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Research Article

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Regulation of Human Immunoglobulin E Synthesis in Acute Graft versus Host Disease

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ABSTRACT Immunoglobulin (Ig) E synthesis was studied in vitro in eight patients who had received transplants of allogeneic bone marrow. Seven of these patients developed acute graft vs. host disease (GVHD) and elevated serum IgE levels, whereas the eighth did not. In vitro synthesis of IgE, but not of IgG, was elevated in cultures of lymphocytes obtained during acute GVHD (17,923±14,607 pg/10^6 cells) but not in cultures of lymphocytes obtained after resolution of the acute GVHD when the serum IgE had returned to normal (106±31 pg/10^6 cells). In contrast, lymphocytes from the patient with no acute GVHD, like normal lymphocytes, failed to synthesize IgE in vitro.

The increased in vitro IgE synthesis in acute GVHD was suppressed by normal allogeneic lymphocytes and by autologous lymphocytes obtained after the resolution of the acute GVHD, but not by allogeneic lymphocytes obtained from patients undergoing acute GVHD. The deficiency in functional IgE-specific suppressor cells in acute GVHD occurred in the face of normal or increased percentages of circulating T8+ cells, which in normal subjects contain the IgE-specific suppressor cells.

In two patients studied, there was evidence of activated IgE-specific, circulating helper T cells. T cells from these two patients, but not normal T cells, secreted spontaneously upon culture in vitro a factor that induced IgE, but not IgG, synthesis by normal B cells.

Finally, a survey of 21 bone marrow transplant recipients revealed that acute GVHD was a necessary requirement for the development of elevated serum IgE levels in recipients of bone marrow transplants. These results suggest that acute GVHD is accompanied by an imbalance in IgE-specific immunoregulatory T cells consisting of activated helper T cells and deficient suppressor cells.

INTRODUCTION

There is considerable evidence in experimental animals to indicate that immunoglobulin (Ig) E synthesis by B lymphocytes is regulated by both helper and suppressor T lymphocytes (1–8). Much less is known about the regulation of IgE synthesis in man. Studies from several laboratories indicate that peripheral blood lymphocytes (PBL) from atopic patients synthesize large amounts of IgE spontaneously in vitro, whereas normal PBL fail to do so (9–12). The basis for this difference is not completely clear but has important implications for the understanding of the human reaginic response and the potential manipulation of this response. Studies by Fiser and Buckley (12) and Saxon and co-workers (13) have indicated that in many instances, normal T lymphocytes are capable of suppressing IgE synthesis when co-cultured with lymphocytes from atopic patients and suggested that IgE-specific suppressor T cells may be deficient in hyper IgE states. In a recent study, patients with the hyper IgE syndrome were shown to be deficient in circulating T8+ cells, a subset that in normal subjects was responsible for the suppression of IgE synthesis (14).

Increased IgE synthesis may result not only from a deficiency of IgE-specific suppressor T cells but also from increased IgE-specific helper T cells. In the present work we examined the regulation of IgE synthesis

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in patients with acute graft vs. host disease (GVHD)[1] after transplantation of allogeneic bone marrow. Our experience has indicated that these patients frequently exhibit elevated levels of serum IgE (15). The data presented here suggest that a deficiency in IgE-specific suppressor cells as well as activation of IgE-specific T helper cells underlie the increased production of IgE in patients with acute GVHD.

METHODS

Subjects. Eight patients undergoing bone marrow transplantation (BMT) were studied. The clinical characteristics of these patients are shown in Table I. All eight patients received allogeneic bone marrow from HLA-A, B, C, and D histocompatible siblings. Patients transplanted for leukemia were prepared with cytosine arabinoside, cyclophosphamide (CTX), and total body irradiation (800 rad). The patient with aplastic anemia and the patient with paroxysmal nocturnal hemoglobinuria were prepared with rabbit antithymocyte serum, procarbazine, and CTX. The patient with Wiskott-Aldrich syndrome was prepared with antithymocyte serum, CTX, and busulfan. Patients were maintained in protective isolation, received a diet low in bacterial content, and were administered nonabsorbable oral antibiotics. Six patients (1–4, 6, and 8) received methotrexate, 10 mg/m^2, as prophylaxis against acute GVHD on days 1, 3, and 11. The remaining two patients (5 and 7), whose donors were identical twins, did not receive methotrexate.

