (~45 cpm) from the total counts per minute. All determinations were done in triplicate.

in PBS, then extensively washed in PBS. B cell-enriched cell suspensions (4×10^6 cells/ml) depleted of monocytes by adherence overnight over plastic petri dishes were then allowed to incubate in the IgG-coated dishes for 1 h at room temperature, after which the nonadherent cells were gently aspirated, counted, and resuspended in culture medium.

Statistical analysis. Statistical analysis was performed by the two-tailed Student's *t* test.

RESULTS

Characteristics of the anti-Id Antisera. The characteristics of the three anti-Id antisera used in the present study are shown in Fig. 2. 1 mg of rabbit anti-Id IgG precipitated a sizeable fraction (34–41%) of the ^{125}I -labeled IgG F(ab')₂ anti-TT, used for immunization (Fig. 2). In contrast, rabbit anti-Id IgG precipitated negligible amounts of ^{125}I -labeled TT nonreactive IgG F(ab')₂ derived from the "idiotype" donor (Fig. 1) and negligible amounts (<2%) of pooled ^{125}I -IgG and negligible amounts (<3%) of ^{125}I IgG F(ab')₂ anti-TT obtained from three unrelated donors (data not shown).

Each of the three anti-Id IgG reacted with the antigen binding site of IgG from the corresponding idiotype donor as demonstrated by their capacity to inhibit the binding of ^{125}I -TT, but not of ^{125}I -diphtheria toxoid, to IgG obtained from that donor 1 wk after the recent booster immunization (Fig. 2). Rabbit anti-Id IgG did not inhibit (<3% inhibition) the binding of ^{125}I -TT to IgG derived from unrelated donors (data not shown).

FIGURE 2 Characteristics of rabbit anti-Id IgG. The upper panels depict the binding of ^{125}I -IgG F(ab')₂ anti-TT "idiotype" (~60,000 cpm, ~10 ng) and of ^{125}I -TT nonreactive IgG F(ab')₂ designated TT negative (~80,000 cpm, ~10 ng) to anti-Id IgG. The lower panels depict the inhibition of ^{125}I -TT (~60,000 cpm, ~10 ng) and ^{125}I -DT (~35,000 cpm, ~10 ng) binding to IgG under conditions of limiting amounts of IgG resulting in the binding of ~70% of the radiolabeled antigen. All three subjects were immune to DT antigen.

The effect of rabbit anti-Id IgG on the binding of IgE to TT and ragweed antigen is shown in Fig. 3. The serum was obtained 1 wk after the recent booster immunization. Rabbit anti-Id IgG inhibited in a dose-dependent fashion the binding of TT but not of AgE to IgE derived from the idiotype donor. The binding of TT to IgE from an unrelated donor was not affected by rabbit anti-Id IgG (data not shown).

In vitro synthesis of total IgE and of antigen-specific IgE. At the time of the present study, peripheral blood lymphocytes (PBL) from all three subjects studied synthesized significant amounts of IgE in vitro compared to PBL derived from normal nonallergic subjects (Table I). 6 mo after the present study PBL from the two allergic subjects continued to synthesize significant amounts of IgE in vitro, whereas PBL from the third subject synthesized very small amounts of IgE (100–200 pg/ml).

In all three subjects part of the *de novo* made IgE had specificity for TT antigen, and in the two ragweed allergic donors (subjects 1 and 2) *de novo* made IgE had specificity for AgE (Table I). The specificity of the IgE anti-TT and of the IgE antiragweed antibodies present in the supernatants was confirmed by demonstrating that TT (10 $\mu\text{g}/\text{ml}$) completely blocked the detection of IgE antibodies to TT but not of IgE antibodies to AgE and vice versa (data not shown). The IgE antibodies detected in the culture supernatants were made *de novo* in culture because addition of cycloheximide to the cultures reduced the IgE in the supernatant to the level of IgE detected in supernatants of cultures of frozen and thawed cells (data not shown). The IgE antibodies detected in our assay could not have been due to nonspecific aggregation of IgE and antigen-specific IgG secreted in the supernatants

FIGURE 3 Inhibition of antigen binding to IgE by rabbit anti-Id IgG. In these experiments serum IgE was bound to anti-IgE-coated disk, the disks were washed and ^{125}I -TT (~60,000 cpm, ~10 ng) or ^{125}I -AgE (~45,000 cpm, ~10 ng) were added and the binding to the disks was assessed in the presence or absence of various amounts of rabbit anti-Id IgG (ref. 9). The experiments were carried under conditions of limiting amounts of IgE resulting in the binding of ~70% of the radiolabeled antigen.

