Deficiency of Suppressor T Cells in the Hyperimmunoglobulin E Syndrome

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ABSTRACT The status of suppressor T cells (Ts) was assessed in seven children with the hyper IgE syndrome (recurrent staphylococcal infections, eczematous skin rash, and elevated serum IgE) to determine whether a deficiency in Ts is associated with increased IgE synthesis. When circulating T cells and their subsets were enumerated with the aid of monoclonal antibodies that identify T cells (T3), helper/inducer T cells (T4), and suppressor/cytotoxic T cells (T8), there was a selective deficiency of T3+ cells (51.7±11.2% vs. 66±5% for normal controls) and of T8+ cells (7.5±4.4% vs. 22±4% for normal controls) but not of T4+ cells (36.5±7.5% vs. 37±3% for normal controls).

Suppressor T cell function was assessed by examining the ability of mononuclear cells incubated for 48 h with concanavalin A to suppress the proliferation of fresh autologous mononuclear cells in response to the mitogens phytohemagglutinin and pokeweed mitogen. All seven patients were severely deficient in concanavalin A-inducible suppressor cells.

In vitro *de novo* synthesis of IgE in 6-d cultures of peripheral blood lymphocytes was measured in four patients by a solid-phase radioimmunoassay. Mononuclear cells from all four patients synthesized spontaneously increased quantities of IgE in vitro (4,950 ±3,760 pg/10^6 cells vs. 250±215 pg/10^6 cells for eight normal controls). IgE synthesis was suppressed by the addition of parental T cells to the culture. Elimination of the T8+ subset, but not of the T4+ subset, by complement-dependent lysis resulted in the loss of the capacity of parental T cells to suppress IgE synthesis.

These results suggest that a deficiency of Ts underlies the elevated IgE levels observed in the hyper IgE syndrome.

INTRODUCTION

The hyper IgE syndrome is associated with recurrent infections, chronic pruritic dermatitis, and elevation of serum IgE antibodies, some of which are directed to antigens on *Staphylococcus aureus* (1–10). The etiology of this elevation is unclear, although many atopic conditions, including dermatitis and allergic rhinitis associated with increased serum IgE, have been associated with a decrease in the number of circulating total T cell number (11, 12). In this regard, however, there is considerable evidence in the murine system to indicate that IgE secretion is closely regulated by suppressor T cells (13). Thus, selective depletion of suppressor cells by either use of low dose cytoxan or total body irradiation results in marked augmentation of IgE synthesis (14).

Recently it has become possible to define inducer and suppressor T cell populations in man with monoclonal antibodies directed at subset-specific cell surface glycoproteins (15). Analysis of T lymphocytes from patients with a variety of autoimmune and immunodeficient states have provided increasing evidence to suggest that a balance among these immunoregulatory populations plays a critical role in maintaining immune homeostasis. Previous work in man have suggested that suppressor T cells play a role in the regulation of IgE synthesis because addition of normal peripheral blood lymphocytes (PBL)[1] to PBL from

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Con A, concanavalin A; HBSS, Hanks' balanced salt solution; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

patients with elevated serum IgE levels often results in suppression of in vitro IgE synthesis (16, 17). Furthermore, IgE levels are elevated in at least two conditions (atopic dermatitis and acute graft vs. host disease) in which the subset of suppressor T cells recognized by mouse monoclonal antibodies to T cells is absent (18, 19). To determine whether aberrations of immunoregulatory T cells might result in the elevated IgE levels found in the hyper IgE syndrome, we studied the regulation of IgE synthesis in a series of patients. In the results to be reported below, we demonstrate that a severe deficiency in suppressor T cells underlies the increased production of IgE in this syndrome.

METHODS

Patients. Seven children with the hyper IgE syndrome were studied. They all gave history of chronic dermatitis, recurrent infections, predominantly with *S. aureus*, and all had elevated levels of serum IgE and a positive radioallergosorbent test for *S. aureus*. Their clinical characteristics are shown in Table I. Consent was obtained from all subjects or from their parents before obtaining blood for these studies.

Determination of serum IgE. Serum IgE concentrations were determined using Phadebas PRIST reagent kits obtained from Pharmacia, Inc., Piscataway, N. J. Results are expressed as international units (IU) per milliliter, according to standards provided by the manufacturer.