Normal subjects, whose serum IgE levels were <100 IU/ml, were used as a source of normal PBL in co-culture experiments. Written consent was obtained from all subjects after the purposes of the study were explained to them.

Scoring of acute GVHD. The diagnosis of GVHD was made clinically by the characteristic skin rash substantiated by skin biopsy, elevation of hepatocellular enzymes, and diarrhea. The severity of the GVHD was scored by the Seattle criteria (16).

Determinations of IgE levels. Serum IgE levels were determined by using the commercially available paper radioimmunosorbent test (PRIST) assay (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ). Results are expressed as international units (IU) per milliliter according to standards provided by the manufacturer.

Isolation of PBL. Mononuclear cells were obtained from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals) density gradient centrifugation. Cells were washed five times in Hanks' balanced salt solution (HBSS) (Microbiological Associates, Bethesda, MD) and resuspended in RPMI 1640 (Microbiological Associates) supplemented with penicillin (100 U/ml), streptomycin (50 μg/ml), and 10% heat-inactivated fetal calf serum (FCS; complete medium).

Preparation of T cell-rich and B cell lymphocyte populations. T cell- and B cell-rich lymphocyte populations were obtained by rosetting peripheral blood mononuclear cells with sheep erythrocytes (E) pretreated with neuraminidase (Behring Diagnostic, American Hoechst Corp., Somerville, NJ) as previously described (14) and sedimenting the mixture over a Ficoll-Hypaque gradient. Cells from the bottom of the gradient were collected, erythrocytes were lysed by suspending the pellet in 50% fresh human serum (containing natural antibodies to E) and incubating them for 15 min at 37°C. The resulting lymphocytes were washed and suspended in culture medium. These suspensions contained >92% E rosette-forming cells. Cells that failed to form rosettes with E were collected from the interface of the gradient, washed, and used as B cell-rich population of cells. They contained <5% E rosette-forming and 50–60% surface Ig-positive cells (14).

Cell cultures. PBL were cultured at a final concentration of 1×10^6 cells/ml in a final volume of 1 ml in 12×75 round-bottom tubes (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, CA). Cultures were done in duplicate and were incubated in a humidified atmosphere at 37°C in 5% CO_2 for 7 d. Cycloheximide (100 μg/ml) was added to one set of cultures to inhibit protein synthesis. After 7 d, cell cultures were centrifuged at 3,000 rpm for 10 min and the supernatants were stored at −20°C until assayed for their content of IgE and IgG.

Radioimmunoassay for IgE. The radioimmunoassay for IgE was performed in flexible flat-bottom microtiter plates (Cooke Laboratory Products, Alexandria, VA) as previously described (14). The wells were filled with 0.1 ml of a 0.4-mg/ml solution of an immunoabsorbent-purified rabbit anti-human IgE. This was derived from the IgG fraction of the serum of a rabbit immunized with purified myeloma from patient P.S. This rabbit IgE was absorbed with insolubilized human myelomas IgG, IgM, and IgA and with K and L Bence Jones proteins, then immunoabsorbent-purified over an IgE P.S. (a kind gift of Dr. Ishizaka, Johns Hopkins University, Baltimore, MD) column. It reacted solely with ^{125}I-IgE (patients P.S. and N.D., the latter a gift of Dr. Johansson, Uppsala, Sweden) but not with ^{125}I-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 coating solution was removed and the wells were washed three times with phosphate-buffered saline (PBS) containing 1% horse serum (HS) (PBS-1% HS). The microtiter wells were then filled with 10% HS in PBS for 2 h then washed three times with PBS-1% HS. One-tenth of a milliliter of culture supernatant or of IgE standard was then added to each of triplicate wells and incubated for 16 h in a humidified chamber at room temperature. The wells were then washed three times with PBS-1% HS and 0.1 ml of ^{125}I-anti-rabbit human IgE (Pharmacia Inc., 12 μCi/μg sp act) was added to each well. 6 h later, the radiolabeled anti-IgE was removed and the wells were washed three times with PBS-1% HS, and several times with running distilled water. The wells were cut out and counted in a Gamma spectrometer (Tracor Analytic Inc., Elk Village, IL). Standard curves were constructed by using the Pharmacia standards. The concentration of IgE in the supernatants was read from the standard curve. The lower limit of sensitivity of this assay varied from 75 to 150 pg/ml. Net IgE synthesis in individual cultures was calculated by subtracting the value of IgE in cycloheximide-treated cultures from the value of IgE in untreated cultures.

