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Research Article

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Hyper IgM Immunodeficiency

A PRIMARY DYSFUNCTION OF B LYMPHOCYTE ISOTYPE SWITCHING

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ABSTRACT Immunological evaluations (lymphocyte markers, B cell differentiation, T cell function) were performed on peripheral blood mononuclear cells from four individuals with hyper IgM immunodeficiency. Number, proportion, and proliferation of T lymphocytes and T lymphocyte subpopulations were relatively normal in affected individuals. The percentage and number of B cells expressing surface IgM and IgD were either normal or elevated in both blood and lymph nodes. However, surface IgG- and IgA-bearing B lymphocytes were completely absent. In vitro stimulation of blood lymphocytes with both T cell-dependent and T-cell independent polyclonal B cell activators resulted in normal numbers of IgM plasma cells and IgM secretion in cultures, but failed to induce any IgG- or IgA-producing cells. This failure of isotype switching was intrinsic to the B cell population and did not involve aberrant T cell help or suppression. Therefore, individuals with this disorder possess an intrinsic B cell dysfunction that is not related to abnormal T cell regulation.

INTRODUCTION

IgM is the first antibody class to be expressed during B cell development in all of the animal species examined (1). Initially, the μ heavy chain isotype can be detected without light chains in pre-B cells (2, 3); although pre-B cells do not normally switch heavy chain isotypes, some malignant pre-B-like cells appear capable of producing both γ and α chains (4, 5, 6). Cells producing each of the heavy chain classes are derived from IgM-bearing B lymphocytes (7). Isotype switching may occur either during expansion of surface immunoglobulin (sIg^+)[1] B lymphocytes or upon differentiation to plasma cells with subsequent secretion of a single Ig class (8). T cells can preferentially enhance or inhibit the differentiation of plasma cells to produce certain Ig isotypes in an antibody response (9–12).

An immunodeficiency syndrome characterized by elevated serum levels of IgM and virtually undetectable quantities of IgG and IgA presents as either an X-linked or an acquired disorder (13, 14). Recent evidence suggests that individuals with this immunodeficiency possess normal numbers of IgM-bearing B lymphocytes, which can be stimulated by pokeweed mitogen (PWM) to secrete IgM but not IgG (15). Variable abnormalities of T helper and suppressor cell function have also been described in blood cells from these patients (16–19). Because PWM normally does not induce substantial isotype switching by peripheral blood B cells (20–22), the significance of this finding in terms of heavy chain

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[1] *Abbreviations used in this paper:* Bc, control B cell fraction; Bp, patients' B cell fraction; Con A, concanavalin A; EBV, Epstein-Barr virus; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; SA, *Staphylococcus aureus* Cowan I; sIg, surface immunoglobulin; Tc, control T cell fraction; Tp patients' T cell fraction.

class commitment and expression in hyper IgM immunodeficiency is unclear.

We have analyzed the phenotype of peripheral blood lymphocyte subpopulations from individuals with hyper IgM immunodeficiency and their function after culture with both T cell-dependent and T cell-independent B cell activators. Our findings indicate that B cells from individuals with this immune disorder are unable to switch heavy chain classes, even after stimulation by several polyclonal activators. The data also suggest that this defect is independent of T cell help or suppression.

METHODS

Patients. Four patients with elevated serum IgM and low IgG and IgA were studied. Patient 1 is a 17-yr-old black male with congenital deafness, a renal cyst, and recurrent fevers. Initial evaluation during a systemic infection with *Pseudomonas aeruginosa* revealed agranulocytosis, high serum IgM, and low serum IgG and IgA (Table I). Skin tests were positive against mumps. He is presently well and being treated with intravenous gamma globulin. His granulocyte levels are now normal.

Patient 2 is a 2-yr-old black male with a history of recurrent otitis media and upper respiratory infections. During an episode of pneumonia, he was evaluated for immune deficiency and studies revealed striking neutropenia, elevated IgM, and depressed IgG and IgA (Table I). Bone marrow aspiration showed a promyelocytic arrest, and epinephrine and hydrocortisone stimulation tests induced a two- and fivefold increase in peripheral neutrophil counts. He is currently receiving intramuscular gamma globulin, and his neutropenia persists.

