Specific Anti-influenza Virus Antibody Production In Vitro by Lymphocytes from a Subset of Patients with Hypogammaglobulinemia

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ABSTRACT Specific anti-influenza virus antibody production in vitro was studied in peripheral blood mononuclear cells from 17 patients with hypogammaglobulinemia. Cells obtained from 6 of 12 patients with common variable hypogammaglobulinemia produced anti-influenza virus antibody, predominantly of the IgM isotype, when cultured in vitro with type A influenza virus. No antibody was produced in vitro, however, by cells from either of two patients with Bruton's type X-linked hypogammaglobulinemia or by cells from any of three patients with X-linked hypogammaglobulinemia and isolated growth hormone deficiency. These studies demonstrate that peripheral blood mononuclear cells from a subset of patients with common variable hypogammaglobulinemia retain the potential to produce specific antibody in response to antigenic stimulation.

INTRODUCTION

The primary immunodeficiencies associated with hypogammaglobulinemia are a heterogeneous group of disorders that share the common features of low serum immunoglobulin levels, impaired in vivo antibody responses to antigens, variable in vivo delayed type hypersensitivity reactions, and an increased susceptibility to infections. Patients with X-linked hypogammaglobulinemia, including a recently described family with associated isolated growth hormone deficiency (1, 2), usually lack circulating surface immunoglobulin positive B cells, while patients with common variable hypogammaglobulinemia (CVH)[1] have either decreased or normal numbers of circulating B cells (3).

Much has been learned in recent years about the cellular mechanisms underlying these disorders by studying in vitro immunoglobulin production in response to polyclonal activators. Using this approach, cultured lymphocytes from many of these patients fail to produce significant amounts of immunoglobulin of any class when stimulated with pokeweed mitogen (PWM) (2, 4–8), the Epstein-Barr virus (2, 8), or lymphocyte mitogenic factor (9). In some of these cases, the patients' B cells fail to produce immunoglobulin even when normal T cells are added as a source of T helper cells, and these patients are believed to have an intrinsic B cell defect (2, 6). In other patients who fail to produce immunoglobulin in vitro, there appears to be a disorder of T cell regulation with an excess of suppressor T cell activity and/or a lack of helper T cell activity (4, 6, 7).

Whereas many patients with hypogammaglobulinemia fail to secrete immunoglobulin in response to polyclonal activators, certain patients with this disorder do synthesize immunoglobulin in vitro (predominantly IgM) when stimulated with PWM or Epstein-Barr virus (5–8, 10, 11). Immunoglobulin production by lymphocytes in response to polyclonal activators, however, may not reflect their ability to produce specific antibody in response to antigenic challenge. In

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[1] *Abbreviations used in this paper:* A/HK, A/Hong Kong/8/68-X31 (H3N2) influenza virus; A/Aichi, A/Aichi/2/68 MN25241 (H3N2) influenza virus; CVH, common variable hypogammaglobulinemia; ELISA, enzyme-linked immunosorbent assay; GHD, isolated growth hormone deficiency; KLH, keyhole limpet hemocyanin; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; XLHG, X-linked hypogammaglobulinemia.

this paper, we define a subset of patients with CVH whose peripheral blood mononuclear cells (PBMC) are able to produce anti-influenza virus antibody in vitro when cultured with influenza virus. These studies demonstrate that at least some patients with hypogammaglobulinemia have in their peripheral circulation the necessary cellular elements to mount a humoral immune response to antigenic challenge.