The specificity of the assay was established by demonstrating that no radioactivity was detectable in wells that received, instead of IgE, large amounts (up to 100 μg/ml) of human IgG, IgM, or IgA myelomas, or of K or L Bence Jones proteins.

Radioimmunoassay for IgG. The assay for IgG in the supernates of cell cultures was a competitive radioimmunoassay as previously described (14). First, it was determined that 0.1 ml of a 1:1,000 dilution of a gamma chain-specific

[1] *Abbreviations used in this paper*: BMT, bone marrow transplantation; CTX, cyclophosphamide; E, sheep erythrocytes; FCS, fetal calf serum; GVHD, graft vs. host disease; HS, horse serum.

rabbit anti-human IgG (N. L. Cappel Laboratories, Inc. Cochranville, PA) precipitated 70% of the radioactivity from 10 ng of ^{125}I-labeled human IgG (3,000 cpm/ng). Subsequently, 0.1 ml of culture supernate (or of its dilutions) or of standard IgG solutions was added to 0.1 ml of 1:1,000 rabbit antiserum in a Beckman plastic microfuge tube (Beckman Instruments, Inc., Fullerton, CA). After an incubation of 1 h at 37°C, 10 ng ^{125}I-IgG were added. The tubes were incubated for 1 h at 37°C, then normal rabbit serum and goat anti-rabbit IgG (preabsorbed against Sepharose-bound human IgG) were added at equivalence. After a 1-h incubation at 37°C and an overnight incubation at 4°C, the immune precipitates were washed three times in ice-cold PBS in a Beckman microfuge, and counted in a Tracor Analytic gamma spectrometer. IgG present in an individual culture supernate was read from the standard curve. The specificity of the assay for human IgG was demonstrated by the failure of large excesses of myeloma IgM, human IgA, or of K and L chains to inhibit the binding of ^{125}I-IgG to rabbit anti-IgG.

Enumeration of T cells and T cell subsets. Monoclonal antibodies reactive with the T cell surface antigens T3, T4, and T8 were purchased from Ortho Diagnostics (Raritan, NJ). These antibodies have been previously shown to be restricted in their reactivity to cells of T cell lineage (17, 18). Anti-T3 reacts with 90% of E-rosette-positive peripheral T cells and 10% of thymocytes. In contrast, anti-T4 and anti-T8 react with the majority of thymocytes and with 60 and 30% of peripheral T cells, respectively. Functionally, anti-T4 defined the human inducer (helper) T cell subset, whereas anti-T8 defined the human suppressor/cytotoxic population (17, 18).

Immunofluorescent analysis of unfractionated cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC, N. L. Cappel Laboratories, as previously described) (19). Fluorescent cells were counted by using a Zeiss fluorescent microscope (Carl Zeiss, Inc., New York, NY). A minimum of 200 cells were counted. Background fluorescence reactivity was determined with a control mouse ascites and never exceeded 3%. All analyses were performed without knowledge of the patient's clinical status.