TABLE I
In Vitro Synthesis of IgE by Unfractionated PBL

Source of PBL	IgE Synthesis		
	Total	Anti-TT	Anti-AgE
	pg/ml	U/ml	U/ml
Donor 1	2,120±128	178±13	385±37
Donor 2	2,680±173	216±25	369±22
Donor 3	382±28	142±14	Not detectable
Normal nonallergic donors, n = 6	61±77	Not detectable	Not detectable

Values represent the mean±SD of triplicate cultures containing 2×10^6 cells/ml. Similar results are obtained in two other separate experiments.

because prior absorption of supernatants with *Staphylococcus aureus* (Cowan I strain) did not affect the amount of antigen-specific IgE antibodies detected in the assay (<10% reduction).

Binding of IgE B cells to anti-Id coated plates. To determine if B cells committed to antigen-specific IgE antibody synthesis bear idiotypic determinants we studied the effect of adsorbing B cells over anti-Id-coated plates on their ability to synthesize antibodies. We first established that enriched population of B cells from the three subjects studied synthesize IgE in the absence of additional autologous T cells (Table II). Addition of autologous T cells (at a 1:1 ratio) resulted in a minimal increase in IgE synthesis averaging $21 \pm 9\%$ for the three subjects. B cells absorbed for 1

TABLE II
Adsorption of B Cells over Anti-Id-coated Plates

Source of B cells	Immunosorbent	IgE Synthesis, pg/ml		
		Total	Anti-TT	Anti-AgE
		pg/ml	U/ml	U/ml
Donor 1	Preimmune IgG	3,711±265	232±15	473±22
	Anti-Id IgG	3,625±360	107±10 ($P < 0.01$)	519±23
Donor 2	Preimmune IgG	4,303±458	330±18	447±34
	Anti-Id IgG	4,190±173	184±13 ($P < 0.01$)	421±21
Donor 3	Preimmune IgG	586±24	160±13	—
	Anti-Id IgG	508±22	87±9 ($P < 0.01$)	—

Values represent the mean±SD of triplicate cultures containing 2×10^6 cells/ml. Similar results were obtained in two other separate experiments.

h at room temperature over plastic dishes coated with anti-Id (1 mg/ml) synthesized significantly less IgE antibodies to TT than control B cells absorbed over plates coated with preimmune rabbit IgG (Table II). In contrast, the synthesis of IgE antibodies to AgE was not affected. The decrease in the synthesis of IgE antibody to TT was not due to interference by carried over anti-Id with the assay for IgE anti-TT. This is because supernatants of cycloheximide-treated cultures of anti-Id-absorbed B cells did not interfere with the assay for IgE anti-TT when they were added to supernatants of cultures of untreated B cells or to dilutions of serum derived from the idiotype donor (data not shown).

Effect of anti-Id on IgE synthesis by unfractionated PBL. The effect of anti-Id on IgE synthesis by unfractionated PBL derived from the idiotype donor is shown in Table III. In these experiments 500 µg/ml of anti-Id IgG were added during the first 48 h of culture then the cells were washed and recultured for 7 d. This was done to remove anti-Id IgG from the cultures because it could interfere with the assay of TT-specific IgE. The results obtained in these experiments are shown in Table III. They indicated that rabbit anti-Id IgG, but not preimmune rabbit IgG, inhibited the synthesis of TT-specific IgE antibodies. This inhibition was TT-specific because the synthesis of IgE antibodies to AgE was not affected (Table II). The inhibition of IgE anti-TT synthesis by anti-Id IgG was idiotype-specific because anti-Id IgG did not inhibit IgE anti-TT synthesis by PBL from subjects unrelated to the idiotype donor (data not shown).

TABLE III
Effect of Anti-Id IgG on IgE Synthesis

Source of PBL	Addition to the culture	IgE Synthesis		
		Total	Anti-TT	Anti-AgE
		pg/ml	U/ml	U/ml
Donor 1	Preimmune IgG	1,870±80	232±17	357±20
	Anti-Id IgG	1,607±115	163±8 ($P < 0.02$)	326±18
Donor 2	Preimmune IgG	2,446±85	228±16	273±21
	Anti-Id IgG	2,377±101	121±13 ($P < 0.02$)	284±14
Donor 3	Preimmune IgG	318±37	120±10	—
	Anti-Id IgG	258±25	77±8 ($P < 0.05$)	—

Values represent mean±SD of triplicate cultures. Rabbit IgG was added at a concentration of 1 mg/ml for the first 48 h of culture, then the cells were washed and recultured at a concentration of 2×10^6 viable cells/ml for 5 additional d.