IgE binding to S. aureus. *S. aureus* Wood 46 strain was a gift of Dr. Kenneth Ault (Harvard Medical School, Boston). This strain was used because of its very low content of protein A. Bacteria was cultured in serial overnight passes at 37°C in trypticase-soy broth. Cultures were harvested by centrifugation at 3,000 *g* for 10 min at 4°C, washed twice with 0.15 M phosphate-buffered saline (PBS), pH 7.4, and stored in suspension at −20°C.

For IgE binding studies, the bacterial suspensions were diluted in PBS to a protein concentration of 1 mg/ml. Aliquots of 1 ml of this suspension were placed in 12 × 75-mm plastic tubes and washed twice with 2 ml of PBS by vigorous vortexing followed by centrifugation at 2,500 *g* for 5 min. The pellet was resuspended in 0.5 ml of PBS and 0.1 ml of serum or serum dilution was added. The mixture was vortexed vigorously and then incubated at room temperature for 3 h. After this incubation, the pellets were washed three times with PBS, 0.1 ml of human immune globulin (Cohn Fraction II, 160 mg/ml, Massachusetts Biological Laboratories) was added to each tube, and the contents were incubated at room temperature for 3 h. The pellets were washed three times with PBS, and then 50 μl of ^{125}I-immunosorbent purified rabbit anti-IgE (purchased as the radioallergosorbent test reagent from Pharmacia, Inc.), specific activity 5 μCi/0.8 μg, as added and the incubation was continued overnight at room temperature. The pellets were finally washed three times with PBS, and the bound radioactivity was determined with a Packard gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The results are expressed as the percentage of total ^{125}I added that remained bound to the pellets. All determinations were performed in duplicate, and values for controls in which PBS were used in place of serum were subtracted to correct for nonspecific binding. This nonspecific binding was always <2% of the amount of ^{125}I-anti-IgE added.

Isolation of PBL. Mononuclear cells were obtained from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Inc.) density gradient centrifugation. The cells were washed five times in Hanks' balanced salt solution (HBSS) and resuspended in RPMI 1640 (both from Microbiological Associates, Walkersville, Md.) supplemented with penicillin (100 μg/ml), streptomycin (50 μg/ml), and with either 10% fetal calf serum or 10% heat-inactivated human serum obtained from donors with type AB, Rh+ erythrocytes (complete medium).

Preparation of T cell-rich lymphocyte suspensions. Lymphocyte suspensions rich in T cells were obtained by rosetting peripheral lymphocyte suspensions with sheep erythrocytes pretreated with neuraminidase (Calbiochem-Behring Corp., American Hoechst Corp., Somerville, N. J.) (50 U/ml) for 30 min at 37°C and sedimenting the lymphocyte erythrocyte mixture over a Ficoll-Hypaque gradient. The sheep erythrocyte rosette-forming cells were collected, the erythrocytes were lysed in 0.87% Tris-buffered NH_4Cl, and the resulting lymphocytes were washed and suspended in culture medium. These suspensions contained >92% sheep erythrocyte rosette-forming cells.

Production of monoclonal antibodies and analysis of lymphocyte populations with fluorescence-activated cell sorter. Production and characterization of monoclonal antibodies anti-T3, anti-T4, and anti-T8 were the subjects of earlier reports (15–20). In brief, these antibodies were shown to be restricted in their reactivity to cells of T lineage. Anti-T3 reacted with 100% of peripheral T cells and ~10% of thymocytes. In contrast, anti-T4 and anti-T8 reacted with the majority of thymocytes and with 60 and 30% of peripheral T cells, respectively (15, 20). Functionally, anti-T4 defined the human inducer (helper) T cell subset, whereas anti-T8 defined the human suppressor/cytotoxic population (15, 20).

Cytofluorographic analysis of cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC, Meloy Laboratories Inc., Springfield, Va.) on a fluorescence-activated cell sorter (FACS-I, Becton, Dickinson & Co., Mountain View, Calif.), as previously described (13). Background fluorescence reactivity was determined with a control ascites obtained from mice immunized with nonsecreting hybridoma clones. All analyses were performed without knowledge of the patient's clinical status.