Effect of allogeneic cells on in vitro synthesis of IgE by the patient's PBL. In these experiments, 1 million normal allogeneic PBL, T cells, and T cells depleted of either T4+ or T8+ cells were mixed with 1 million PBL from the patients and cultured in a final volume of 1 ml/culture. The value of IgE observed in the supernatants of the mixed cell culture was compared with the expected value of IgE, which was calculated to be the sum of the IgE synthesized in individual cultures of the patient's PBL (1×10^6 cells in 1 ml) and of allogeneic cells (1×10^6 cells in 1 ml). The assumption that the expected amount of IgE in co-cultures should equal the sum of IgE present in the individual cultures was verified previously by showing that the amount of IgE present in the supernatant of a 1-ml culture containing 2×10^6 cells/ml was approximately twice the amount of IgE present in the supernatant of a 1-ml culture containing 1×10^6 cells/ml.

Percentage of suppression of IgE synthesis by normal cells was calculated as: 1 − (observed value of IgE/expected value of IgE) × 100.

Complement-dependent lysis of T cell subsets. This was performed as previously described (14). Briefly, 20 million T lymphocytes were suspended in 0.8 ml of a 1:250 dilution of anti-T8 or anti-T4 in PBS and incubated for 1 h at room temperature. 0.2 ml rabbit serum (Pel-Freeze Biologicals, Inc., Rogers, AR) was then added and the suspension was incubated an additional 60 min at 37°C. Control cells were incubated with PBS and with rabbit serum. The cells were then washed three times in HBSS and adjusted to 2×10^6 viable cells/ml in RPMI with 10% FCS. Cell recoveries after lysis varied from 65 to 80%. Immunofluorescence of the lymphocytes before and after treatment with anti-T8 or anti-T4 antibody and complement demonstrated complete (>95%) removal of the corresponding T cell subset from the cell suspension.

Generation of T cell supernatants. Purified populations of T cells were suspended in complete culture medium (RPMI 1640-10% FCS) at a concentration of 1×10^7/ml and incubated for 24 h at 37°C in 5% CO_2 air. The cultures were then centrifuged (400 *g* 10 min) and the supernatants were collected and frozen at −20°C until tested.

Statistical analysis. Statistical analysis was performed by the *t* test.

RESULTS

Clinical characteristics and serum IgE levels. The clinical characteristics of the eight patients studied are shown in Table I. All eight patients underwent BMT from HLA-A, B, C, and D histocompatible siblings. Patients 5 and 7 received BMT from identical twin donors. Acute GVHD developed in seven patients and varied in severity from mild (+1) to severe (+4) as assessed by the Seattle criteria (16). None of the patients had a previous history of allergic disorders. Serum IgE levels were normal in five patients before BMT, moderately elevated in one patient (5), and borderline high in two patients (1, 8).

Serum IgE levels rose in all seven patients with acute GVHD but not in the patient without acute GVHD. Serum IgE started to rise beginning day 14 to day 28 after BMT, peaked between days 20 and 40 postBMT, and in six of the seven patients returned to within normal range by 12 wk posttransplant. Fig. 1 illustrates the time course of the rise and fall of serum IgE levels in two patients (1 and 5) and its relationship to acute GVHD.

Serum IgE levels in the BMT donors were <200 IU/ml in all cases except one, the donor for patient 4, whose serum IgE was 475 IU/ml.

In vitro synthesis of IgE. The spontaneous secretion of *de novo*-made IgE in 7-d cultures of unfractionated PBL from the patients and from six nonallergic healthy subjects is shown in Table II. PBL were obtained from patients within a few days of the time at which their serum IgE levels peaked and 4 wk posttransplant from patient 8. Net IgE synthesis by PBL obtained from seven patients during acute GVHD was markedly increased and averaged 17,923±14,607 pg/10^6 cells (Table II). PBL from the patient without GVHD synthesized negligible amounts of IgE in vitro (Table II). PBL from five of six normal controls made either no detectable IgE (five of six cases) or negligible amounts of IgE (Table II). In contrast to the increased

TABLE I
Clinical Characteristics of the Patients

Patient	Age	Sex	Diagnosis	Severity of acute GVHD	Serum IgE levels		
					Pretransplant	Peak IgE*	Posttransplant‡
	yr					*IU/ml*	
1	16	M	Aplastic anemia	4+	220	3,950	215
2	13	M	CML	3+	5	1,950	19
3	22	M	AML	3+	9	3,850	5
4	16	M	T cell ALL	3+	60	3,600	15
5	42	M	AML	1+	395	24,000	2,000
6	1½	M	Wiskott Aldrich	2+	170	9,000	150
7	45	M	CML	1+	5	6,400	140
8	31	F	PNH	0	230	160	12