METHODS

Patients. 12 patients, ages 31 to 67 yr, with CVH were studied. All had serum IgG levels of 2.4 mg/ml or less and decreased IgA and IgM levels as determined by radial immunodiffusion (Table I). Isohemagglutinins (done by the NIH Clinical Center Blood Bank) were decreased in each of those patients in whom they could be determined (Table I). None of the patients with CVH had an X-linked pattern of inheritance, thymoma, or other illness known to cause hypogammaglobulinemia, and all had the onset of symptoms after 2 yr of age. These patients were selected only on the basis of their clinical diagnosis. Two unrelated male patients with Bruton's type X-linked hypogammaglobulinemia (XLHG), ages 7 and 22 yr, each had low or undetectable serum Ig levels, undetectable isohemagglutinins (Table I), absent tonsils and circulating B cells, a brother with hypogammaglobulinemia, and the diagnosis of their illness in early childhood. Three additional male patients, ages 5, 7, and 31 yr, with X-linked hypogammaglobulinemia and isolated growth hormone deficiency (XLHG-GHD) have been previously described (2). Except where noted, all patients were receiving gamma globulin replacement therapy and prophylactic antibiotics at the time of the study. Controls for in vitro studies and resting in vivo anti-influenza virus antibody titers consisted of 14 randomly selected healthy adult volunteers. Controls for in vivo antibody responses consisted of 25 healthy young adult volunteers. With the exception of patient 1 who had had yearly vaccinations with inactivated influenza virus for the past 10 yr, neither patients nor controls had been recently immunized with influenza virus.

In vivo antibody responses. Serum samples from patients and controls were obtained at the time of and at three weekly intervals following intramuscular immunization with 2.5 mg of keyhole limpet hemocyanin (KLH) and/or intradermal immunization with 1 floculation U of diphtheria and tetanus toxoids. The pre- and post- (maximum of the three samples) titers of antibody to these antigens were determined by standard hemagglutination technique (2).

Cell preparation and culture conditions. PBMC obtained from patients and normal controls were prepared and cultured as previously described (12, 13). Briefly, PBMC isolated by Ficoll-Hypaque centrifugation of heparinized peripheral venous blood were depleted of cytophilic immunoglobulin by a 1-h incubation at 37°C in RPMI 1640 medium supplemented with 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Gibco Laboratories, Grand Island, NY), followed by two 10-min centrifugations through gradients of fetal calf serum. This procedure has been shown to effectively remove cytophilic antibody, as the antibody released into culture by cells treated in this manner is cycloheximide inhibitable (12, 13). 2 million viable PBMC were cultured in triplicate in RPMI 1640 medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 4 mM L-glutamine, and 10% fetal calf serum (Armour Pharmaceutical Co., Kankakee, IL) in a final volume of 2 ml in 16-mm well diam cluster tissue culture plates (Costar, Data Packaging, Cambridge, MA). Cultures were stimulated with 0.03 hemagglutinin antigen units of live A/HK influenza virus (A/Hong Kong/8/68-X31 [H3N2]) as a dilution of infectious allantoic fluid, 0.5 hemagglutinin antigen units of purified formalin-inactivated A/Aichi influenza virus (A/Aichi/2/68 MN25241 [H3N2]), PWM (Gibco Laboratories) diluted 1:100 vol:vol, or media alone. Preliminary studies revealed these concentrations of viruses and mitogen to yield maximal antibody production. The viruses were the gift of Dr. Brian R. Murphy, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. Some cultures received in addition 15 μg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO). The cultures were incubated for 10 d at 37°C in humidified air enriched with 5% CO_2, the supernatants harvested, and the latter stored at −20°C until assayed.

Enzyme-linked immunosorbent assays. IgG and IgM anti-influenza virus antibody in the culture supernatants was assayed by a two-step enzyme-linked immunosorbent assay (ELISA) in flat-bottomed microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) as previously described (12, 13). Briefly, the ladder of reagents from the solid-phase up consisted of purified formalin-inactivated A/Aichi influenza virus, dilutions of the culture supernatant, rabbit anti-human IgG or IgM antisera (13), goat anti-rabbit IgG conjugated to alkaline phosphatase (Dynatech Diagnostics, So. Windham, ME), and *p*-nitrophenol phosphate (Sigma Chemical Co.) as the substrate. The specific antibody in the culture supernatant was determined by comparison of the optical density read at 405 nm with a curve of absorbance vs. concentration obtained with dilutions of a reference serum as previously described (13). The concentration of IgG and IgM anti-A/Aichi antibody in this reference serum was estimated using a modification of the technique of R. H. Stevens (14) as described (12, 13). Results presented are the arithmetic means of the triplicate cultures (13). As A/Aichi and A/HK influenza viruses are nearly identical antigenically (13), the former was used to measure specific antibody elicited by either virus.