The inhibition of TT-specific IgE antibody synthesis by anti-Id IgG was not due to the interference of carried over anti-Id IgG with the assay for IgE anti-TT because supernatants of cells cultures treated for 48 h with anti-Id IgG, then washed and recultured for 7 d in the presence of cycloheximide, did not interfere with the assay of IgE anti-TT when mixed with supernatants of 7-d cultures of untreated autologous cells (Table IV) or with dilutions of serum derived from the idiotype donor (data not shown).

Effect of anti-Id on B and T cells. The anti-Id-mediated inhibition of IgE anti-TT by unfractionated PBL could have resulted from idiotypic antiidiotypic interactions involving B cells, T cells, or both subpopulations of cells. To answer these questions the effects of pretreatment of B and T cell-enriched populations with anti-Id were examined.

Since B cell-rich populations synthesized IgE in the absence of additional T cells, B cells were incubated with rabbit anti-Id IgG or preimmune rabbit anti-IgG (500 μ g/ml) for 48 h at 37°C, washed extensively, then cultured without addition of T cells. After 7 d the culture supernatants were assayed for IgE. The results of these experiments, as depicted in Table V, indicate that anti-Id IgG specifically inhibited IgE anti-TT synthesis by B cells derived from the idiotype donor. This inhibition was antigen specific (Table V) and idiotype

TABLE IV
Effect of "Carried over" Anti-Id IgG on the Determination of IgE Anti-TT in PBL Culture Supernatants

Material assayed	Addition*	IgE anti TT U/ml
0.1 ml supernatant of 7 d PBL culture	0.1 ml of medium	127±18
0.1 ml supernatant 7 d PBL culture	0.1 ml of supernatant from PBL cultures treated for 48 h with preimmune rabbit IgG, washed, then cultured for 5 d with cycloheximide§	136±15
0.1 ml supernatant 7 d PBL culture	0.1 ml of supernatant from PBL cultures treated for 48 h with anti-Id IgG washed then cultured for 5 d with cycloheximide§	120±13

* None of the materials added contained any detectable IgE anti-TT.

† Mean±SD of triplicate determinations.

§ PBL were all derived from the same donor.

TABLE V
Effect of Pretreatment of B Cells with Anti-Id on IgE Synthesis

Source of B cells	Pretreatment	IgE Synthesis		
		Total pg/ml	Anti-TT U/ml	Anti-AgE U/ml
Donor 1	Preimmune IgG	2,556±96	177±24	298±33
	Anti-Id IgG	2,302±103	102±15 (<i>P</i> < 0.07)	317±28
Donor 2	Preimmune IgG	2,932±163	202±20	303±17
	Anti-Id IgG	2,883±211	135±9 (<i>P</i> < 0.05)	282±27
Donor 3	Preimmune IgG	408±47	122±11	—
	Anti-Id IgG	350±48	72±12 (<i>P</i> < 0.05)	—

Values represent mean±SD of triplicate cultures. B cell rich suspensions were incubated for 48 h with rabbit IgG, washed extensively then cultured at 2×10^6 viable cells/ml for 5 d.

specific in that it was not exerted on B cells from subjects unrelated to the idiotype donor (data not shown). Furthermore, as described in the preceding section the observed inhibition of IgE anti-TT synthesis was shown not to be due to the interference of carried over anti-Id in the assay for IgE anti-TT.

