Modulatory Effects on Immunoglobulin Synthesis and Secretion by Lymphocytes from Immunodeficient Patients

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ABSTRACT Incubation of peripheral lymphocytes (PBL) from normal donors with pokeweed mitogen (PWM) induced terminal differentiation by B lymphocytes to immunoglobulin (Ig) synthesizing and secreting plasma cells. B cells from hypogammaglobulinemic patients with different primary immunodeficiencies failed to undergo functional differentiation after similar treatment with PWM. Co-cultures of PBL from normal donors and hypogammaglobulinemic patients often resulted in deviations, both positive and negative, from expected levels of PWM-stimulated intracellular Ig biosynthesis. Suppression of B-cell differentiation was manifested by PBL from patients with several different primary immunodeficiencies, including infantile sex-linked agammaglobulinemia. Immunoregulatory activities were noted to vary with the normal donor used in co-culture experiments and with time. Cell populations that were active in influencing B-cell differentiation to functional plasma cells did not have an appreciable modulatory effect on T-lymphocyte responses to mitogens. These observations may provide a functional subclassification for immunoregulatory cells in man.

INTRODUCTION

Modulation of the normal immune response requires a delicate balance between immunologic-enhancing activities and inhibitory effects. With regard to the latter, cells that can suppress humoral or cell-mediated immune functions have been extensively described in a number of animal models (for review, see reference 1). The recent association of suppressor cells in several human disease states involving immune dysfunction (2–5) suggests a role for these cells in the pathogenesis of immunodeficiency in man.

Waldmann and his colleagues (2) showed that T lymphocytes isolated from patients with hypogammaglobulinemia inhibit immunoglobulin (Ig)[1] production by pokeweed mitogen (PWM)-stimulated normal lymphocytes in co-cultures. It was proposed that these suppressor cells subserve a pathogenetic basis for the hypogammaglobulinemia of common variable immunodeficiency (CVI). Other investigations, however, demonstrated that suppressor activity in both benign and neoplastic diseases may also be manifested by inhibition of T-lymphocyte functions, namely, allogeneic stimulation (3) and blastogenic responses to mitogens and antigens (5). The latter noted that no suppression of Ig production occurred when patient lymphocytes known to inhibit proliferative responses were co-cultured with normal cells.

In this paper, we demonstrate that the molecular basis of the Ig suppressor effect after PWM induction is the inhibition of Ig biosynthesis as contrasted to a block in transmembrane secretion of intracellular Ig and that suppressor cell populations in several immunodeficiency diseases characterized by hypogammaglobulinemia are selectively active on B cell-derived functions of normal lymphocytes. We further studied the role of suppressor as well as enhancing activity in both cell-mediated and humoral immunity in different immunodeficiency diseases.

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; CVI, common variable immunodeficiency; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; MC, mitomycin C; PBL, peripheral blood lymphocytes; PBS, phosphate buffered saline; PHA, phytohemagglutinin-P; PWM, pokeweed mitogen; TKM, 0.05 M Tris HCl (pH 7.6), 0.025 M KCl, 0.005 M $MgCl_2$.

METHODS

Patients and normal donors. Diagnosis and classification of immunodeficiency states were made using the guidelines of the World Health Organization Expert Committee (6, 7). A profile of clinical and immunologic parameters for the patients under investigation is presented in Table I. All patients for these studies were under the care of physicians of the Immunobiology Clinic of the Memorial Sloan-Kettering Cancer Center, and informed consent was obtained in accordance with Department of Health, Education, and Welfare and institutional policies as approved by our Clinical Investigation Committee. Specimens from pediatric patients were obtained with the authorization and supervision of the responsible pediatrician.

Isolation of lymphocytes. Peripheral blood lymphocytes (PBL) were prepared from fresh heparinized blood (20 U heparin/ml) by a modification of the density gradient technique of Böyum (8). The mononuclear cell band was harvested and washed three times with Ca^{++}-and Mg^{++}-free Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) and resuspended in RPMI 1640 medium (Grand Island Biological Co.) containing 20% heat-inactivated fetal calf serum (Associated Biomedic Systems, Inc., Buffalo, N. Y.), 8.0 μg gentamicin (Schering Corp., Kenilworth, N. J.), and 300 μg of glutamine/ml.