Anti-A/Aichi influenza virus antibody in serum samples was measured in a similar manner as previously described (12); serum antibody to influenza virus as determined by this method has been shown to correlate with the more conventional hemagglutinin inhibition assay in serum samples from a group of normals (12).

Total IgG was measured using a "sandwich" ELISA as previously described (13). Here, the sequence of reagents from the solid-phase up consisted of goat anti-human IgG antibody, dilutions of the culture supernatant, alkaline-phosphatase conjugated goat-anti-human IgG Fc (Sigma Chemical Co.), and *p*-nitrophenol phosphate. Again, the concentration of IgG in the test sample was determined by comparison of the optical density read at 405 nm with a curve of absorbance vs. concentration of IgG obtained with dilutions of a reference standard. Total IgM immunoglobulin was determined in a similar manner as previously described (15).

Statistics. Correlations between serum antibody levels and in vitro antibody production and between the total IgM in PWM-stimulated cultures and specific IgM antibody in virus-stimulated cultures were determined using the Spearman's rank correlation coefficient (16). The first result obtained with any given patient or control was used for these correlations.

RESULTS

In vivo anti-A/Aichi influenza virus antibody levels. Each of the 14 normal adults tested had detectable resting serum anti-A/Aichi influenza virus antibody levels with IgG being the predominant isotype. A/Aichi influenza virus and related H3N2 type A viruses have circulated widely since 1968, and these antibody titers are presumably the result of prior infection. As compared to these normals, most of the patients with hypogammaglobulinemia had low levels of IgG- and IgM-anti-A/Aichi influenza virus antibody (Table I). A few patients, however, had normal or near normal resting anti-A/Aichi influenza virus antibody levels. In the case of IgG, this was possibly due to acquired antibody from gamma globulin injections; indeed, patients 7, 15, and 16, who were not receiving gamma globulin had the lowest IgG antibody levels. This was not likely the case for IgM however, as gamma globulin contains little IgM, and these antibody levels most likely represent in vivo production as a result of prior infection or immunization with influenza virus. Interestingly, patient 1, who had yearly vaccinations with inactivated influenza virus for the past 10 yr, had an IgM antibody level higher than that of the normals.

In vivo antibody responses in patients with CVH. In vivo antibody responses to the antigens KLH, diphtheria toxoid, and tetanus toxoid were studied in the patients with CVH (Table II). When immunized with KLH, an antigen not likely to have been previously encountered, none of the eight patients tested had a fourfold or greater rise in antibody titer. In contrast, 24 of the 25 controls tested had a fourfold or greater titer increase. When the recall antigens diphtheria toxoid and tetanus toxoid were studied, these patients also had diminished responses as compared to normals with low postimmunization antibody levels and/or only small rises in titers. As noted (Table II), most of these patients were receiving replacement therapy with gamma globulin (which contains anti-diphtheria toxoid and anti-tetanus toxoid antibody) and the measured antibody levels in these patients likely includes a component of passively acquired antibody.

In vitro immunoglobulin and antibody production by PBMC from patients with CVH. When stimulated with the polyclonal activator PWM, PBMC from 5 of the 12 patients with CVH failed to make over 40 ng/

TABLE I

Clinical Characteristics of Patients with Hypogammaglobulinemia and Serum Antibody Levels