Patient 3 is a 21-yr-old white male with a history of severe infections (*Haemophilus influenza* meningitis, overwhelming Herpes zoster, pneumococcal meningitis, and osteomyelitis), lymphadenopathy, and splenomegaly since age seven. He also experiences severe protein-losing enteropathy as well as anaphylactoid reactions when injected with intramuscular gamma globulin. He is currently treated with a different preparation of gamma globulin intravenously and is doing well.

Patient 4 is a 12-yr-old white male with a family history of an uncle who died in infancy after developing agranulocytosis and *Candida* sepsis, and whose autopsy revealed atrophic lymphoid tissue. The patient developed bilateral pneumonia at 7 mo of age. No palpable lymph nodes were detected and he was significantly neutropenic (Table I). Serum immunoglobulins revealed low IgG and IgA, but an elevated IgM. He subsequently developed *Pneumocystis carinii* pneumonia from which he recovered and is presently in relatively good health. He is treated with intramuscular gamma globulin every 3 wk.

Isolation of peripheral blood mononuclear cell (PBMC) populations. Heparinized venous blood obtained from normal adult volunteers (ages 18–50 yr) and patients was separated on Ficoll-Hypaque cushions (Pharmacia Fine Chemicals, Piscataway, NJ). The washed mononuclear leukocyte (PBMC) were either analyzed for cell phenotype or were depleted of monocytes (peripheral blood lymphocytes [PBL]) by adhering cells to plastic or Sephadex G-10 (23). To separate B cell-enriched (B) from T cell-enriched (T) fractions, PBL were incubated with aminoethylisothiouronium bromide-treated sheep erythrocytes (24). After centrifugation on Ficoll-Hypaque cushions, B cell-enriched fractions (65±12% sIg^+ cells, 25% nonspecific $esterase^+$ cells, 2±2% $OKT3^+$ cells) were harvested from the interphase. T cells were collected from the pellet after lysis of erythrocytes with NH_4Cl buffer (96±2% $OKT3^+$ cells).

Cell culture. Total PBL were cultured at 10^6 cells/ml. For lymphocyte subpopulations, 5×10^5 cells from the B fraction were combined with 5×10^5 T cells in 1 ml of medium (RPMI-1640 plus 10% fetal bovine serum, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 μg/ml streptomycin sulfate, and 100 U/ml penicillin). Cells were incubated for 7 d (37°C; humidified 5% CO_2-air) in the presence or absence of the indicated activator and media were harvested. In some experiments, cells were collected and prepared for fluorescent staining. Cells were washed with phosphate-buffered saline (pH 7.4) containing 0.5% bovine serum albumin and 0.01% NaN_3, and centrifuged onto glass slides. Cells were fixed in ice-cold 95% ethanol–5% acetic acid for 15 min.

Quantitation of plasma cell differentiation and Ig secretion. Fixed cells were stained with fluorescein or rhodamine conjugates of goat anti-human μ, γ, or α (25). Brightly stained plasma cells were counted on a Leitz (E. Leitz, Inc., Rockleigh, NJ) fluorescent microscope and the number of

TABLE I

Ig Levels and Leukocyte Counts in Patients with Hyper IgM Immunodeficiency

	Serum Ig				Differential				
Patient	IgM	IgG	IgA	Leukocyte	PMN	Lymphocytes	Monocytes	Eosinophil	Basophil
	*mg/dl**			*/mm³*			%		
1	2,000	<6	<1.2	2,000	75	25	0	0	0
2	175	23‡	<1.2	14,400	1	86	10	2	1
3	620	63‡	<2	21,800	48	37	0	12	3
4	600	240‡	<2	6,400	10	76	10	2	2

PMN, polymorphonuclear leukocyte.

* Serum Ig were quantitated by laser nephelometry in the clinical pathology laboratories of the Children's Memorial Hospital and the University of Alabama, Birmingham.

