Antihelper T Cell Autoantibody in Acquired Agammaglobulinemia

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ABSTRACT A patient with acquired agammaglobulinemia had an antihelper T cell factor that was identified as an immunoglobulin of the IgG class. The factor specifically bound to the TH_2^- T cell subset and, in the presence of complement, abolished the helper effect of normal T cells. The antihelper T cell antibody preceded by several years the appearance of suppressor $TH_2^+Ia^+$ T cells, at which time the clinical course rapidly deteriorated. Plasmapheresis resulted in lymphocytosis and reappearance of a functionally intact helper T cell population. It did not affect the suppressor cells. Conversely, total thymectomy resulted in a temporary disappearance of the $TH_2^+Ia^+$ suppressor cells, but did not decrease the levels of the autoantibody to helper T cells. Neither of these treatments reversed the state of agammaglobulinemia.

INTRODUCTION

Several different processes may lead to agammaglobulinemia in animals and in man. Although most congenital immunodeficiencies have been related to an inherent lymphocyte differentiation arrest, aberrant immunoregulatory mechanisms have been implicated in acquired agammaglobulinemia (1-6). It has been shown that a subset of T cells is responsible for the suppression of gammaglobulin secretion by normal B cells (1, 3-6). In some instances, in vitro removal of suppressor cells resulted in reestablishment of gammaglobulin secretion (1, 3-6). However, similar in vivo manipulations of suppressor cells, including treatment with cytotoxic drugs and steroids (1, 4), did not reverse the state of agammaglobulinemia, suggesting that much

remained to be learned about immunoregulatory processes in man. The initial immunological aberration in our patient was an autoantibody to the TH_2^- helper T cell subset (7), followed several years later by suppressor T cells characterized as being TH_2^+, Ia^+ cells (6). We report here the characterization of the antihelper T cell antibody and the changes in the T cell phenotype that resulted from various therapeutic interventions.

Case report. D.L. was a 17-yr-old white male with no family history of immunodeficiency or autoimmune diseases. Two of his three healthy siblings were HLA B and D identical with him. Recurrent infections started in the patient in the 1st yr of life with conjunctivitis, dacryocystitis, rhinitis, and otitis media, causing a progressive hearing loss. Frequent episodes of bronchitis and bronchopneumonias appeared at 3 yr, soon followed by diffuse bronchiectasis. However, childhood illnesses including measles, mumps, and varicella between the ages of 6-8 yr were uneventful. Moreover, immunizations with diphtheria, tetanus, and pertussis as well as with live oral polio vaccine at 1-4 yr caused no adverse reactions. Only smallpox vaccination at 4 yr caused a severe localized reaction, with ulceration persisting for several weeks. Agammaglobulinemia was diagnosed at 7 yr, and regular prophylactic treatment with gammaglobulin was instituted at 8 yr. Skin tests for delayed-type hypersensitivity were positive, and in vitro mitogenic responses of lymphocytes to phytohemagglutinin (PHA)¹ were normal. Growth and weight proceeded along the third percentile until 10 yr, but came to a complete halt at age 13.

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¹ *Abbreviations used in this paper:* Con A, concanavalin A; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; MLC, mixed lymphocyte culture; PBL, peripheral blood lymphocyte; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SRBC, sheep erythrocyte.

The patient was followed at the Albert Einstein College of Medicine from the age of 11. At 15 yr a sudden deterioration occurred, hallmarked by progressive interstitial pneumonitis with respiratory failure, malabsorption (lactose intolerance), recurrent parotitis, epileptic seizures, and a rapidly spreading erythematous and exfoliative maculopapular rash. On biopsy, the skin lesion consisted of an infiltrate with atypical blastlike lymphocytes, some of which had visible nucleoli. At this time, suppressor T cells were first noted and later on identified as TH_2^+Ia^+ cells (6).