Complement-dependent lysis of T cell subsets. 20–40 million T lymphocytes were suspended in 0.8 ml of a 1:250 dilution of anti-T8 or anti-T4 ascitic fluid in PBS and incubated for 1 h at room temperature. Then, 0.2 ml rabbit serum (Pel-Freeze Biologicals Inc., Rogers, Ark.) was 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% fetal calf serum. Immunofluorescence of the lymphocytes before and after treatment with anti-T8 or anti-T4 antibody and complement demonstrated complete (>99%) removal of the corresponding T cell subset from the cell suspension.

Proliferative response to mitogens. Lymphocyte suspensions were cultured in complete culture medium at a concentration of 1 × 10^6 cells/ml in plastic microtiter culture wells (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in the presence or absence of mitogens. Cultures were incubated for 3 d with the mitogens phytohemagglutinin (PHA) (1:100 dilution of PHA-P, Difco Laboratories, Detroit, Mich.) or pokeweed mitogen (PWM) (1:500 dilution of PWM, Gibco Diagnostics Laboratories, Gibco Invenex Div., Lawrence, Mass.). Cultures were pulsed with [^{3}H]-thymidine (2.0 Ci/mmol, New England Nuclear, Boston, Mass.), and 16 h later the degree of [^{3}H]thymidine incorporated into DNA was determined with the aid of a tissue culture automatic harvester (Flow Laboratories, Inc., Rock-

ville, Md.). Results were expressed as counts per minute of [^{3}H]thymidine incorporated per culture.

Generation of suppressor cells with concanavalin A (Con A) treatment. PBL in complete medium at 5×10^6 cells/ml were cultured in the presence or absence of Con A (50 μg/ml) for 48 h. At the end of the incubation, Con A (50 μg/ml) was added to the control cells, and control lymphocytes were washed twice with HBSS supplemented with α-methyl-D-mannoside (25 mg/ml), treated with mitomycin C (25 μg/ml at 37°C for 45 min, both from Sigma Chemical Co., St. Louis, Mo.), washed three times in HBSS, and resuspended in complete culture medium at a concentration of 1×10^6/ml.

Assay of suppressor activity. Untreated autologous lymphocytes suspended at 1×10^6 cells/ml in complete culture medium were mixed with an equal volume of Con A-treated or control lymphocytes at 1×10^6 cells/m and cultured together as described above. Suppression of the proliferation was expressed as percentage of suppression:

$$\% \text{ Suppression} = \left(1 - \frac{\text{cpm (stimulated} - \text{unstimulated) in the presence of Con-A-treated cells}}{\text{cpm (stimulated} - \text{unstimulated) in the presence of control cells}} \right) \times 100.$$

Synthesis of immunoglobulins in vitro. Mononuclear cells isolated by Ficoll-Hypaque centrifugation were washed five times in HBSS containing 1% fetal calf serum and then cultured at 1×10^6 cells/ml in 12×75-mm round-bottom plastic tubes (Falcon Labware) in medium RPMI 1640 containing 10% fetal calf serum and in an atmosphere of 5% CO_2 in air. All cultures were made in duplicate. 6 d later the supernates were collected, millipored, and frozen at −80°C until tested. At the initiation of the cultures, 1-ml aliquots of cell suspension were frozen and thawed three times, spun at 200 *g* for 10 min, and the resultant supernate was collected, millipored, and stored at −20°C. The IgE content of the supernate of frozen and thawed cells was taken to represent the amount of IgE that remained bound to the cells in the cultures and was subtracted from the values of IgE obtained from the supernates of 6-d cultures. Parallel 6-d cultures were carried in the presence of cycloheximide (Sigma Chemical Co., 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. The radioimmunoassay for IgE was performed in flexible flat-bottom microtiter plates Dynatech, Labs, Inc., Alexandria, Va.). The wells were filled with 0.1 ml of a 0.6-mg/ml solution of immunosorbent purified rabbit anti-human IgE, and incubated overnight at room temperature in a humid chamber. 18 h later the rabbit anti-human IgE was removed and the wells were washed three times with PBS containing 1% bovine serum albumin (PBS 1% BSA). The microtiter wells were then incubated with 0.2 ml 10% BSA in PBS for 1 h at room temperature, then washed three times with PBS/1% BSA. 0.1 ml of either IgE standard (range 12–24 ng/0.1 ml) or of samples to be measured were added to the microtiter wells, and the plates were incubated for 16 h at room temperature in a humidified chamber. The next day the wells were washed three times with PBS/1% BSA, filled with 0.1 ml ^{125}I-anti-human IgE 25–30,000 cpm (sp act, 3–5 μCi/μg) in 5% BSA and 6 h later, the ^{125}I-anti-IgE was removed, and the wells were washed three times with PBS/1% BSA, and eight times with tap water. Finally, the wells were cut out with scissors and counted in a gamma scintillation counter (Packard Instrument Co., Inc.). The activity bound for each IgE standard was plotted on semi-logarithmic paper. The IgE content of individual samples was read from the standard curve. The lower limit of sensitivity of this assay was 15 to 20 pg.