‡ These patients had received either blood transfusions or gamma globulin.

plasma cells per well determined by: (% plasma cells) × (cell number) per well/100.

Ig secretion into culture media was determined using a quantitative solid phase enzyme-linked immunosorbent assay (20). This assay is sensitive and linear between 10–80 ng/ml for IgM, 5–50 ng/ml for IgG, and 50–400 ng/ml for IgA.

Immunofluorescence. B lymphocyte subpopulations were determined by staining viable PBMC with fluorochrome-conjugated affinity-purified monospecific goat antibodies to human μ, δ, γ, α, ϵ, κ, or λ (26). Plasma cells were analyzed by reacting fixed cytocentrifuge preparations with 1:5 and 1:10 dilutions of antibodies that were used for cell surface fluorescent staining (26). Total T lymphocytes and T cell subpopulations were analyzed after reacting cells with either OKT_3 or Leu-1 (Pan T cell), OKT_4 or Leu-3 (helper/inducer T cell), or OKT8 or Leu-2 (suppressor/cytotoxic T cell) followed by fluorescein-conjugated goat anti-mouse IgG (absorbed with human gamma globulin) (27). Monocytes were enumerated by nonspecific esterase staining (28). Evaluation of fluorescent-stained surface markers was performed by using both the fluorescence-activated cell sorter (FACS-IV, Becton-Dickinson & Co., Orangeburg, NY) and Leitz fluorescent microscope equipped with epi-illumination and selective filters for fluorescein and rhodamine. Variation between the two methods was <15%. Cytoplasmic Ig in plasma cells was detected using fluorescence microscopy.

Proliferative responses. Proliferation of PBMC was assessed after stimulation with concanavalin A (Con A; 1–10 μg/ml, Sigma Chemical Co., St. Louis, MO), phytohemagglutinin M (1–10 μl/ml, Difco Laboratories, Inc., Detroit, MI), PWM (1:200–1:800, vol/vol, Gibco Laboratories, Grand Island, NY), and mitomycin-treated allogeneic lymphocytes as previously described (29).

RESULTS

Peripheral blood B cells only express IgM and IgD. We analyzed the heavy chain isotypes expressed by PBMC obtained from four individuals with the hyper IgM syndrome. In all patients and on every preparation of their PBMC, the only heavy chain classes that were detectable either on cell surfaces or intracellularly were μ and δ (Table II). After carefully examining between 7.5 and 10×10^8 lymphocytes, we have been unable to identify any IgG, IgA, or IgE B lymphocytes or plasma cells. In one individual (patient 1), a lymph node was evaluated and revealed 40% $sIgM^+$ cells and 35% $sIgD^+$ cells, but < 0.1% sIgG or sIgA B cells. Bone marrow from a second child (patient 2) revealed 8% $sIgM^+$ cells, 1% pre-B cells (25), and 15% IgM plasma cells (normal values: pre-B cells, 0.69±0.38%; $sIgM^+$ cells, 1.8±0.60%; IgM plasma cells, 0.10±0.04%). No IgG^+ or IgA^+ cells were detected. These results indicate that failure to detect heavy chain isotypes other than μ or δ on peripheral blood B cells was not due to sequestering of such cells in bone marrow or lymph nodes.

In contrast to B cells, no abnormalities were discernible in either the percentage or absolute numbers of T cells or T cell subpopulations (Table II). Relative proportions of monocytes in PBMC were somewhat elevated; this is probably indicative of the chronic inflammatory state of these individuals.

Induction of Ig isotypes in vitro. To determine the nature of the cellular defect in hyper IgM immunodeficiency, cultured PBMC and their subpopulations were stimulated by several polyclonal activators (PWM, *Staphylococcus aureus* Cowan I [SA], Epstein-Barr virus [EBV]) to determine the quantities and isotype distribution of Ig in plasmacytes, and secreted into supernatant fluids. Despite repeated attempts, no IgG or IgA plasma cells were ever detected by immunofluorescent staining after stimulation with any B cell activator (T cell-dependent or T cell-independent; Table III). Furthermore, no significant amounts of IgG and IgA could be measured in culture supernatants (Table IV). Generation of IgM plasma cells and secretion of IgM were either normal or slightly increased (Tables III and IV).