Pt*	Diagnosis	Age	Sex	Serum Ig levels			Blood type	Isohemagglutinins (normal > 1:32)	Anti-A/Aichi influenza virus antibody level	
				IgG	IgA	IgM			IgG	IgM
		yrs		*mg/ml*	*mg/ml*	*mg/ml*			*ng/ml*	*ng/ml*
1	CVH	62	M	0.5	<0.1	0.2	A+	1:1 anti-B	16.24	19.6
2	CVH	67	F	0.8	<0.1	<0.1	O−	1:1 anti-A & anti-B	12.68	0.33
3	CVH	57	F	2.2	0.3	0.2	B+	<1:1 anti-A	6.27	0.13
4	CVH	60	M	1.4	<0.1	<0.1	B+	1:4 anti-A	12.77	0.12
5	CVH	34	M	1.6	0.1	0.1	B+	1:4 anti-A	20.92	0.71
6	CVH	32	M	1.6	<0.1	<0.1	A+	<1:1 anti-B	8.06	<0.11
7	CVH	31	M	0.6	0.2	0.2	A+	1:8 anti-B	0.96	0.59
8	CVH	31	M	1.9	<0.1	<0.1	B+	1:8 anti-A	12.27	0.13
9	CVH	32	F	0.9	<0.1	<0.1	O−	<1:1 anti-A & anti-B	8.15	<0.11
10	CVH	48	F	2.4	<0.1	<0.1	O+	1:4 anti-A; 1:1 anti-B	14.05	1.51
11	CVH	37	M	0.7	<0.1	0.2	AB+	−	18.86	2.23
12	CVH	33	F	2.3	0.9	0.1	A+	1:4 anti-B	24.53	0.76
13	XLHG	7	M	<0.3	0.1	<0.1	O+	<1:1 anti-A & anti-B	25.26	<0.11
14	XLHG	22	M	2.0	<0.1	<0.1	O−	<1:1 anti-A & anti-B	8.65	<0.11
15	XLHG-GHD	5	M	1.3	<0.1	<0.1	A+	<1:1 anti-B	0.96	<0.11
16	XLHG-GHD	7	M	0.3	<0.1	<0.1	A+	<1:1 anti-B	0.59	0.11
17	XLHG-GHD	31	M	2.1	<0.1	<0.1	AB+	—	16.84	<0.11
Controls‡									51.72 (25.62–104.47)	3.18 (1.01–9.99)

* All patients (Pt) were receiving gamma globulin when serum samples were obtained for the antibody titers except for patients 7, 15, and 16.

‡ Geometric mean (67% confidence interval) of the serum anti-A/Aichi influenza virus antibody levels in 14 normal adult controls.

TABLE II

Specific In Vivo Antibody Production Following Immunization in Patients with CVH

Patient*	KLH		Diphtheria toxoid		Tetanus toxoid	
	Pre	Post	Pre	Post	Pre	Post
	Titer	*Titer*	*IU/ml‡*	*IU/ml*	*IU/ml*	*IU/ml*
1	<1:2	<1:2	0.75	0.75	1.3	1.3
2	<1:2	<1:2	<0.2	<0.2	<0.01	<0.01
3	<1:2	<1:2	0.2	24	1.3	5
4	<1:2	<1:2	24	48	5	10
5	ND§	ND	ND	ND	ND	ND
6	<1:2	<1:2	6	6	10	10
7	1:64	1:64	0.05	0.38	0.04	2.2
8	ND	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	ND	ND
10	ND	ND	0.75	1.5	0.63	0.63
11	<1:2	<1:2	0.19	0.09	0.16	0.16
12	1:2	1:4	ND	ND	2.5	1.25
Controls‖	1:7 (3.3)	1:1020 (4.9)	7.3 (6.2)	286 (4.7)	14.0 (4.0)	160 (3.3)

* All patients except for 2 and 7 were receiving gamma globulin replacement therapy when serum samples for antibody titers was obtained. Patient 3 was started on gammaglobulin after the preimmunization serum was obtained.

‡ Antidiphtheria toxoid or antitetanus toxoid antibody in International Units/milliliter.

§ ND, Not done.

‖ Geometric mean (standard deviation) of antibody levels in adult controls.

ml of either IgM or IgG immunoglobulin (Table III). The other seven patients each made over 40 ng/ml of IgM and of these, three also made measurable IgG. In comparison, each of the 14 controls' PBMC made over 40 ng/ml of both IgM and IgG when stimulated with PWM.