Transfusions of fresh-frozen radiated plasma and injection of thymosin fraction V (Hoffman-La Roche; Nutley, N. J.) failed to alter the downhill course. The same applied to an 8-wk course of biweekly extensive plasmapheresis, each time followed by a transfusion of irradiated fresh-frozen plasma. Although the serum antihelper T cell activity was removed, no clinical change was noted. Attempts to modify the suppressor T cell activity by the use of three daily infusions of up to 20 mg/kg of an anti- TH_2^+ immunoglobulin (prepared by the Upjohn Co., Kalamazoo, Mich.) and by a 1-mo course of daily 2 mg/kg body wt prednisone were successful, but had no lasting effect, and serum immunoglobulins never increased. A total thymectomy was performed on 21 March 1979. Within 2 wk after this procedure, the maculopapular skin rash disappeared, and the patient remained afebrile for 5 wk (the longest interval without temperature since 1977). Moreover, the anorexia and malabsorption disappeared, the dyspnea decreased, and no TH_2^+Ia^+ cells were detectable in the peripheral blood. 2 mo later, however, the initially beneficial response to thymectomy was reversed by the development of osteomyelitis of the sternum. Although massive antibacterial treatment, drainage of the abscess, and finally a sternotomy temporarily brought the infection under control, the patient progressively lost ground. In September 1979, a bone marrow transplant was attempted as a last resort; shortly after the transplant, however, the patient succumbed to sepsis. Autopsy permission was refused.

METHODS

Serum immunoglobulins

IgA, IgG, IgD, and IgM were measured by radial immunodiffusion, using specific antisera. Peripheral blood lymphocytes (PBL) were isolated on a Ficoll-Hypaque density gradient. Blast transformation after stimulation with PHA, concanavalin A (Con A), pokeweed mitogen (PWM), *Candida*, streptodornase-streptokinase staphylococcus A, tetanus toxoid, purified protein derivative, Herpes Zooster, and in the mixed lymphocyte cultures was measured by the incorporation of tritiated thymidine as previously reported (8). T cells were detected by rosetting with sheep erythrocytes (SRBC). Rosettes were read in a fluorescence microscope after staining with euchrysin (8). Complement receptor positive lymphocytes were counted by rosetting with SRBC coated with

Forssman rabbit anti-SRBC serum and mouse complement (8). Surface immunoglobulin on lymphocytes was detected by rhodamine-conjugated F(ab')_2 antisera to human immunoglobulins according to Preud'homme and Labaume (9). TH_2^+ , TH_2^- , and Ia^+ lymphocytes were counted on the fluorescence-activated cell sorter (FACS II).

In brief, isolated T cells were incubated with a horse anti-serum to T cells, or with a rabbit anti-p23,30 serum to detect Ia-like antigens (6). The cells were then washed and reacted with fluorescein isothiocyanate (FITC)-conjugated F(ab')_2 rabbit anti-horse IgG, or with FITC-goat IgG fraction anti-rabbit IgG as developer for the anti-Ia serum. FACS analysis was performed as described previously (6, 10). Principally, the percentage of TH_2^+ cells was calculated automatically on the FACS II by setting the window to the right of the notch (foot of the second peak) of the bimodal fluorescence-staining pattern.

Suppressor cell assay

SRBC rosettes were used to separate T and B cells. Rosetted cells were fractionated on a Ficoll-Hypaque gradient. The pellet containing rosetted T cells was treated with cold ammonium chloride to lyse SRBC, and rosetted to obtain further enriched T cells in a second Ficoll-Hypaque gradient. Interface cells were harvested as a source of B cells and Null cells. Monocytes (adherent cells) were removed by adherence to Falcon plastic flasks (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) for 2 h at 37°C. X-irradiation of T cells was performed on a gammator for a total of 1,000 rad. Immunoglobulin secretion was measured by the double antibody precipitation according to Litwin (11, 12). Principally, co-culture experiments were conducted for 7 d in RPMI 1640 containing 1% PWM. At the end of the culture period the cells were pulsed with [^3H]leucine, centrifuged, and the supernate reacted with a rabbit anti-human kappa antiserum, followed by precipitation in equivalence with goat anti-rabbit antiserum. The immunologic precipitate was extensively washed on a Millipore sampling manifold (Millipore Corp., Bedford, Mass.) and the radioactivity on the filter paper monitored. Precipitation with normal rabbit serum was used as a control and the counts subtracted from those obtained with rabbit anti-kappa antiserum. Suppressor cells were also identified by the reverse plaque assay as previously reported (6).

Nature of the lymphocytotoxic serum factor

In most experiments, patient's serum was used. In some, an IgG serum fraction was studied. The IgG-enriched fraction was obtained by ammonium sulfate precipitation followed by fractionation on a G-200 Sephadex column, and by separation of the IgG fraction by DE-52 ion-exchange chromatography. The obtained IgG fraction was extensively concentrated (up to 300 mg% of IgG) to obtain a T cell reactivity identical to that of native serum with IgG levels of ~50 mg%. The used serum or its IgG fractions were free of aggregates as measured by the Raji cell assay, according to Theofilopoulos, and did not react with Fc receptors of T cells, as suggested by the lack of inhibition of T_{FCY} and T_{FCR} rosettes (8) after preincubation of T cells with the patient's serum or its IgG fraction.