Radioimmunoassay for IgG. IgG in the supernates of cell cultures was assayed by a competitive radioimmunoassay. First, the amount of rabbit anti-human IgG needed to precipitate 70% of ^{125}I-labeled human IgG (10 ng in 0.1 ml, 9,000 cpm/ng) was determined to be 0.1 μl. Subsequently, 0.1 ml of culture supernate (or of its dilutions) or of solutions of known IgG content (standards) was added to 0.1 μl of rabbit antiserum in a Beckman plastic microfuge tube (Beckman Instruments, Inc., Fullerton, Calif.). After an incubation of 1 h at 37°C, ^{125}I was added (10 ng in 0.1 ml). 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. Following 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 Packard gamma spectrometer. A standard curve was constructed from the samples with known IgG content. IgG present in an individual culture supernate was read from the standard curve.

Effect of parental cells in in vitro synthesis by the patient's PBL. In none of the three families available for the co-culture studies (families of patients 1, 2, and 3) was there a histoidentical sibling. For this reason co-cultures were carried with parental cells. Parental unfractionated lymphocytes, T cell-rich lymphocytes, and T cells depleted of either T4+ or T8+ cells were mixed in a 1:1 ratio with PBL from the patients with the hyper IgE syndrome and cultured at a concentration of 1×10^6 cells/ml in a final volume of 1 ml/culture. The value of IgE observed in the supernates of the mixed cell culture was compared with the expected value of IgE, which was calculated to be half the sum of the IgE present in supernates of the culture of the child's PBL and of the IgE present in the supernates of the cultures of parental cells. Individual cultures of PBL from patients and parents contained 1×10^6 cells/ml. Percentage of suppression of IgE synthesis by parental cells was calculated as:

$$1 - \frac{\text{observed value of IgE}}{\text{expected value of IgE}} \times 100.$$

To assess the effect of mixing PWM-induced immunoglobulin histoidentical cells by synthesis, a set of cultures was stimulated with PWM (1/500 dilution, Laboratories Gibco) for 6 d and IgG was measured in the supernates of these cultures.

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

RESULTS

Clinical characteristics and serum IgE levels. The clinical characteristics of our seven patients with hyper IgE syndrome are shown in Table I. As indicated, their age ranged from 2 to 19 yr and they comprised five males and two females. Serum IgE levels were markedly elevated in all seven patients and demonstrated some specificity in binding for *S. aureus* as previously reported by Schopfer et al. (9) and Berger et al. (10). In contrast, Table II shows that immunoglobulin of the IgE isotype did not bind to *S. aureus* in patients with other conditions associated with elevated serum IgE levels, including individuals with atopic dermatitis,