* The level of IgE shown is the highest level measured within 8 wk of transplants.
‡ The level of IgE was measured 8 wk or later after acute GVHD had subsided in patients 1–7 and 8 wk after transplant in patient 8.
CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; PNH, paroxysmal nocturnal hemoglobinuria.

spontaneous synthesis of IgE by PBL observed during acute GVHD, there was no concomittant increase in the spontaneous secretion of IgG compared with normal controls (404±78 ng of IgG/10^6 cells for normal PBL, $n = 6$, vs. 460±81 ng of IgG/10^6 cells for patient's PBL in acute GVHD, $n = 4$). Negligible amounts of *de novo* IgE were made by PBL from three patients (1, 4, and 6) studied before BMT (66±69 pg/10^6 cells). PBL from five patients were studied after the resolution of the acute GVHD and the return of serum IgE towards normal and were found to secrete negligible amounts of IgE (106±31 pg/10^6 cells).

Suppression of IgE synthesis by normal PBL and by PBL obtained after the resolution of acute GVHD. The production of human IgE in vitro has been shown to be regulated by normal circulating suppressor cells (14). We therefore performed a series of mixing experiments to determine whether circulating IgE-specific suppressor cells were deficient during acute GVHD and whether they reappeared after the resolution of acute GVHD and the return of the IgE levels to normal. Table III demonstrates that the in vitro IgE synthesis in acute GVHD could be suppressed by normal allogeneic (line 1), by normal haploidentical (line 2), or histoidentical PBL (line 3), but not by allogeneic PBL from other patients with acute GVHD (lines 4 and 5). More importantly, PBL obtained from two patients after the resolution of their acute GVHD and the return of their serum IgE levels to normal were capable of suppressing IgE synthesis by autologous lymphocytes obtained during acute GVHD and frozen under liquid nitrogen until tested (Table III, lines 5 and 6). These findings suggested that circulating IgE-specific suppressor cells were deficient during acute GVHD but reappeared after the resolution of acute GVHD and may have contributed to the return of serum IgE levels to normal.

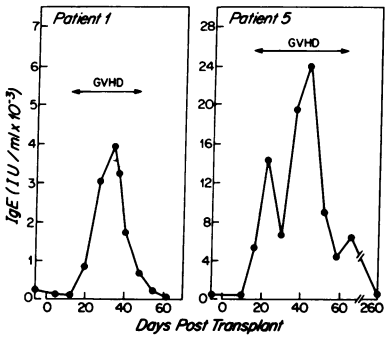

FIGURE 1 Time course of serum IgE elevation in relation to bone marrow transplantation and to the presence of acute GVHD in two patients.

Circulating T cells and T cell subsets. In a previous study we have found that normal IgE-specific suppressor cells resided in the T8+ subset of normal T cells (14). We therefore proceeded to enumerate circulating T cells and T cell subsets in patients with acute GVHD to determine if they were deficient in cells belonging to the T8+ subset.

The results of these studies are shown in Table IV. In the control group of normal subjects, 66±5% of PBL were T cells as determined by reactivity with anti-T3, whereas 37±3% were helper T cells as determined by reactivity with anti-T4 and a smaller percentage, 22±4%, were reactive with anti-T8 suppressor/cyto-

TABLE II
IgE and IgG Synthesis In Vitro

	Subjects	IgE			IgG
		Untreated cultures	Cycloheximide-treated cultures	Net synthesis	
		pg/10⁶ cells			*ng/10⁶ cells*
Patients with acute GVHD	1	43,200	9,960	36,240	510
	2	19,900	1,080	18,820	504
	3	10,700	300	10,400	—
	4	8,250	400	7,850	487
	5	4,700	150	4,550	339
	6	4,875	375	4,500	—
	7	43,250	3,050	40,200	—
		Mean±SD		17,923±14,607	460±81
Patient with no acute GVHD	8	825	690	135	—
Normal	1	1,820	1,800	20	305
	2	<75	<75	—	450
	3	<75	<75	—	503
	4	1,020	1,360	—	380
	5	385	490	—	457
	6	<150	<150	—	330
		Mean±SD			404±78