T cell function is normal in hyper IgM immunodeficiency. The PBMC from all patients proliferated normally in response to all mitogens and allogeneic lymphocytes (data not shown). To analyze whether T cells, which are capable of helping normal B cells synthesize and secrete IgG and IgA, were present in PBL

TABLE II
Lymphocyte Markers on PBMC of Patients with Hyper IgM Immunodeficiency

	sIg*					Surface T cell markers*		
Patient	M	D	G	A	κ/λ	OKT3 or Leu-1	OKT4 or Leu-3	OKT8 or Leu-2
1	6.9	5.5	<0.5	<0.5	1.0	38	25	12
2	19.9	19.9	<0.1	<0.1	1.3	74	65	9
3	14.0	11.4	<0.1	<0.1	1.3	49	40	10
4	12.5	10.9	<0.1	<0.1	1.8	43	35	14
Normal	5.7–15.3	4.0–13.4	0.7–2.5	0.5–1.9	1.5–2.9	50–76	29–57	13–23

* Surface fluorescent staining was performed as described in Methods. Results are expressed as percentage of total mononuclear cells.

TABLE III
Plasma Cell Differentiation In Vitro by PBMC from Individuals with Hyper IgM Immunodeficiency

Patient	Stimulus*	No. plasma cells/well × 10^{-4}‡ IgM	IgG	IgA
3	None	1.7	<0.1	<0.1
4	None	0.4	<0.1	<0.1
Controls	None	0.7±0.3	0.3±0.2	0.1±0.1
3	PWM	7.2	<0.1	<0.1
4	PWM	3.3	<0.1	<0.1
Controls	PWM	4.7±2.4	3.2±1.4	2.0±1.8
3	SA	13.7	<0.1	<0.1
4	SA	10.0	<0.1	<0.1
Controls	SA	9.4±2.7	5.0±3.3	6.2±1.4
1	EBV	13.0	<0.1	<0.1
2	EBV	8.0	<0.1	<0.1
3	EBV	22.0	<0.1	<0.1
4	EBV	13.3	<0.1	<0.1
Controls	EBV	15.0±3.3	12.5±9.5	5.2±3.0

* Activators were at optimum concentrations for 10^6 monocyte-depleted PBMC/ml medium. PWM = 1:400 vol/vol final dilution; SA = 1:10,000 vol/vol final dilution; EBV = 1:20 vol/vol of B-95-8 culture supernatant.

‡ After 7 d, cells were harvested, washed, counted, and centrifuged onto slides. After fixing, cells were stained with combinations of fluorescein-conjugated goat anti-human μ, rhodamine-conjugated goat anti-human γ, and rhodamine-conjugated goat anti-human α. Percentage plasma cells were determined on at least 500 cells/slide, and plasma cells per well were determined as follows:

Plasma cells/well = [(% plasma cells) × (cell number)/100]/well.

from patients, co-culture studies were performed (Table V). T cells from patients (Tp) were combined with B cells from normal individuals (Bc) and were then stimulated by polyclonal activators. In repeated experiments, the patients' T cells were capable of providing excellent help for normal B cells to differentiate into IgG- and IgA-secretory cells. In most instances, allogeneic interactions enhanced secretion of all immunoglobulin isotypes when coupled with mitogenic stimulation. Furthermore, when patients' T cells were added to normal B plus T cells, no suppression of IgG or IgA secretion could be detected (Table V); instead, marked increases in total Ig secretion compared with autologous B plus T cell mixtures occurred.

Since T cells stimulated with Con A will suppress plasma cell differentiation in vitro (30, 31), we treated T cells from patient 3 and a normal control with 25 μg/ml Con A for 24 h. Both the patient and control possessed T cells that could suppress antibody synthesis for all isotypes (including IgM) (Table VI). Thus, the elevated levels of IgM associated with this syndrome were not due to diminished T suppressor capacity for lymphocytes committed to IgM production.