TABLE I

Clinical and Laboratory Characteristics of Patients with the Hyper IgE Syndrome

Patient	Age	Sex	Infections	Total IgE	^{125}I-anti-IgE bound when patient's serum was incubated with *S. aureus*
	yr			*IU/ml*	%
1	5	M	*Staphylococcus*: pneumonia, otitis *Candida*: paronychia, cutaneous	27,000	22
2	2	M	*Staphylococcus*: pneumonia, otitis, arthritis, abscesses	19,400	30
3	7	M	*Staphylococcus*: pneumonia, otitis *Hemophilus influenzae*: pneumonia	6,800	24
4	19	M	*Staphylococcus*: pneumonia, otitis *Streptococcus*: pneumonia *Diplococcus pneumonia*: otitis, pneumonia	47,000	32
5	7	F	*Staphylococcus*: pneumonia, otitis *Candida*: paronychia	7,000	15
6	4	M	*Staphylococcus*: pneumonia *Hemophilus influenzae*: otitis	2,500	16
7	6	F	*Staphylococcus*: pneumonia *Candida*: paronychia	2,400	13

allergic rhinitis, acute graft vs. host disease following allergeneic bone marrow transplantation, and Wiscott-Aldrich syndrome. Furthermore, no IgE binding to *S. aureus* was observed in the serum of two patients with chronic granulomatous disease and recurrent staphylococcal infections (data not shown).

In vitro synthesis of IgE. To determine whether the elevation in serum IgE was related to increased synthesis of immunoglobulin of this isotype, we performed a series of in vitro studies. Unstimulated PBL of four of the seven patients with hyper IgE syndrome or of normal controls were placed in culture for 6 d, after which time spontaneously secreted IgG and IgE immunoglobulins were measured. As shown in Table III, IgE production by four individuals with hyper IgE syndrome was markedly increased and averaged

TABLE II

Specificity of IgE Binding to S. Aureus

Diagnosis	Number of patients	Mean serum IgE	^{125}I-anti IgE bound when 100 IU of IgE were incubated with *Staphylococcus*
		IU/ml	%
Atopic dermatitis	7	5,515±5,135 (1,100–17,000)	0.6±0.2
Allergic rhinitis	8	3,537±2,183 (1,200–8,100)	0.3±0.2
Acute graft vs. host disease	4	5,950±2,350 (3,000–9,500)	0±0
Wiskott-Aldrich syndrome	2	1,950±250 (1,700–2,200)	0.2±0
Hyper IgE syndrome	7	16,014±15,242 (2,400–47,000)	16.0±4.4

All sera are diluted in PBS to contain 1,000 IU/ml of IgE. Values represent mean±SD. Values in parentheses represent the range.

TABLE III
In Vitro IgE and IgG Synthesis in the Hyper IgE Syndrome

Patient	IgE	IgG
	pg/10⁶ cells	*ng/10⁶ cells*
1	7,600	215
2	5,300	145
3	750	110
4	2,860	120
Mean±SD of patients	4,950±3,760	147±41
Mean±SD of normal controls (n = 5)	250±215	175±90
P	<0.05	>0.5

4,950 pg/10^6 cells in comparison with 250 pg/10^6 cells produced by normal controls' lymphocytes. In contrast, the amount of IgG produced by the same patients' cells was not greater than that of the controls. Furthermore, the IgE detected in the supernates of the 6-d cultures represented *de novo* production since the addition of cycloheximide resulted in a >90% reduction of secreted IgE (data not shown). Taken together with the clinical findings, these results indicate that the elevated levels of IgE found in the serum of hyper IgE patients are due to increased production by B cells.

Circulating T cells and T cell subsets. Since the production of immunoglobulin in both mitogen- and antigen-activated systems is regulated by T lymphocytes, the above findings suggested that the increased IgE production was due to either autonomous B cell hyperactivity and/or a defect in an immunoregulatory T cell population responsible for controlling B cell immunoglobulin production. To investigate this latter point further, we quantitated the patients' circulating peripheral T cells and individual T cell subsets. As shown in Table IV, in the control group of normal persons, 66±5% of peripheral lymphocytes were T cells, as determined by reactivity with anti-T3, whereas 37±3% were helper T cells reactive with anti-T4, and a smaller percentage, 22±4%, reactive with anti-T8 were suppressor T cells. Lymphocytes from patients with hyper IgE syndrome were abnormal in all cases. In six of seven cases, the percentage of T cells (T3+) was decreased. More importantly, there was a selective diminution in T8+ cells but not in T4+ cells in all individuals tested, which resulted in markedly abnormal ratios of T4+/T8+ cells compared with normal controls (Table IV). The decrease in T8+ cells was absolute, because the number of lymphocytes harvested from these patients' peripheral blood was never greater than that of normals (data not shown). The decrease in total (T3+) T cells in patients with the hyper IgE syndrome was not accompanied by an increase in the number of surface Ig-positive cells (B cells) nor by the presence of circulating immature T cells defined by the monoclonal anti-T6 antiserum, which recognizes the T6 antigen present on the majority of human thymocytes. Thus, it seems that these patients would have an increase in the number of null cells (data not shown).