TABLE III
IgE Synthesis in Co-cultures

Co-cultures		Net IgE synthesis		
PBL from patients with acute GVHD*	Added PBL	Observed	Expected	Suppression
		pg/culture		%
1–5 (n = 5)	Unrelated donor	3,790±4,670	15,540±12,560	76
6	Maternal	2,225	4,500	51
7	Twin donor	18,200	40,200	55
6	Allogeneic subject with acute GVHD‡	15,350	15,850	4
7	Allogeneic subject with acute GVHD§	15,100	17,150	12
6	Autologous, postrecovery	425	2,400	82
7	Autologous, postrecovery	1,200	7,400	84

* Cells from a single subject were frozen at different intervals and then used for different experiments.
‡ The allogeneic subject used in this experiment is subject 7.
§ The allogeneic subject with acute GVHD used in this experiment is not shown in Table I and his PBL were taken when his serum IgE was elevated; 7,500 IU/ml.

TABLE IV
Circulating T Cell Subsets in Acute GVHD

Patient	Cells positive for T3	T4	T8
		%	
1	55	30	29
2	69	33	41
3	44	31	35
4	46	29	24
5	41	27	30
6	64	37	38
7	56	41	44
Mean±SD of patients	53.6±10.5	32.6±4.9	35.6±8.0
Mean±SD of normal controls (n = 20)	66±5	37±3	22±4
P	<0.01	NS	<0.01

toxic T cells. PBL from the seven patients with acute GVHD showed a significantly decreased percentage of T3+ T cells of 53.6±10.5% ($P < 0.01$), a normal percentage of T4+ cells of 32.6±4.9%, and a significantly increased number of T8+ cells of 35.6±8.2% ($P < 0.01$) compared with normal controls.

Release of IgE helper factor by T cells from patients with acute GVHD. Because the synthesis of immunoglobulins by B cells is regulated by T lymphocytes and their products, the finding of increased IgE production in patients with acute GVHD suggested that these patients may possess in vivo activated IgE helper T cells. We therefore examined the capacity of T cells from these patients to release soluble factors into their culture supernatants capable of inducing IgE synthesis by normal B cells. T cell supernatants were tested for their capacity to induce IgE and IgG synthesis in normal B cells by adding them at a final concentration of 50% to cultures of normal B cell cultures. 7 d later, the B cell culture supernatants were collected and assayed for IgE and IgG. Because of the large number of T cells required to generate the supernatants and because of the lymphopenia of the patients during acute GVHD, these experiments were performed in two patients only (6 and 7). Control supernatants were generated from normal T cells including the T cells from the normal bone marrow donor, in one case (7). Normal B cells were derived from unrelated adult nonallergic subjects whose PBL made 100 pg/10^6 cells in culture. As shown in Table V supernatants from the T cells obtained during acute GVHD induced significant *de novo* synthesis of IgE in normal B cells, whereas supernatants from normal T cells did not. Supernatants of T cells obtained from the same two patients (6 and 7) after the resolution of the acute GVHD failed to induce IgE synthesis in normal B cells. T cell supernatants from the patients and the normal controls caused a modest and equivalent increase in the IgG synthesis over "background". The value of background IgG synthesis varied with the lot of FCS used and was reduced to <50 ng/ml by the addition of cycloheximide to the cultures (data not shown).