The possible effects of anti-Id on T cells involved in the regulation of IgE antibody synthesis was examined by studying the effect of anti-Id treated T cells on IgE antibody synthesis. In these experiments purified populations of T cells were incubated for 48 h with preimmune rabbit IgG (500 μ g/ml) or rabbit anti-Id IgG (500 μ g/ml), extensively washed and then added at a 1:1 ratio to freshly obtained autologous B cells. As previously mentioned addition of autologous T cells to B cells resulted in a moderate enhancement of IgE synthesis (data not shown). The results depicted in Table VI demonstrate that T cells preincubated with anti Id IgG selectively suppressed IgE anti-TT synthesis by B cells. Because anti-Id could inhibit IgE anti-TT synthesis by directly interacting with B cells (see Table V), it was imperative to demonstrate that the T cell-mediated suppression was not due to carried over anti-Id. For this reason we examined the capacity of irradiated (3,000 rad) T cells preincubated for 48 h with anti-Id to inhibit IgE anti-TT synthesis and we examined the capacity of T cells preincubated for 1 h with anti-Id to inhibit the synthesis of IgE anti-TT. As shown in Fig. 4, irradiated T cells treated with anti-Id failed to inhibit TT-specific IgG synthesis by B cells. Furthermore, in two instances tested (subjects 1 and 2) T cells incubated with anti-Id for 1 h, washed, then

TABLE VI
Effect of Pretreatment of T Cells with Anti-Id IgG
on IgE Synthesis

Source of cells	Pretreatment of T cells	IgE Synthesis		
		Total	Anti-TT	Anti-AgE
		pg/ml	U/ml	U/ml
Donor 1	Preimmune IgG	2,420±163	178±18	425±13
	Anti-Id IgG	2,401±84	98±15 (<i>P</i> < 0.05)	423±32
Donor 2	Preimmune IgG	2,620±198	227±24	395±48
	Anti-Id IgG	2,528±130	148±16 (<i>P</i> < 0.05)	338±44
Donor 3	Preimmune IgG	407±27	172±20	—
	Anti-Id IgG	352±19	103±16 (<i>P</i> < 0.05)	—

Values represent mean±SD of triplicate cultures. These cultures contained equal numbers of fresh B cells and of autologous T cells that have been preincubated for 48 h with rabbit IgG then extensively washed.

added 48 h later to autologous B cells failed to inhibit IgE anti-TT synthesis (4 and 9 % inhibition, respectively) whereas nonirradiated T cells incubated for 48 h with anti-Id inhibited IgE anti-TT synthesis (38 and

FIGURE 4 Effect of irradiation on the capacity of T cells to suppress IgE anti-TT synthesis. Irradiated (3,000 rad) and nonirradiated T cells were treated with anti-Id IgG or preimmune IgG (500 µg/ml) for 48 h, washed then added at a 1:1 ratio to fresh B cells. 7 d later *de novo* made IgE was assayed in the culture supernatants. Percent inhibition for each subgroup of T cells (nonirradiated and irradiated) was calculated as: $100 \times 1 - \text{units of IgE in cultures with anti-Id-treated T cells} / \text{units of IgE in cultures with control-treated T cells}$. ■, irradiated T cells; □, T cells.

32%, respectively). These results indicate that the precursors of antigen-specific IgE suppressor T cells induced by anti-Id are radiosensitive and that carried over anti-Id did not account for the inhibition of IgE anti-TT synthesis observed when anti-Id-treated non-irradiated T cells were added to B cell cultures.

DISCUSSION

The present study demonstrates that the *in vitro* synthesis of TT-specific IgE in man is accessible to regulation by anti-Id antibodies.

This study was made possible because a sensitive and specific radioimmunoassay enabled us to measure the *in vitro* synthesis of antigen-specific IgE (Fig. 1). Two of the three subjects included in our study were allergic individuals and their sera persistently contained IgE anti-TT antibodies. The third subject had a slightly elevated serum IgE level (250 IU/ml) with no clinical allergies. Her serum mounted a transient IgE response to TT after booster immunization. The transient development of serum IgE antibodies to injected antigens including TT in normal individuals has been previously reported (14, 15). PBL from each of the three individuals synthesized measurable quantities of IgE and IgE anti-TT when studied after booster immunization with TT (Table I). In addition, *in vitro* synthesis of IgE antibodies to ragweed antigen (AgE) by PBL was easily measured in the two ragweed allergic subjects. In contrast, PBL from nonallergic subjects synthesized negligible amounts of IgE and no detectable TT or AgE-specific IgE into their culture supernatants. These results are in agreement with the results of many previous investigators who uniformly have found that normal lymphocytes fail to secrete IgE spontaneously *in vitro* (16–21). The small amount of IgE synthesized by the third subject included in the present study could be explained by her slightly but definitely elevated serum IgE, and by transient activation of her TT-specific IgE B cells. Transient activation of circulating antigen-specific IgE B cells has been well documented by Saxon et al. (22).