Lymphocytotoxicity of patient's serum. A pellet of 1×10^6 peripheral blood lymphocytes or 1×10^6 T cells isolated by rosetting with SRBC was incubated for 1 h at 37° or at 4°C with 0.2 ml of patient's serum. Next, the cells were washed three times in RPMI and incubated with guinea pig complement for an additional 2–18 h at 37°C. Cell-killing was evaluated by two different techniques: (a) trypan blue dye exclusion

according to James et al. (13), and (b) ^{51}Cr -micro cytotoxicity assay according to Brunner et al. (14). Percent cytotoxicity was calculated according to the formula:

$$\frac{(\text{}^{51}\text{Cr released by experiment}) - (\text{}^{51}\text{Cr released spontaneously})}{(\text{}^{51}\text{Cr released by freeze-thaw}) - (\text{}^{51}\text{Cr released spontaneously})} \times 100 = \% \text{ cytotoxicity.}$$

Patient's serum in the absence of added guinea complement, normal human AB serum, and guinea pig complement alone were used as controls. Values of >15% cytotoxicity were considered significant.

Effect of patient's serum on blast transformation to non-specific mitogens (PHA, Con A), to specific mitogens (*Candida*, purified protein derivative), and to allogeneic cells (MLC). PBL, T cells, or B and Null cells separated as mentioned above were preincubated for 2 h with patient's serum or with its concentrated IgG fraction, in the presence of guinea pig complement. After the incubation period the cells were washed extensively and cultured for 3 d in the presence of nonspecific mitogens, or for 5 d with specific mitogens or allogeneic cells. In the MLC, only the responder cells were treated with the patient's serum.

Leukocyte inhibitory factor secretion. Leukocyte inhibitory factor secretion was studied in the agarose microdrop-let assay according to McCoy et al. (15) with *Candida* antigen and streptokinase-streptodornase. Before the reaction with the antigens, PBL were incubated for 2–18 h with patient's serum in the presence of guinea pig complement.

Generation of suppressor cells. Generation of suppressor cells by Con A was studied as reported previously (6, 16). Principally, PBL were incubated for 48 h with or without Con A. Next, cells were washed in buffered saline solution and α -methyl mannoside. Washed Con A-treated cells or untreated cells (for controls) were Mitomycin C treated and added to freshly drawn PBL from the same or from an unrelated donor. PHA, Con A, or *Candida* antigen was added to the co-cultures. PBL were incubated for 2–18 h with patient's serum and guinea pig complement at three different phases of the assay: (a) before the generation of suppressor cells with Con A; (b) with the Mitomycin-treated cells; and (c) with fresh PBL of the second phase of the assay, before their co-culture with Mitomycin C-treated cells. At the end of the culture period, DNA synthesis was measured by [^3H]thymidine uptake. Cells treated with patient's serum were compared with cells preincubated with normal AB serum.

Effect of patient's serum on helper T cells. The in vitro secretion of immunoglobulin kappa chains (IgK) by B cells was studied as mentioned above (see suppressor cell assay). To assess helper T cell activity, co-cultures of B cells were conducted with 1,000 rad-irradiated T cells to eliminate suppressor cells by the method of Siegal and Siegal (17). Co-cultured irradiated and nonirradiated T cells were preincubated for 2–18 h with guinea pig complement in the presence or absence of patient's serum or with the purified autoantibody.

Binding of patient's serum to enriched T cell populations. The reactivity of the patient's serum or its IgG fraction with viable T cells was studied by membrane immunofluorescence using indirect techniques. A pellet of 1×10^6 T cells was first reacted with 0.2 ml of patient's serum for 30 min at 4°C. The cells were then extensively washed and stained for 45 min at 4°C with FITC-conjugated F(ab')₂ antibody to human IgG. Controls included T cells reacted with normal human serum and FITC anti-IgG, and T cells reacted with FITC anti-human IgG alone. The percentage of cells with surface fluores-

cence was scored in a Leitz Orthoplan fluorescence microscope (E. Leitz Inc., Rockleigh, N. J.).