Con A-inducible suppressor cell activity. To determine whether the decrease in the T8+ population was associated with a functional diminution in suppressor cell activity, we used a Con A-inducible suppressor cell assay. As shown in Table V, Con A-treated lymphocytes from all of the seven hyper IgE syndrome patients failed to suppress the proliferative response

TABLE IV
Number of Circulating T Cells and T Cell Subsets in the Hyper IgE Syndrome

	Patient	Cells positive for T3	Cells positive for T4	Cells positive for T8	Ratio T4/T8
			%		
Hyper IgE syndrome	1	50	43	10	4.3
	2	35	31	2	15.5
	3	50	43	10	4.3
	4	61	37	14	2.64
	5	43	29	7	4.14
	6	66	54	4	13.5
	7	57	26	6	4.33
Mean±SD of patients		51.7±11.2	37.5±7.5	7.5±4.4	6.96±5.2
Mean±SD of normal controls* (n = 20)		66±5	37±3	22±4	1.97±0.3
P		<0.01	>0.5	<0.001	<0.02

* Controls were age-matched with the patients.

TABLE V
Con A-inducible Suppressor Cell Activity in the Hyper IgE Syndrome

Patient	Suppression of the proliferative response to PHA	PWM
	%	
1	8	9
2	1	5
3	2	18
4	2	23
5	7	11
6	0	10
7	1	12
Mean±SD of patients	3±3	12.6±5.6
Mean±SD of normal controls (n= 8)	51±18	60±21
P	<0.001	<0.01

TABLE VI
Effect of Cell Density on Spontaneous IgE Synthesis

Source of cells		IgE per milliliter culture of		
Diagnosis	Patient	0.5×10^6 patient cells	0.5×10^6 patient cells and 0.5×10^6 thymocytes	1×10^6 patient cells
		pg		
Hyper IgE syndrome	1	3,100	ND*	5,800
	2	3,850	ND	8,100
	3	630	ND	1,350
Atopic dermatitis	1	780	840	1,650
	2	450	420	860
	3	220	4,260	410

All cultures were made as described in the text in 1-ml vol and incubated for 6 d. Thymocytes were irradiated with 500 rad.
* ND, not done.

of autologous lymphocytes to PHA and PWM, in comparison with normal controls. In addition, it should be noted that the failure to suppress the proliferative response was not due to loss of responsiveness to a suppressor stimulus. Con A-activated cells from the patient's normal mother or father were capable of suppressing the patient's peripheral blood mitogen response. In contrast, Con A-stimulated cells from the patient could not suppress the PHA response of parental peripheral lymphocytes. In four separate experiments parental Con A-stimulated cells suppressed the patient lymphocytes response to PHA by 56±14%. This was not significantly different from the ability of the same Con A-treated parental cells to suppress the PHA response of allogeneic normal peripheral blood (61±16%).

Suppression of IgE synthesis by parental cells. The above findings indicated that patients with hyper IgE syndrome had an elevated spontaneous secretion of immunoglobulin of the IgE isotype and at the same time had a diminution in the T8+ suppressor population associated with the functional loss of Con A-inducible suppression. To determine whether the decrease in the suppressor T cell subset was related to the augmented IgE response seen in vitro, a series of mixing experiments was performed. We first verified the assumption that the expected amount of IgE in cocultures is one-half the sum of the IgE present in each individual cultures by demonstrating that the amount of IgE present in 1 ml of culture containing 0.5×10^6 cells/ml is approximately one-half the amount of IgE present in cultures containing 1×10^6 cells/ml (Table VI). Furthermore, the presence of 0.5×10^6 irradiated thymocytes, used as filler cells, in cultures containing 0.5×10^6 cells from patients with atopic dermatitis was shown not to alter the amount of IgE into the culture fluid.