In vitro anti-A/Aichi influenza virus antibody production following stimulation with PWM, live A/HK/68 (H3N2) influenza virus, and formalin-inactivated A/Aichi/68 (H3N2) influenza virus was studied in PBMC obtained from these same controls and patients (Table III). All but 1 of the 14 normal adult controls' PBMC made detectable IgM and IgG anti-viral antibody when stimulated with inactivated A/Aichi influenza virus, and all but three made measurable antiviral antibody when stimulated with live A/HK influenza virus or with PWM. When studied in the same manner, PBMC from 6 of the 12 patients with CVH (No. 1–6) failed to make anti-A/Aichi influenza virus antibody with any of the stimuli used (Table III). Five of these six patients had also failed to secrete polyclonal immunoglobulin in response to stimulation with PWM. PBMC obtained from the other six patients with CVH (No. 7–12), however, produced measurable anti-A/Aichi influenza virus antibody when stimulated in vitro with live or formalin-inactivated virus (Table III), and five of these produced an IgM response to A/Aichi above the mean of the normal controls on at least one testing. In two of the patients (11 and 12), the IgM antibody produced in response to stimulation by A/Aichi approached in magnitude the total IgM immunoglobulin response to PWM; the reason for this relative unresponsiveness to PWM is not clear but may be due to PWM-induced suppression as has been described in patients with CVH (17). In contrast to the PBMC from normals in which IgG was the predominant class of antibody produced, PBMC from the patients in general produced a predominance of IgM antibody.

This measured anti-A/Aichi influenza virus antibody was secreted by the cells during the culture period rather than being passively carried on the surface of the PBMC since antibody could never be measured in supernatants harvested immediately after the cultures were established. In addition, in each case, there was no antibody released in the absence of mitogenic or antigenic stimulation. Finally, the addition of 15 μg/ml of cycloheximide (a protein synthesis inhibitor) at the beginning of the culture period completely inhibited specific antibody production both in normals and in the six patients whose PBMC made antibody.

While there was considerable variation in the antibody produced among both patients and controls, the results obtained with any given donor were fairly con-

TABLE III

Total Immunoglobulin and Anti-influenza Virus Antibody Produced In Vitro by PBMC Obtained from Patients with CVH

	Ig production		Anti-A/Aichi influenza virus antibody production					
	PWM		PWM		A/HK		A/Aichi	
Patient	IgG	IgM	IgG Ab*	IgM Ab*	IgG Ab*	IgM Ab*	IgG Ab*	IgM Ab*
				ng/ml				
1	<40	<40	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1
2	<40	<40	<2.1	<2.1	<2.1	<2.1	ND‡	ND
3	<40	107	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
4	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
5	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
6	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
7	1,068	913	<2.1, <2.1	<2.1, <2.1	9.3, 9.4	4.6, 4.1	3.8, ND	19.8, ND
8	280	1,485	2.6	<2.1	6.6	<2.1	ND	ND
9	72	177	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	5.7, 7.4	13.2, <2.1	16.6, 2.8
10	<40	1,688	<2.1, 6.5	6.0, 28.4	<2.1, <2.1	4.7, <2.1	<2.1, 14.4	21.7, 5.2
11	<40	268	<2.1	<2.1	<2.1	<2.1	<2.1	71.3
12	<40	47	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	<2.1, <2.1	5.8, 9.0	4.0, 30.0
Controls§	852 (202–3,580)	3770 (524–27,100)	10.1 (1.9–52.1)	3.1 (1.8–5.3)	18.1 (2.6–128)	10.5 (2.8–39.4)	43.7 (9.5–201)	14.2 (3.0–68.0)

* IgG or IgM anti-A/Aichi influenza virus antibody produced in cultures stimulated by PWM, live A/HK influenza virus, or formalin-inactivated A/Aichi influenza virus. When patients were cultured on more than one occasion, the first two results are shown.

‡ ND, not done.

§ Geometric mean of total immunoglobulin and anti-A/Aichi influenza virus antibody produced in cultures of PBMC obtained from 14 normal adult controls. Values in parentheses are the 67% confidence intervals.

sistent when studied on more than one occasion. Four of the six patients (7, 9, 10, 12) whose PBMC produced anti-influenza virus antibody when first tested were restudied and each again produced specific antibody in response to A/HK or A/Aichi influenza virus (Table III); patient 7 produced measurable antibody on each of three occasions over a 2.5-yr period. Furthermore, patient 1 failed to make specific antibody on each of four occasions tested, even following in vivo immunization with inactivated flu vaccine.