Relationship between acute GVHD, cytotoxic regimen used for recipient preparation, and elevated serum IgE. Because administration of cytotoxic drugs in experimental animals can result in the enhancement of IgE synthesis (20, 21) it was necessary to examine the relationship of the cytotoxic drug regimen administered to the patients before BMT and acute GVHD

TABLE V
Effect of T Cell Supernatants on the Synthesis of IgG and IgE by Normal B Cells

Source of T cells	Acute GVHD	IgE synthesis in the presence of* T cell supernatants	T cell supernatants + cycloheximide	Net increase	IgG synthesis in the presence of‡ ——	T cell supernatants	Net increase
		pg/ml				*ng/ml*	
Patient 6	Present	2,375	1,375	1,000	640	750	100
Patient 6	Resolved	<100	<100	Und	——	——	——
Patient 7	Present	7,350	2,400	4,950	210	270	60
Patient 6	Resolved	<100	<100	Und	——	——	——
Normals n = 5		<100	<100	Und	467±296	607±363	140±116

* No IgE could be measured (<100 pg/ml) in supernatants of the unstimulated normal B cells used in these experiments.
‡ Addition of cycloheximide to normal B cell cultures reduced the IgG measured in the supernatants to <50 ng/ml.
Und, Undetectable.

TABLE VI

Relationship between Elevation of Serum IgE Levels and Administration of Cytotoxic Drug Regimen, Acute GVHD

Number of patients	Cytotoxic regimen	Acute GVHD	Engraftment	No. of patients	Patients elevated serum IgE
16	+	+	+	16	14
3	+	−	+	3	0
2*	+	−	−	2	0

* These two patients survived 65 and 109 d, respectively, after transplant.

on one hand and the elevation of serum IgE levels on the other hand. We examined these relationships in 21 consecutive transplant recipients, all of whom received a similar cytotoxic drug before BMT. As shown in Table VI, the presence of acute GVHD was a prerequisite for serum IgE elevation. Indeed, all 14 patients who developed acute GVHD had an elevation of serum IgE. Five patients who received identical cytotoxic drugs and who did not develop GVHD had no elevation of IgE.

DISCUSSION

In the present study we investigated the immunoregulation of IgE synthesis in recipients of allogeneic BMT exhibiting elevated serum IgE levels during acute GVHD. Unlike normal individuals, these patients were found to have a deficiency in IgE-specific suppressor cells and to possess circulating T cells capable of spontaneously releasing an IgE-specific helper factor.

Seven of the patients included in the present study developed transient elevation of serum IgE during their acute GVHD (Table I). The eighth patient had no acute GVHD and her serum IgE levels did not rise. Because of the lack of allotypic markers on the IgE molecule, it is not known whether the elevated IgE is of donor or recipient origin or both.

In comparison with normals, PBL from patients with acute GVHD spontaneously secreted large quantities of IgE in vitro (Table II). IgE synthesis in our patients was suppressed by normal lymphocytes but not by lymphocytes obtained during acute GVHD (Table III). More importantly, cells obtained after the resolution of the acute GVHD, and the return of serum IgE to normal, suppressed IgE synthesis of autologous cells collected during acute GVHD (Table III), suggesting that IgE-specific suppressor cells were relatively deficient during acute GVHD, i.e., not sufficient to oppose IgE-specific T cell help. Normal IgE suppressor cells have been shown to bear the T8 antigen (14). However, despite their deficiency in IgE suppressor cells, patients with acute GVHD were found to have normal or increased percentages of T8+ cells (Table IV) as also found by deBriun et al. (22). This indicates that the mere expression of the T8+ antigen does not imply the presence of intact function. In this regard, Parkman et al.[2] have shown that a sizeable portion of T8+ cells in acute GVHD lack the T3 antigen present on mature T cells because selective lysis of PBL of these patients with anti-T3 and complement has revealed the presence of T8+, T3− lymphocytes. Thus, BMT recipients appear to be deficient in functional T3+, T8+ suppressor T lymphocytes that regulate IgE synthesis.

The mechanism by which functional suppressor T cells are transiently lost in acute GVHD is not understood. In murine systems suppressor cells are short-lived and under a tropic influence from the thymus, so that thymectomy results in subsequent loss of the suppressor subpopulation (23, 24). It is possible that whole-body irradiation, CTX, or other chemotherapeutic agents could cause transient thymic dysfunction. Reconstitution of thymic function could result in the gradual reestablishment of functional suppressor cells, which could then abrogate IgE synthesis, as indeed occurred in our patients. Alternatively it is possible that concomittant infection of our patients by ubiquitous organisms such as cytomegalovirus (25) may have contributed to the T cell subset imbalance seen.