Binding of patient's serum to TH₂⁻ T cells. Normal (SRBC-rosetted) human T cells were reacted with an anti-thymocyte globulin (Upjohn Co., Kalamazoo, Mich.) raised in horses as previously reported (10). The cells were then washed and reacted with FITC-conjugated IgG F(ab')₂ fraction of rabbit anti-horse IgG. The fluorescence staining pattern was recorded on the FACS II. T cells were sorted into TH₂⁻ (weakly reactive) and TH₂⁺ (strongly reactive) cells. Trypsinized and nontrypsinized TH₂⁻ and TH₂⁺ cells were cultured overnight at 37°C, then reacted with patient's serum and developed with an FITC-conjugated rabbit F(ab')₂ IgG anti-human IgG. The fluorescence pattern of these cells was then analyzed on the FACS II as mentioned earlier.

Isolation of the T cell subpopulation reactive with the patient's autoantibody. Normal human T cells were preincubated with patient's serum, then rosetted with erythrocytes coated by the CrCl₃ technique with rabbit anti-human IgG. Rosetted cells were fractionated on a Ficoll-Hypaque gradient. Pelletted rosettes were treated with 0.87% NH₄Cl to lyse erythrocytes. Unfractionated T cells, rosetted T cells, and cells from the interface of the Ficoll gradient were then reacted with the antithymocyte globulin, developed with FITC-F(ab')₂ rabbit anti-horse IgG, and their fluorescence pattern analyzed on the FACS as above.

RESULTS

Serum immunoglobulins were undetectable or extremely low since the patient was first seen in 1970, at age 7 (Table I). Complement receptor positive cells and cells with surface immunoglobulins were present in normal numbers (Table I). Specific antibodies to tetanus-toxoid, polio virus vaccine, measles, mump, rubella, Epstein-Barr virus, streptococci, staphylococci, and pseudomonas antigens were absent. Isohemagglutinin titers to blood group B were not detectable. Peripheral blood lymphocytes yielded normal percentages and numbers of rosettes with SRBC. TH₂⁻ cells were diminished, whereas TH₂⁺ cells were increased (Table I); all TH₂⁺ cells were Ia positive as previously reported (6).

In vitro lymphocyte blast transformation in response to nonspecific mitogens and to specific antigens was first tested at 8 yr. Since 12 yr these tests were performed 6–14 times yearly. The responses were practically absent only in the last 2 yr of the patient's life. Table I includes the range of mitogenic responses recorded in the last 4 yr. The upper levels of the range were recorded in the years 1975–1978 before suppressor T cells became detectable. The background cpm remained unchanged and ranged between 218–1,422 for the 3-d cultures, with a mean of 651 in 43 cultures.

In vitro secretion of immunoglobulins (PWM assay)

Isolated patient's B cells were capable of secreting gammaglobulins when freed of autologous T cells and

TABLE I
Immunological Parameters before Treatment

Parameter	n	Patient data		Normal controls	
		Mean	Range	Mean	Range
IgG, mg%	11	34	16-70	1,158	680-1,493
IgA, mg%	11	0.2	0-4	208	81-232
IgM, mg%	11	0.8	0-9	99	57-308
Complement receptor positive cells, %	10	8	6-15	11	6-18
Surface Ig ⁺ cells, %	4	9	3-15	13	5-24
Cytoplasmic Ig ⁺ cells, %	3		3,6,7	—	8,14,9
Lymphocytes/cm ³	21	1,225	790-2,200	>1,000	
T cells, %	33	72	60-88	77	73-91
T cells/cm ³	21	1,063	632-2,469	>780	
TH ₂ ⁺ cells, %	5	65	56-80	18	11-32
Lymphocyte mitogenic responses (mitogenic index)					
PHA	32	5.6	1.9-31	31	14-104
Con A	32	4.5	1.5-10	23	11-46
PWM	21	5.6	1.1-10	19	11-31
Antigens	17	1.4	1-2.1	8	3-58
Mixed leucocyte culture	8	1.9	1.7-5	11	7-24

Range control values were obtained from 50-100 separate controls; for cytoplasmic Ig⁺ cell values, 3 age-matched simultaneous controls were used, and 14 simultaneous controls were used for TH₂⁺ cell values. The mitogen index represents the uptake of [³H]thymidine with mitogen stimulation vs. uptake without stimulation. "Antigens" refers to purified protein derivative, *Candida*, streptokinase-streptodornase, and tetanus toxoid. Data collected from 1975-August 1978.

co-cultured with allogeneic normal T cells or with T cells from a histoidential sibling. After plasmapheresis, irradiated patient's T cells were also capable of supporting a measurable gammaglobulin secretion by autologous B cells, yet to a smaller extent than were normal T cells (Table II).