In the group of 14 normal controls, there was a correlation between resting in vivo IgG anti-A/Aichi influenza virus antibody titers and the IgG antibody produced in vitro in response to inactivated A/Aichi ($r_s = 0.60$, $P < 0.05$). This correlation, however, did not hold for IgM antibody ($r_s = 0.06$, $P > 0.05$) in this group. In addition, there was no significant correlation between in vivo and in vitro antibody of either IgG ($r_s = 0.12$, $P > 0.05$) or IgM ($r_s = 0.41$, $P > 0.05$) isotypes in the patients with CVH. It should be noted that the in vivo IgG antibody levels in the patients were likely affected by the gamma globulin injections. There was, however, a strong correlation between the total IgM immunoglobulin produced in response to PWM and the IgM anti-viral antibody produced in vitro in response to A/Aichi influenza virus in the patients with CVH ($r_s = 0.90$, $P < 0.01$), suggesting that the specific and polyclonal in vitro responses may measure parallel potentials for B cell activation albeit by different mechanisms.

In vitro immunoglobulin and antibody production in patients with XLHG. The results of the in vitro studies of PBMC obtained from the patients with XLHG are shown in Table IV. With the exception of patient 17, who made a barely detectable total IgM response with PWM, none of these patients made any detectable immunoglobulin or antibody with any of the stimuli used. The small quantity of IgM antibody produced by patient 17 in response to PWM may be due to an incomplete deficiency in B cells as has been described in this patient's brother who also has XLHG-GHD. The lack of antibody production in these patients with XLHG was not likely to be due to a lack of exposure to influenza virus, as the patients were able to mount a normal proliferative or cytotoxic response in vitro to type A influenza virus (2).

DISCUSSION

Previous studies have revealed that PBMC obtained from the majority of normal adults produce anti-influenza virus antibody in vitro when cultured with type

TABLE IV

Total Immunoglobulin and Anti-influenza Virus Antibody Production In Vitro by PBMC from Patients with XLHG or with XLHG-GHD

		Ig Production		Anti-A/Aichi influenza virus antibody production					
		PWM		PWM		A/HK		A/Aichi	
Patient	Dx.	IgG	IgM	IgG Ab*	IgM Ab*	IgG Ab*	IgM Ab*	IgG Ab*	IgM Ab*
				ng/ml					
13	XLHG	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
14	XLHG	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
15	XLHG-GHD‡	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
16	XLHG-GHD‡	<40	<40	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
17	XLHG-GHD	<40	72	<2.1	<2.1	<2.1	<2.1	<2.1	<2.1
Controls§		852 (202–3,580)	3770 (524–27,100)	10.1 (1.9–52.1)	3.1 (1.8–5.3)	18.1 (2.6–128)	10.5 (2.8–39)	43.7 (9.5–201)	14.2 (3.0–68)

* IgG- or IgM-anti-A/Aichi influenza virus antibody produced in cultures stimulated with PWM, live A/HK influenza virus, or formalin-inactivated A/Aichi influenza virus.

‡ Patients 15 and 16 were studies on three separate occasions and failed to produce anti-A/Aichi influenza virus each time.

§ Geometric mean of immunoglobulin and antibody produced in cultures of PBMC obtained from 14 normal adult controls. Values in parentheses are the 67% confidence intervals.

A influenza viruses (13, 18). As shown here, there is a correlation between the "resting" in vivo IgG antibody in these normals and the in vitro antibody response to A/Aichi virus. The antibody secreted is predominantly directed at the stimulating type of influenza virus, but not an irrelevant (type B) influenza virus (13, 15, 18). This in vitro antibody response requires the cooperative interaction of B and $OKT4^+$ T cells (13, 15, 18). Unlike the case with PWM, both positive and negative allogenic immunoregulatory effects are observed in influenza virus-stimulated co-cultures of allogenic B and T cells (19, 20). In addition, studies with T cell lines that do not manifest these allogenic effects have suggested that there are HLA-DR linked restrictions on the cooperation between B and T cells (21).