B cells from hyper IgM immunodeficient patients cannot switch isotypes. When B cells from patients were stimulated with either a T cell-independent polyclonal activator (EBV) or T cell-dependent activators (PWM, SA) plus normal T lymphocytes, only IgM plasma cells and IgM secretion developed (Table VII). Allogeneic suppression of IgG or IgA production did not influence this observation, since a second (normal) person's T cells permitted expression of all isotypes when cultured with normal (but not the patients') B cells. Therefore, B cells from individuals with hyper IgM immunodeficiency failed to express IgG or IgA independent of the signals used to induce these isotypes.

DISCUSSION

In this report, we have confirmed the results of Geha et al., (15) who demonstrated that B lymphocytes from

TABLE IV
Secretion of Ig by Cultured PBMC from Individuals with Hyper IgM Immunodeficiency

Patient	Stimulus*	Ig secreted‡ IgM	IgG	IgA
			ng/ml	
3	None	30	<10	<62
4	None	<10	<10	<62
Controls	None	38	<10	<10
3	PWM	2,400	<10	<10
4	PWM	420	<32	<62
Controls	PWM	560	2,100	220
		504	312	1,056
3	SA	4,800	<10	<62
4	SA	692	<10	<62
Controls	SA	8,800	6,400	1,500
		3,360	1,088	2,016
1	EBV	5,400	<32	<32
2	EBV	3,560	<32	<32
3	EBV	9,240	<10	<62
4	EBV	1,220	<10	<62
Controls	EBV	4,120	104	360
		7,350	6,500	2,030
		5,215	4,380	1,010

* Cultures were established and treated identically to those in Table III: media was harvested on day 7 for PWM and SA and on day 10–12 for unstimulated and EBV-treated cultures.

‡ Solid phase enzyme-linked immunosorbent assay was performed as previously described. Sensitivity of assays varied between 10 and 128 ng/ml culture supernatant.

TABLE V
Effect of Patient's T Cells on Ig Secretion by Normal B Cells

Patient*	Stimulus‡	Ig secreted§ IgM	IgG	IgA
			ng/ml	
Bc_3	None	60	<10	<62
$Bc_3 + Tc_3$	None	30	44	104
$Bc_3 + Tp_3$	None	280	230	136
$Bc_3 + Tc_3 + Tp_3$	None	220	250	144
Bc_3	PWM	90	<10	<62
$Bc_3 + Tc_3$	PWM	560	2,100	220
$Bc_3 + Tp_3$	PWM	2,800	>10,000	440
$Bc_3 + Tc_3 + Tp_3$	PWM	3,100	>10,000	650
Bc_3	SA	120	62	<62
$Bc_3 + Tc_3$	SA	8,800	6,400	1,500
$Bc_3 + Tp_3$	SA	12,800	>10,000	2,500
$Bc_3 + Tc_3 + Tp_3$	SA	10,120	>10,000	2,500
Bc_4	None	22	<10	104
$Bc_4 + Tc_4$	None	460	46	352
$Bc_4 + Tp_4$	None	500	108	304
$Bc_4 + Tc_4 + Tp_4$	None	500	62	344
Bc_4	PWM	62	<10	216
$Bc_4 + Tc_4$	PWM	504	312	1,056
$Bc_4 + Tp_4$	PWM	1,240	272	312
$Bc_4 + Tc_4 + Tp_4$	PWM	1,800	1,232	2,624
Bc_4	SA	160	124	304
$Bc_4 + Tc_4$	SA	3,360	1,088	2,016
$Bc_4 + Tp_4$	SA	4,400	1,432	3,680
$Bc_4 + Tc_4 + Tp_4$	SA	15,360	>8,000	>4,000

Abbreviations used in this table: C_3, normal control for patient 3; C_4, normal control for patient 4; P_3, patient 3; P_4, patient 4.
* B = 5×10^5 B cell fraction; T = 5×10^5 T cell fraction.
‡ See Table III.
§ See Table IV.