The capacity of parental peripheral lymphocytes to suppress IgE synthesis by the patient's B cells was examined in three different patients. In all three cases, the parent whose peripheral blood lymphocytes were used shared at least one human histocompatibility antigen A, B, C, or Dr haplotype with the patients. Moreover, all parents had a normal distribution and number of circulating T cells and synthesized <200 pg of IgE per culture. As shown in the representative case presented in Table VII, the patient's lymphocytes secreted 3,400 pg of IgE per culture. When peripheral lymphocytes from the normal parent were added, there was a diminution of IgE secretion to 1,050 pg, which was significantly less than the expected 1,800 pg (42% suppression). That this suppressor cell influence was contained within the T lymphocyte subpopulation was demonstrated by the observation that the parent's T cells suppressed the patient's IgE production, whereas the non-T cells from the same parent did not (46 and 14%, respectively). To determine whether this suppressor T cell effect resided within the T4+ or T8+ subset of T cells, isolated subsets of T lymphocytes from the parent were added to the patient's lymphocytes. As shown, the addition of T4+ enriched cells from the normal parent did not result in suppression of IgE synthesis (6%). In contrast, addition of T8+ enriched T cells from the normal parent markedly suppressed the patient's IgE production (53%). Data similar to that shown in Table VII were obtained in a total of six experiments using the cells from three different patients who were studied, respectively, thrice, twice, and once. In these six experiments the average suppression of IgE synthesis by parental cells was

39% with unfractionated PBL, 44% with T cells, 11% with non-T cells, 8% with T4+ enriched T cells, and 51% with T8+ enriched T cells. It should be noted that there was no detectable suppression of spontaneous PWM-induced IgG synthesis upon co-culture of parental cells with the patient's peripheral blood lymphocytes. In five different experiments involving three patients the average suppression of IgG synthesis by parental PBL was 5% for spontaneous IgG synthesis and 12% for PWM-induced IgG synthesis.

DISCUSSION

In the present study, we characterized immunoregulatory T cell functions in individuals with the hyper IgE syndrome. Unlike normal individuals, patients with hyper IgE syndrome were found to have an overall diminution in T cells (T3+). More importantly, there was a selective decrease in the T8+ suppressor T cell subset in all patients. Additional studies demonstrated that the decrease of T8+ cells was associated with a marked reduction in Con A-inducible suppressor cell function. This was not surprising, because previous studies indicated that the T8+ subset of T cells alone was activated by Con A to suppress the immune response (11).

In comparison with normals, lymphocytes from hyper IgE patients spontaneously secreted large quantities of IgE antibody in vitro. This hyper secretion was isotype restricted, as concurrent IgG secretion was not increased. Perhaps more importantly, a series of mixing experiments showed that the patient's augmented IgE synthesis in vitro could be significantly reduced by normal parental T8+ T cells in co-culture. In contrast, parental T4+ inducer cells were incapable of decreasing secretion of IgE by the patient's B cells. From these results, it would appear that the T8+ subset of T cells can regulate IgE production by B cells, and that in the setting of the hyper IgE syndrome there is a diminution in T8+ suppressor cells in association with the elevation of serum IgE.

All seven patients included in the present report fulfilled the clinical and laboratory criteria of hyper IgE syndrome. Specifically, they had extremely elevated serum antibodies of the IgE isotype of which some showed specificity for the *S. aureus*. Given that such patients had recurrent infections and relapsing dermatitis, it was necessary to consider the possibility that the decrease in T8+ cells might be secondary to infection and/or skin injury and not involved in the primary pathophysiologic events giving rise to elevation in their IgE isotype levels. This does not appear to be the case, however, since many patients with recurrent infections, including those associated with common variable hypogammaglobulinemia, tend to have a marked increase in the T8+ population,[2] and since we have recently shown that patients with a variety of skin diseases, excluding atopic dermatitis, have a normal T8+ population (15).

To date, the T8+ subset of T cells has been shown to mediate a wide variety of suppressor cell functions. Thus upon activation with Con A in vitro or viral infections in vivo, T8+ cells suppress autologous T cell proliferative responses to mitogen and antigen. In addition, these same cells suppress B cell immunoglobulin production induced by T4+ helper T cells (14). The present study indicates that a population of

[2] Reinherz, E., M. Cooper, S. Schlossman, and F. S. Rosen. Manuscript in preparation.