We show in this study that PBMC from a subset of patients with CVH are able to produce anti-influenza virus antibody in vitro when stimulated with type A influenza viruses. Previous papers have reported that lymphocytes from certain patients with this disorder can produce immunoglobulin (4–8, 10, 22, 23) or antibody (11) in vitro in response to polyclonal activators such as PWM. In such studies, however, it was probable that the polyclonal activators were stimulating lymphocytes via mechanisms that are different from those operational during antigenic stimulation. More recently, Pawha et al. (17) have shown that a third of patients with CVH could produce a specific antibody response to sheep erythrocytes in cultures supplemented with supernatants of lipopolysaccharide-stimulated adherent cells and with the polyclonal activator Cowan I strain of *Staphylococcus aureus* (17). Their results again indicate that B cells of patients with CVH can produce antibody under certain conditions, but the need for an exogenous factor and polyclonal activator in their system makes further interpretation difficult. In this study, these caveats are not relevant and one must conclude that at least some patients with CVH have all the necessary elements in their peripheral circulation to mount an antigen-induced specific humoral response. This conclusion is in accord with preliminary data showing that PBMC from certain patients with CVH can respond to particulate antigens in vitro (24, 25).

Having ruled out an absolute defect of either B or T cells in this subset of patients, the question remains as to the basis for their low immunoglobulin levels and their impaired in vivo antibody responses to antigens. Certain of these patients might have an immune defect that is operational in vivo but is not expressed in a culture of their peripheral blood. For example, their in vivo immunodeficiency could be accounted for by a serum suppressor factor such as has been described by Geha et al. (9) or circulating suppressor cells that do not survive or manifest themselves under these in vitro conditions, as have been described by Dosch et al. in patients with congenital aggammaglobulinemia (26, 27). The patients who produced antibody in vitro in this study generally made at least as much IgM antibody as IgG antibody. This was in contrast to normals, in whom IgG was the predominant isotype pro-

duced. This preferential production of IgM by patients with CVH is consistent with previously reported studies using polyclonal activators in which IgM, but little IgG or IgA immunoglobulin was produced (5, 7, 8, 22, 23). This preferential production of IgM antibody suggests that some patients might have an impairment in generating IgG "memory" cells, either as a primary B cell defect or secondary to a defect in T cells mediating this process. The possible role of an impairment in the production of IgG "memory" cells in these patients is supported by the studies of Stevens et al. who showed that while some patients with CVH could produce IgM anti-tetanus toxoid antibody following stimulation with PWM, they failed to develop circulating IgG-antibody secreting cells or PWM-inducible IgG-antibody producing memory cells following in vivo immunization with tetanus toxoid (11).

The ability of the patients' PBMC to produce antibody in vitro indicates that the underlying defect or defects in these patients are only partial and that most patients with CVH retain some humoral immune function. In agreement with previously reported results (11), CVH patients were not absolutely impaired in their ability to make antibody in vivo and many had measurable serum IgM anti-A/Aichi influenza virus antibody. It is quite possible that as a result of low levels of anti-influenza virus antibody in their respiratory tract, these patients develop infection with influenza virus more frequently than normals (28) and are thus relatively hyperimmunized to influenza virus. This antigen, then, may be particularly effective in stimulating their residual B cell function. Further studies will be required to identify the relative roles of the defects hypothesized above in these patients. Co-culture experiments with normals have classically been one approach used for this purpose; with antigenic stimulation, however, such experiments are complicated by allogenic effects and restrictions on B and T cell cooperation (19–21, 29).

In contrast to the findings in patients with CVH, PBMC from the two patients with Bruton's type XLHG and from the three patients with XLHG-GHD failed to produce specific antibody in vitro. While consistent with other studies showing a lack of B cell function in these patient groups (2, 6), these results contrast with those of Dosch et al. who found that four of five male patients with classic congenital agammaglobulinemia who lacked circulating B cells and whose PBMC failed to respond to PWM could generate a specific hemolytic plaque-forming response in vitro when cultured with either sheep erythrocytes or ovalbumin (26).

In conclusion, PBMC from a subset of patients with CVH, but none of the patients studied with XLHG, were able to produce antibody in vitro in response to antigenic stimulation. It is possible that as we better understand the regulation of B cell activation, we will be able to more effectively treat this subset of patients by enhancing their existing capability for specific antibody formation.

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