T cell suppression of gammaglobulin secretion

Only after the age of 15 did co-cultures of normal allogeneic and histoidential PBL with patient's T cells result in suppression of gammaglobulin secretion (Table III). Similar results were obtained in the reverse

plaque assay (6). On two occasions after plasmapheresis, patient's T cells augmented the secretion of gammaglobulin when co-cultured in a 1:1 ratio with normal PBL. At a ratio of 2:1, suppression again prevailed, as shown in Table III.

Lymphocytotoxic effect of patient's serum

Both assays used to measure the cytotoxic effect of the autoantibody in the presence of guinea pig complement gave similar results. In 10 unrelated donors the percentage of killed cells as measured by the trypan blue dye exclusion test ranged from 16 to 41%, with a

TABLE II
Secretion of IgK by Patient's B Cells Co-cultured with T Cells (Double-antibody Precipitation)

Co-cultured cells	[¹⁴ C]Leucine
	<i>net cpm</i>
T _{RL}	200
T _{C1}	112
T _{C2}	84
T _{DL}	8
T _{DL} [*]	59

Cells were co-cultured at a ratio of 2:1. DL, patient; RL, histoidential sibling; T^{*}, radiated T cells (1,000 rad); C₁, C₂, normal unrelated controls.

TABLE III
Suppression of PWM-induced IgK Secretion by PBL after Co-culture with Patient's T Cells

PBL	[¹⁴ C]Leucine		Suppression
	Along	Plus T cells (DL) (2:1)	
	<i>cpm</i>		<i>%</i>
Histoidential sibling (RL)	411	5	98
Control	1,969	28	98
Control	354	131	63
Patient (DL)	0	—	—

Double-antibody immunoprecipitation.

TABLE IV
Patient's Serum Lymphocytotoxicity: ⁵¹Cr Release

	⁵¹ Cr release	
	PBL	Total T cells
	%	
Histoidential sibling (CL)	28	34
Unrelated controls		
1	19	28
2	31	43

$$^{51}\text{Cr release} = \frac{(^{51}\text{Cr released by experiment}) - (^{51}\text{Cr released spontaneously})}{(^{51}\text{Cr released by freeze and thaw}) - (^{51}\text{Cr released spontaneously})} \times 100.$$

mean of 34%. Table IV represents one experiment in which the cytotoxic effect was measured simultaneously on PBL and on T cells: the most marked cytotoxicity was noted on enriched T cell populations, whereas the ⁵¹Cr release from B and Null cells (not shown) did not exceed that of the untreated control (<15%). Normal human serum or its IgG fraction also did not induce a cytotoxic effect >15% on T cells.

Suppression of mitogenic responses. Response suppression was noted with unfractionated serum and with its IgG fraction, as represented in Table V. Nonspecific as well as specific mitogenic responses to antigens and to allogeneic cells were suppressed. B and Null cells that were contaminated by up to 5% T

cells were unaffected by patient's serum, whereas in most instances T responses were suppressed to <25%. None of the other serum fractions obtained during the purification of IgG had any suppressive effect on mitogenic responses.

The secretion of leukocyte inhibitory factor as well as the generation of suppressor cells was unaffected by unfractionated patient's serum or by its IgG fraction.

The effects of the lymphocytotoxic factor on helper T cells are shown in Table VI. Patient's serum of its IgG fraction markedly suppressed the helper effect of histoidential and allogeneic T cells. The suppression was most prominent in co-cultures of irradiated T cells with B cells (91 and 96% suppression).

The binding of patient's serum to PBL, B and Null cells, and enriched T cells was studied by indirect fluorescence. Less than 3% of the B and Null cells fluoresced with the patient's serum, whereas the highest percentage was noted with T cells (Table VII). The cell surface fluorescence density was lower than noted with B cells reacted with FITC-labeled anti-immunoglobulin. The patient's autoantibody for T cells was regularly detectable from the age of 12, when the first sample was tested. In the following years two to six samples per year were tested and demonstrated to contain the autoantibody. As shown in Table VII, only serum obtained after extensive plasmapheresis lost the binding to T cells. This serum sample also failed to block the in vitro T cell help for gammaglobulin secretion.