peripheral blood of individuals with hyper IgM immunodeficiency cannot be stimulated to produce IgG in response to PWM. Such patients lack IgG- and IgA-bearing B cells in their blood. We have also stimulated PBL with several other mitogens, both T cell-dependent (SA, SA plus PWM) and T cell-independent (EBV) activators, and have shown a total failure of patients' cells to produce IgG or IgA. Recent studies (20–22) have demonstrated that PWM induces little if any isotype switching by B cells before plasma cell differentiation and that it is unknown whether SA, SA plus PWM, or EBV can stimulate isotype switching by peripheral blood B cells in vitro. Therefore, the failure of cells from patients with hyper IgM immunodeficiency disease to become IgG- and IgA-secreting plasma cells could be due to the virtual absence of γ^+ and α^+ B cells in blood as a result of an earlier isotype switch aberration.

Despite a great deal of effort, few primary antibody deficiency disorders have been reproducibly associated with specific cellular defects. X-linked agammaglobulinemia represents a failure of B lymphocyte development past the pre-B cell stage (32). The common variable immunodeficiencies and IgA deficiency have been linked to functional abnormalities of B cells (33), T cells (34–37), monocytes (38, 39), or all of these subpopulations (40). However, the gross percentages and phenotypes of B cells and T cells in these disorders appear relatively normal (41, 42).

No T cell defects could be demonstrated in blood lymphocytes from hyper IgM patients. Proliferative responses were normal. T cells from affected individuals permitted normal B cells to produce IgG and IgA in vitro. In fact, increased Ig secretion occurred with mixtures of Bc plus Tp when compared with autologous combinations (Table V); this suggests that allogeneic interactions enhanced mitogen-induced plasma cell development (43). Since the small number of T cells in B cell fractions (<4%) were not capable of providing

TABLE VI
Suppression of Ig Secretion by Con A-treated T Cells from Individuals with Hyper IgM Immunodeficiency

Patient*	Stimulus‡	Ig secreted‡ IgM	IgG	IgA
			ng/ml	
$Bc_3 + Tc_3$	PWM	560	2,100	220
$Bc_3 + Tc_3 + Tp_3$	PWM	3,100	>10,000	560
$Bc_3 + Tc_3 + Tc_3$ Con A	PWM	64	110	<62
$Bc_3 + Tc_3 + Tp_3$ Con A	PWM	32	<10	<62
$Bc_4 + Tc_4$	PWM	504	312	1,056
$Bc_4 + Tc_4 + Tp_4$	PWM	1,800	1,232	2,624
$Bc_4 + Tc_4 + Tc_4$ Con A	PWM	110	<10	<62
$Bc_4 + Tc_4 + Tp_4$ Con A	PWM	60	65	<62
$Bc_3 + Tc_3$	SA	8,800	6,400	1,500
$Bc_3 + Tc_3 + Tp_3$	SA	10,120	>10,000	2,500
$Bc_3 + Tc_3 + Tc_3$ Con A	SA	560	475	<62
$Bc_3 + Tc_3 + Tp_3$ Con A	SA	560	220	<62
$Bc_4 + Tc_4$	SA	3,360	1,088	2,016
$Bc_4 + Tc_4 + Tp_4$	SA	15,360	>8,000	>4,000
$Bc_4 + Tc_4 + Tc_4$ Con A	SA	360	60	<62
$Bc_4 + Tc_4 + Tp_4$ Con A	SA	220	115	92

Abbreviations used in this table: C_3, normal control for patient 3; C_4, normal control for patient 4; P_3, patient 3; P_4, patient 4.
* B = 5×10^5 B cell fraction; T = 5×10^5 T cell fraction; T Con A = 2.5×10^5 T cells treated with 25 μmg/ml Con A for 24 h.
‡ See Table III.