TABLE VII

Suppression of IgE Synthesis in Cultures of PBS from Patient 1 by Parental Cells

Parental cells added to the culture	IgE synthesis		Suppression	*P*
	Observed	Expected		
	pg/culture		%	
—	3,400±250	—	—	
PBL	1,050±145	1,800	42	<0.0001
T cells	900±55	1,700	46	<0.0001
Non-T cells	1,500±210	1,800	14	0.24
T4+ enriched T cells	1,600±120	1,700	6	0.40
T8+ enriched T cells	800±65	1,700	53	<0.001

Values represent mean±SD of duplicate cultures. Individual cultures of child's cells and parental cells contained 1×10^6 cells/ml. Co-cultures also contained 1×10^6 cells/ml with equal numbers of parental and child's cells. Cultures of parental PBL and parental non-T cells contained 200 pg of IgE, whereas cultures of parental T cells and T cell subsets contained no detectable IgE.

cells contained within the T8+ subset is also responsible for specific IgE isotype regulation in man.

As noted above, lymphocytes from patients with hyper IgE syndrome spontaneously synthesized large quantities of IgE in vitro, without the need for additional stimulation. These results are in agreement with those of other investigators who studied in vitro IgE synthesis in atopic patients with elevated serum IgE levels (16, 17, 21–23), and suggest that IgE-secreting B cells are activated in vivo. In light of the present findings, this activation could be secondary to loss of functional T8+ suppressor T cells. Furthermore, the selective elevation of the IgE isotype is most likely due to loss of a unique subpopulation of T8+ suppressor cells that regulate IgE expression. The IgE-specific suppressor cells but not IgG-specific suppressor cells were included in the subpopulation of T8+ cells that was deficient in patients with the hyper IgE syndrome. Indeed, had IgG specific suppressor cell influence been lost as well, then elevated serum IgG levels in vivo and elevated IgG synthesis in vitro should have been observed. Clearly this was not the case.

Isotype-specific suppressor cells have been shown to exist in experimental systems where IgE and IgG synthesis can be differentially controlled by immunoregulatory T cells (13, 14, 24). The observation that serum IgE levels are often elevated in the presence of normal or low IgG levels in many human T cell deficiencies (25, 26), including selective deficiency of suppressor T cells (27), further supports this view. Furthermore, some but not all authors (11, 12, 28) have found a depression of the number of circulating total T cells in patients with atopic diseases, and have suggested that this is a reflection of a suppressor T cell deficiency especially because normal T cells could suppress IgE synthesis by the PBL of atopic patients (16, 21, 29). Also, Canonica et al. (30) have suggested on the basis of their enumeration of T cells with Fcγ receptors that patients with atopic diseases are deficient in suppressor cells, but in view of the demonstration by Reinherz et al. (31) that most of T cells are monocytes, the conclusion of Canonica et al. remained unverified.

An increase in activated suppressor cells is commonly found in infectious diseases of both viral and bacterial origin (32, 33). The detection of diminished T8+ cells suppressor cells in patients with hyper IgE syndrome suggests that the immune response in these patients may be aberrant, given the fact that they frequently suffer from recurrent bouts of infection. The production of significant amounts of IgE antibody directed at *S. aureus* bacterial antigens further supports this view.

Loss of the population of suppressor cells is not specific to the hyper IgE syndrome. Indeed, it has been shown to occur during the active stages of systemic lupus erythematosus, multiple sclerosis, acute graft vs. host disease (19), and atopic dermatitis (18), although the elevated IgE levels in the hyper IgE syndrome, acute graft vs. host disease, and atopic conditions suggest that these diseases are associated with a common decrease in IgE specific suppressor cells. Although addition of normal T8+ T cells did not totally abrogate IgE production by patient's B cells in the present study, probably as a result of preactivation of IgE-secreting B cells (29), it did markedly decrease IgE secretion. In view of the above findings, our results would indicate that a rational therapeutic intervention in patients with hyper IgE syndrome might include a method aimed at restoring the suppressor cell population and/or its function.

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