The binding of patient's sera with variable lymphocytotoxic activity to normal TH₂ cells sorted on the

TABLE V
Suppression of Normal PBL Blast Transformation by Preincubation with Patient's Serum and Complement

Cells	Antigens	GPC	GPC plus patient's serum	GPC plus IgG fraction of patient's serum*
		cpm	cpm	cpm
PBL	—	384	381	428
PBL	<i>Candida</i>	3,486	539	940
PBL	Purified protein derivative	7,025	2,051	1,829
T cells (rosetted)	—	801	774	925
T cells (rosetted)	PHA	51,884	12,557	12,345
T cells (rosetted)	Con A	12,674	1,369	3,393
B and Null cells	—	627	473	
B and Null cells	PHA	8,260	10,776	
B and Null cells	Con A	1,852	1,823	
PBL-MLC	Control ₁ ^M			
	+ Control ₂	54,020	36,644	10,871
PBL-MLC	Control ₁ ^M			
	+ Control ₂ ^M	2,023	3,033	1,940

Values are net counts per minute [³H]thymidine uptake. GPC, guinea pig complement.

* Concentrated to 300 mg% IgG.

TABLE VI
Suppression of T Cell Help for B Cell IgK Secretion by Patient's Serum

	Suppression	
	Patient serum	IgG fraction of serum
	%	
B _C plus T _C	59	66
B _C plus T _{RL}	76	96
B _{RL} plus T _{RL}	48	
B _{RL} plus T _{DL}	91	
B _{DL} plus T _{RL}	64	

T cells were incubated 2 h at 37°C with guinea pig complement with or without (GPC) patient's serum before co-culture with B cells.

Percent suppression

$$= 100 - \frac{\text{B cells + T cells (pretreated with GPC + serum) cpm IgK}}{\text{B cells + T cells (pretreated with GPC) cpm IgK}} \times 100.$$

RL, histoidential normal sibling; DL, patient; C, normal unrelated control; T^x, irradiated T cells (1,000 rad).

FACS II is given in Table VIII. The binding activity to TH₂⁺ cells was retained in sera obtained from the patient after treatment with anti-TH₂⁺ serum, after hydrocortisone treatment, and after thymectomy. In fact, serum obtained 7 wk after thymectomy shifted the fluorescence binding curve the farthest to the right (higher fluorescence intensity). At this time a normal population of TH₂⁺ cells and no TH₂⁺Ia⁺ cells were recorded. Only one serum sample obtained after extensive plasmapheresis did not bind to TH₂⁺ cells.

T cells that bound the patient's serum lymphocyto-

TABLE VII
Binding of Patient's Serum to T Cells

T cell source	Fluorescing T cells
	%
Normal control 1	9
Normal control 2	21
Normal control 3	31
Histoidential sibling (CL)	14
Histoidential sibling* (RL)	31
Histoidential sibling†	16
Histoidential sibling§	2

Background fluorescence using FITC F(ab')₂ rabbit anti-human IgG without D.L. serum was present in <3% of T cells.

* Patient's serum before plasmapheresis.

† Patient's serum after plasmapheresis twice.

§ Patient's serum after plasmapheresis five times.

TABLE VIII
Binding of Patient's Serum to TH₂⁺ Cells

Serum condition	TH ₂ ⁺ cells binding autoantibody
	%
Control serum	0
Before plasmapheresis	39
After plasmapheresis	2
After anti-TH ₂ ⁺ serum treatment	34
After hydrocortisone	35
After thymectomy	42

Analysis performed on FACS II. The sequence of therapies over a period of 11 mo was plasmapheresis, anti-TH₂⁺ serum, hydrocortisone, and, finally, thymectomy.

toxic factor could be enriched by binding to erythrocytes coated with an anti-human IgG antiserum. Rosetted cells were pelleted on a Ficoll-Hypaque gradient, freed of erythrocytes by NH₄Cl lysis, and re-analyzed on the FACS. 95% of rosetted T cells were TH₂⁺, as compared with 82% of the unfractionated T cells. Nonrosetted cells were enriched for TH₂⁺ cells (76%), but still contained 24% of TH₂⁺ cells (Fig. 1).