TABLE VII
Ig Secretion by B Cells from Individuals with Hyper IgM Immunodeficiency

Patient*	Stimulus‡	Ig secreted‡ IgM	IgG	IgA
			ng/ml	
$Bp_3 + Tp_3$	None	38	<10	<62
$Bp_3 + Tc_3$	None	76	<10	<62
$Bp_3 + Tp_3$	PWM	2,400	<10	<62
$Bp_3 + Tc_3$	PWM	800	<10	<62
$Bp_3 + Tp_3$	SA	4,800	<10	<62
$Bp_3 + Tc_3$	SA	9,920	<10	<62
Bp_3	EBV	7,620	<10	<62
$Bp_3 + Tp_3$	EBV	6,450	<10	<62
$Bp_3 + Tc_3$	EBV	6,980	<10	<62
$Bp_4 + Tp_4$	None	<128	<10	<62
$Bp_4 + Tc_4$	None	352	<10	<62
$Bp_4 + Tp_4$	PWM	420	<10	<62
$Bp_4 + Tc_4$	PWM	1,120	<10	<62
$Bp_4 + Tp_4$	SA	692	<10	<62
$Bp_4 + Tc_4$	SA	3,600	<10	<62
Bp_4	EBV	2,630	<32	<62
$Bp_4 + Tp_4$	EBV	1,220	<32	<62
$Bp_4 + Tc_4$	EBV	1,870	<10	<62

Abbreviations used in this table: C_3, normal control for patient 4; P_3, patient 3; C_4, normal control for patient 4; P_4, patient 4.
* B = 5×10^5 B cell fraction; T = 5×10^5 T cell fraction.
‡ See Table III.

significant help for Ig secretion with either PWM or SA, it is likely that the patients' T cells were stimulated by control B cells (or macrophages) to produce allogeneic helper factors (44). In total, these data suggest that neither diminished T cell help for IgG and IgA expression nor excessive suppression exists in cells from these patients. When provided with proper signals (allogeneic interactions, mitogen stimulation), substantial help for Ig production by normal B cells can be generated.

Excessive T suppressor cell activity has been associated with certain immune deficiency disorders (33). Individuals with common variable panhypogammaglobulinemia may possess T cells capable of blocking plasma cell differentiation of B cells for all isotypes (34). Individuals with selective IgA deficiency can express class-specific (IgA) T suppressor activity (9, 37). In mice, cloned T suppressor cells or hybridomas that are specific for IgE production have been characterized (45). Our patients did not demonstrate abnormal T suppressor activity in vitro, and their Con A-stimulated T cells could suppress IgM synthesis as well as other isotypes.

It has been postulated that T helper cells may be specific for individual isotypes and may determine the preferential association of certain antibody classes with specific types of antigenic or mitogenic stimulation (46, 47). A deficiency of the T cells that are needed for IgG and IgA expression did not exist in our patients; all four individuals possessed T cells that provided substantial help for normal B cells to secrete the three major immunoglobulin classes. While T cells from patient 4 provided poor help for IgA synthesis by normal B cells after PWM stimulation (Table V), they were able to significantly enhance IgA secretion by this same B cell fraction after culture with SA. Although the ability of SA to induce plasma cell differentiation by human B cells in the absence of T cells is controversial (48–50), we have found that these bacteria require T cells to provide satisfactory help for Ig secretion. (Beckmann, E., and Levitt, D., manuscript submitted for publication; Bard, J., and Levitt, D., manuscript submitted for publication.)

The defect in hyper IgM immunodeficiency may then be attributed to at least two potential B cell abnormalities. First, B cells could lack receptors capable of receiving specific helper signals from T cells that would induce antibody switching. This proposal could be evaluated once a system is developed in which B cells clearly undergo heavy chain class switching in vitro. Second, B cells from patients might lack mechanisms that promote DNA recombination at switch sites for the heavy chain loci. These defects could involve either DNA deletions or mutation at switch sites, or reduced amounts of specific recombinase or switching enzymes (51, 52). Detailed information on the nucleotide sequences of heavy chain constant region genes, especially switch-recombination sites, could help to define a disease mechanism in this instance. However, in view of the association of this immunodeficiency with X-linked inheritance, it seems more likely that a regulatory gene defect is the basis for the failure of heavy chain isotype switching.

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