The anti-Id antibodies used in the present study reacted after appropriate absorptions with only a fraction of the immunizing idiotype (Fig. 2) reflecting the fact that only some of the idiotypic determinants in the heteroclonal anti-TT response were present in sufficient quantities to induce an anti-Id response in the rabbit (10). Although the IgG F(ab')₂ anti-TT used for immunization contained no detectable IgE, the rabbit anti-Id antisera showed idiotype- and antigen-specific reactivity with anti-TT antibodies of both IgG and IgE isotypes (Figs. 2 and 3) indicating that the two isotypes shared idiotypic determinants. In this regard rabbit anti-Id antibody raised against idiotype from subject

1 was previously shown to elicit a Prausnitz-Kustner reaction in skin sites of normal recipients injected 48 h previously with the IgE-enriched fraction of serum from the idiotype donor (8).

Absorption of B cells over anti-Id-coated plates specifically reduced their subsequent synthesis of IgE anti-TT (Table II) indicating that the surface immunoglobulin of B cells committed to the synthesis of IgE anti-TT shared Id determinants with serum antibody to TT. These findings also suggested that IgE B cells can serve as the targets for regulation by anti-Id antibody.

The signal delivered by Id anti-Id interactions to the IgE B cells was an inhibitory signal because addition of anti-Id antibody to cultures of unfractionated PBL or of B cell-rich populations resulted in a specific inhibition of IgE anti-TT synthesis (Table III). This inhibition was not merely the result of antibody-dependent complement-mediated lysis of TT-specific B cells because (a) there was no exogenous source of complement in the cultures, (b) it was previously demonstrated that IgG anti-TT synthesis induced by nonspecific T cell helper factor was not affected by anti-Id (5), thus making it unlikely that anti-Id-dependent complement lysis was operative in the *in vitro* cultures. Furthermore, the inhibition of IgE anti-TT synthesis by anti-Id could not have resulted from the potentially suppressive effects of histamine (23) liberated by the interaction of anti-Id and IgE anti-TT bound to the basophils that separate with mononuclear cells on Ficoll-hypaque. This is because the suppressive effects of histamine mediated by a radiosensitive T cell are nonspecific (24), yet the synthesis of IgE antibodies to AgE was not reduced in those cultures that received anti-Id.

The inhibition of TT-specific IgE synthesis by anti-Id operated via complex mechanisms. Two possible mechanisms were demonstrated in the present study. The first mechanism appeared to be T cell-independent and occurred when anti-Id antibody was added to B cell-rich cultures (Table V). Because the majority of IgE B cells have been shown to bear both surface IgE and IgM (25, 26) it is not known whether idiotypic interactions involving either or both of these two surface idiotypes resulted in the inhibitory signal. Inhibition of immunoglobulin production of IgG and of IgM by anti-Id in man has been previously reported by us (5, 27) and by Bona and Fauci (28). The mechanism of such inhibition is not well established. At least in one instance it appeared to be dependent on the presence of an intact Fc portion of the anti-Id antibody (27).

A second mechanism for the inhibition of TT-specific IgE synthesis by anti-Id was shown to be T cell dependent. Incubation of T cells with anti-Id resulted

in the generation of antigen-specific suppressor T cells that caused inhibition of IgE anti-TT synthesis (Table VI). The precursor of this T suppressor cell was shown to be radiosensitive and presumably was idiotype positive. Indeed, it is unlikely that the preparation of rabbit anti-Id IgG contained antibody that was directed against an idiotype-negative T cell-derived material that copurified from the serum with the IgG F(ab')₂ anti-TT used to raise the anti-Id antibody. In this regard, there is previous data in experimental animals and in man to indicate the presence of idiotype-positive T cells that are activated by anti-Id antibody into idiotype-specific suppression (29-31).

The present work has important implications for the regulation of the IgE antibody response. First, it demonstrates that IgE antibody synthesis is subject to regulation by antiidiotypic antibody. Second, in view of our recent demonstration that auto-antiidiotypic antibodies arise during the course of the normal human immune response (9), the present study raises the possibility that IgE antibody synthesis *in vivo*, as well as *in vitro*, can be regulated by anti-Id. Third, it appears that anti-Id antibody can regulate *in vitro* IgE synthesis in nonallergic as well as in allergic subjects making it important to determine if there are *in vivo* differences between these two groups in so far as regulation of IgE synthesis by anti-Id antibody.

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