TH₂⁺ cells diminished (7%) most significantly after thymectomy (see also Fig. 2; 5/31/79 was 70 d after thymectomy). However, 71 d after surgery the percentage of TH₂⁺ cells abruptly returned to its original high levels (Fig. 2: 6/1/79, 42%; 6/4/79, 65%). The suppression of allogeneic B cells by patient's T cells disappeared after hydrocortisone treatment as well as after total thymectomy. It did not recur even after TH₂⁺Ia⁺ cells reappeared 71 d after thymectomy. On the other hand, the suppression of histoidential B cells remained unchanged after thymectomy and after hydrocortisone treatment. On only one occasion after treatment with the TH₂⁺ antiserum did this suppression disappear (6). However, at no time was an in vivo increase of gammaglobulin levels noted. After plasmapheresis, the activity of the lymphocytotoxic autoantibody dimin-

FIGURE 1 Separation of normal T cells by binding to patient's serum, then rosetting with SRBC coated with anti-IgG.

FIGURE 2 FACS analysis of patient's T cells reacted with anti-T cell globulin (ATG).

ished (Table VII and VIII), and a rapid increase of total lymphocytes and T cell counts up to 4.2-fold over the preplasmapheresis level was noted. The autoantibody remained unchanged or even increased during treatments with an anti- TH_2^+ serum or hydrocortisone, and after thymectomy (Table VIII).

DISCUSSION

Most patients with acquired agammaglobulinemia probably have an intrinsic B cell defect. In some, suppression of B cell differentiation by T cells was considered an important pathogenetic mechanism (1, 3-6). However, manipulation of suppressor T cells has at most reconstituted B cell function in vitro (1, 4), and many patients with acquired agammaglobulinemia who had nonfunctional circulating B cells lacked detectable suppressor T cells (1-5). Consequently, regulatory factors other than suppressor T cells may have been involved in these patients. Indeed, the lack of a factor responsible for B cell maturation was reported in a patient with thymoma and hypogammaglobulinemia (18, 19), whereas in other instances a serum factor inhibiting in vitro gammaglobulin secretions was documented (20). Such inhibitory factors may exert their influence by virtue of their lymphocytotoxicity, as has been postulated in patients with episodic lymphopenia and immunologic amnesia (21). Lymphocytotoxins have been identified in a variety of disorders directly or indirectly related to the immune system, such as systemic lupus erythematosus (22, 23), juvenile rheumatoid arthritis (22, 24), infectious mononucleosis, and after live rubella virus vaccine (25). We have reported a non-HLA lymphocytotoxic factor in a patient with acquired agammaglobulinemia. This factor peaked during an episode of acute graft-vs.-host reaction (26). The nature of this antibody and its specificity could not be

further defined. However, Tursz et al. (27) identified in a patient with hypogammaglobulinemia a factor that turned out to be an autoantibody to B cells, and in their patient, a dramatic increase in B cells occurred after plasmapheresis (27). In the patient presented here, the detected autoantibody reacted with a subset of T cells, but not with B cells.

Our patient's serum or its IgG fraction not only bound to T cells (Table VII), but killed a subset of T cells in the presence of complement (Table IV). This subset was further characterized by the use of heteroantisera to human T cells. It has been recently shown that the fluorescence pattern of T cells reacted with these FITC-conjugated heteroantisera is bimodal when analyzed on the FACS (10). 80% of T cells show low intensity and are termed TH_2^- , whereas 20% demonstrate high fluorescence intensity and are termed TH_2^+ . Human suppressor T cells are found in the TH_2^+ population, whereas helper cells are TH_2^- (10, 28, 29). The lymphocytotoxic antibody from our patient's serum reacted with the TH_2^- subset (Table VIII). Furthermore, TH_2^- cells that were reacted with the lymphocytotoxic factor could be rosetted out with erythrocytes coated with an anti-human IgG (Fig. 1).

Several observations clearly indicated that this autoantibody played a central causative, if not the primary role in the development of agammaglobulinemia in our patient. First, the TH_2^- subset, with which the autoantibody reacted in vitro, was also diminished in vivo. Second, functions mainly related to the TH_2^- subset (6, 10, 24) such as mitogenic responses to antigens, PHA, Con A, and allogeneic cells were abolished in vitro and in vivo by the autoantibody (Tables I and V). Third, irradiation of patient's T cells to obtain a functional helper T cell population freed of the concomitantly existing suppressor T cells (17) failed to provide help for gammaglobulin secretion by normal B

cells (not shown). This finding further supported the hypothesis that not only were the helper T cells diminished numerically by the antibody, but they were also deficient functionally. Fourth, normal irradiated T cells, when preincubated with the patient's serum or autoantibody in the presence of complement, lost their previously intact helper function (Table VI). Fifth, plasmapheresis resulted in an increase of TH_2^- cells in the peripheral blood and in a several-fold increase in total lymphocyte and T cell numbers. Moreover, after plasmapheresis, irradiated patient's T cells regained some of their helper potential for gammaglobulin secretion by autologous B cells.