In light of the severe depression in suppressor T cell function in acute GVHD, the selective elevation of the IgE isotype was thought to reflect the in vivo presence of activated IgE-specific helper cells. In this regard, activated T cells expressing Ia antigens have been shown to be present in the circulation in acute GVHD (26). Circulating activated IgE-specific helper T cells were clearly demonstrated in acute GVHD because freshly isolated T cells from the patients spontaneously released in vitro an IgE-specific helper factor (Table V). Although T cells from only two patients were studied for their capacity to release an IgE helper factor, a similar factor has been identified recently by us in

[2] Parkman, R., J. Rappeport, J. Lipton, and D. G. Nathan. Submitted for publication.

supernatants of T cells obtained from patients with the hyper-IgE syndrome and from patients with atopic dermatitis and elevated serum IgE levels (27). Previous attempts by several investigators (12, 13) at demonstrating induction of IgE synthesis in normal B cells by T cells from atopic patients have generally met with failure. This may have been due to several factors such as differences in the source of the T cells, use of relatively low number of T cells, and the possible induction of suppressor effects in a mixed lymphocyte culture.

The selectivity of the T cell-derived helper factor in inducing IgE but not IgG synthesis in normal B cells suggests that the conditions and/or the T cells required to generate IgE-specific help in man may differ from those required to generate IgG-specific help. Indeed, had IgG-specific helper T cells been activated in our patients, then elevated IgG levels in vivo and elevated IgG synthesis in vitro should have been observed. Clearly, this was not the case. Actually, serum IgG levels steadily fell in our patients after their preparation for BMT and remained low for a few months afterwards. Prolonged depression of IgG synthesis in vivo and in vitro after BMT has been well documented by Ringden et al. (28).

IgE-specific helper factors have been shown to exist in experimental animal systems where IgE and IgG synthesis can be differentially controlled by immunoregulatory T cells (6). Ishizaka and co-workers (29) have described the spontaneous release of an IgE-specific helper factor by T lymphocytes isolated from the lymph nodes of rats infected with *Nippostrongylus braziliensis*. This IgE potentiating factor had affinity for IgE and could also be detected by its ability to inhibit rosette formation of Fc receptor-bearing cells with IgE-coated erythrocytes. We are currently attempting to define the characteristics of the human IgE helper factors.

In our experience, serum IgE elevation in BMT has not occurred in the absence of acute GVHD (Table VI). It appears that the cytotoxic conditioning regimens administered to patients before BMT are not sufficient to induce a rise in serum IgE levels because five patients without acute GVHD (three with engraftment, two without engraftment) had no elevation of serum IgE. Furthermore, two patients with severe combined immunodeficiency who developed acute GVHD secondary to intrauterine maternal transfusion had elevation of serum IgE levels without administration of any cytotoxic drugs (30 and unpublished observations). IgE helper T cells in acute GVHD could be activated by antigens expressed in the recipient but not in the donor (non-HLA antigens, tumor or viral antigens). In this regard, human lymphocytes activated in vitro have been reported to release IgE binding factors (31) and IgE regulatory factors (32). It is not clear at present why the IgE regulatory factors released in vitro during a mixed lymphocyte reaction are predominantly suppressor factors for IgE (32), whereas those released by in vivo activated T cells are helper factors for IgE. This may have to do with the nature of the activating stimulus and the immunoregulatory status of the responder cells.

Selective increase of a particular isotype, such as IgE in acute GVHD, may reflect the differential perturbation of isotype-specific T cell help and/or suppression. Experiments currently in progress are aimed at establishing T cell lines and clones from patients with acute GVHD in an attempt to isolate IgE-specific helper T cells. This should help to determine whether IgE helper T cells are exclusively isotype specific and to characterize their mode of actions.

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