In addition to the autoantibody to TH_2^- cells, our patient was also noted to have an abnormal TH_2^+ cell population. The percentage of TH_2^+ cells was markedly increased, and most of these cells were activated, as indicated by the presence of the Ia^+ marker (6). Moreover, the in vitro secretion of gammaglobulin was abolished when normal lymphocytes were co-cultured with the patient's T cells (Table III). The pathogenetic role of the T-suppressor cells vs. the autoantibody to helper T cells could, by and large, be clarified by the temporal analysis of both phenomena. Initially the autoantibody alone was detectable, whereas late in the course of the disease both existed side by side. In fact, the autoantibody preceded by several years the detection of suppressor cells by the PWM assay. The appearance of detectable suppressor cells coincided with a rapid decay of cell-mediated immunity and with a clinical deterioration evinced by progressive pulmonary failure, malabsorption, and a skin rash consisting of extensive infiltrates with blastlike lymphocytes. The relationship of this symptomatology to the suppressor cells was further supported by its reversal during treatments aimed against the suppressor cells. Introduction of an anti- TH_2^+ serum and the administration of steroids treatment (29), as well as total thymectomy, each caused a regression of the skin rash and the dyspnea and the disappearance of the symptomatology related to the malabsorption syndrome.

Taken together, the above data suggested that the suppressor cells were a secondary phenomenon in this patient. It became also apparent, however, that the clearance of the autoantibody by plasmapheresis could be of benefit for the patient only if combined with a permanent reduction of the suppressor cell population. Because steroids and the use of an anti- TH_2^+ serum failed to induce a long-lasting and appropriate control of the suppressor T cells, we resorted to a total thymectomy to achieve this goal.

Several studies in humans and in animals suggested that thymectomy may reduce the suppressor cell subset. In mice the maturational impulse for the suppressor cells bearing the $\text{Ly}_{2,3}$ surface marker, the corollary of the TH_2^+ cells in humans, is provided by the

intrathymic environment. Thymectomy in mice results in the loss of functional $\text{Ly}_{2,3}$ cells (30). In humans, thymectomy may lead to a decay of cell-mediated immunity (31) and to de novo autoimmune diseases such as myasthenia gravis and lupus erythematosus (32, 33); in both diseases a suppressor T cell defect was documented (34, 35). Conversely, thymomas may be accompanied by an abundance of suppressor T cells and acquired agammaglobulinemia (12). Our patient had an enlarged thymic shadow on tomography. After total thymectomy a gradual decline of TH_2^+ cells to normal values was noted (Fig. 2, 5/31/79). TH_2^+Ia^+ cells were also not detectable at that time. Concomitantly, the patient's T cells lost their suppression for allogeneic, but not for histoidentical B cells (29). This finding suggested that a small but potent suppressor cell subset, which could not be identified on the FACS by its TH_2 and Ia phenotype, may have persisted. Moreover, 71 d after thymectomy, the TH_2^+Ia^+ subset re-emerged, probably because of infection (Fig. 2, 6/1 and 6/4/79); it was functionally different from the prethymectomy TH_2^+Ia^+ subset. Namely, after thymectomy the patient's T cells did not regain their suppressor effect on allogeneic B cells. The lack of allogeneic B cell suppression after thymectomy may be related to the absence of the thymic microenvironment, as has been suggested in thymectomized and congenitally athymic (nude) mice (30). In such mice, a variety of substances that increase intracellular cyclic AMP levels were capable of producing changes in prothymocytes similar to the thymus itself. Those changes included the expression of Ly markers, which seem to be the analogue of the TH_2 markers in humans. These generated cells were endowed only with a moderate response to Con A and a minimal allogeneic response, however. It was thus postulated that in the absence of the thymus, the T cell precursor goes into a pathway of differentiation that generates nonfunctional T cells (30). The same hypothesis may apply to the post-thymectomy TH_2^+Ia^+ cells in our patient.

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