Cell culture and preparation of lysates. All cultures consisted of a total of 5×10^6 lymphocytes in 5 ml RPMI containing 50 μg of PWM (Grand Island Biological Co.) in a polypropylene tube (Falcon Plastics, Oxnard, Calif.). Autologous control cultures contained 5×10^6 total cells and the co-cultures were performed by adding 2.5×10^6 lymphocytes from patients with hypogammaglobulinemia to an equal number of lymphocytes from healthy, volunteer donors. The cells were cultured for 7 days in a humidified

TABLE I

Immunologic Parameters of Patients Studied for Immunoregulatory Functions

			Immunoglobulins			Lymphocytes				Mitogen responses		
							Markers					
Patient	Age	Sex	G	A	M	No.	ERFC	PV	IgM	PHA	ConA	PWM
	yr		*mg/dl*				%			*net cpm**		
A. Common variable immunodeficiency												
L. Ar.	13	F	420	2	18	1,609	82	10	7	13,856	14,791	1,915
M. D'A.	10	M	195	288	326	1,217	90	8	7	15,361	13,805	11,141
M. Ed.	20	M	40	<3	<3	2,052	94	6	2	15,753	18,402	16,236
P. En.	22	F	66	0	5	2,519	83	27	6	29,461	22,437	12,315
V. Ka.	53	F	360	0	136	884	97	6	5	11,885	12,132	1,482
B. McD.	4	M	500	12	76	5,212	80	31	2	18,568	13,110	5,239
F. Ro.	35	F	58	80	156	1,520	90	9	7	34,324	35,612	20,981
M. Ro.	30	F	180	0	80	2,233	75	11	8	26,215	23,356	18,734
G. Sca.	52	F	245	45	284	1,000	89	4	1	1,426	4,385	ND
T. Sc.	13	M	142	<1	6	873	87	15	4	24,322	15,548	4,507
B. Bruton's agammaglobulinemia												
R. Ar.	9	M	260	3	5	2,349	92	1	0.5	29,135	33,827	21,793
G. Be.	18	M	92	0	0	1,774	88	0.5	0	23,741	11,318	5,043
L. Ha.	8	M	340	<5	<5	3,370	90	6	0	8,863	9,389	5,836
M. Ri.	24	M	44	0	0	1,339	80	0	0	21,832	18,449	7,143
C. Congenital agammaglobulinemia												
K. Wh.‡	9	F	560	0	24	3,756	89	6	0	30,354	32,298	29,982
R. Wh.‡	4	F	400	0	16	2,862	88	0	ND	28,738	36,616	30,845
D. IgG and A deficiency + hyper IgM												
C. Di.	14	M	132	<2	>1,000	7,000	89	14	10	17,949	13,914	3,464
E. Thymoma and agammaglobulinemia												
F. Ka.	52	M	100	0	<2	1,368	89	0	0	28,583	9,097	13,089
W. St.	46	M	540	280	580	800	75	0	0	2,538	1,820	994
F. Severe combined immunodeficiency												
M. Ru.	2	M	20	0	0	~300	35	0	0	6,242	6,886	1,142

Abbreviations used in this table: ERFC, sheep erythrocyte rosette-forming cells; ND, not determined; PV, cells staining with a polyvalent fluoresceinated anti-human immunoglobulin.

* A normal response to PHA has been determined to be >17,000 cpm; normal responses to PWM and Con A are ≤PHA response (see reference 12).

‡ Siblings.

TABLE II

Serospecificity of the Radioimmunoassay for Immunoglobulin Production

Subject	Serologically precipitable radioactivity (cpm/2.5 × 10^6 cells)							
	Intracellular Ig				Secreted Ig			
	Specific*	Non-specific‡	Net Ig§	Inhibition	Specific*	Non-specific‡	Net Ig§	Inhibition
				%				%
Normal donor	37,293	1,198	36,095	—	61,654	327	61,327	—
(N)	37,213	1,033	36,180	—	58,557	317	58,240	—
Patient (R. Ar.)‖ [X-linked agammaglobulinemia]	845	849	0	—	237	208	29	—
	980	1,052	0	—	234	173	61	—
Co-culture	7,633	1,091	6,542	63.9	7,320	152	7,168	76.4
(N + R. Ar.)	7,249	740	6,509	(18,069)¶	7,167	159	7,008	(29,915)¶

* Rabbit anti-human $F(ab')_2$ plus unlabeled carrier human immune serum globulin.

‡ Rabbit anti-KLH plus unlabeled carrier KLH.

§ Specific reaction minus nonspecific reaction.

‖ 12 y-old ♂ with X-linked agammaglobulinemia.

¶ Predicted value for net Ig based upon sum of Ig from individual cultures of normal donor and patient lymphocytes divided by 2.

atmosphere of 5% CO_2, 95% air. At the end of the 7th day, the cells were assayed for number and viability by trypan blue dye exclusion, collected by centrifuging at 850 *g*, and resuspended in 2 ml of leucine-free Dulbecco's Modified Eagle medium supplemented with 25 μCi L-[4,5-^{3}H(N)]leucine ([^{3}H]leucine, 1.0 mCi/ml; New England Nuclear, Boston, Mass.) for the last 4 h of incubation. After sedimenting the cells from the supernate, they were lysed with 2 ml of 1% solution of Nonidet P-40 (Particle Data, Inc., Elmhurst, Ill.) in 0.05 M Tris HCl (pH 7.6), 0.025 M KCl, and 0.005 M $MgCl_2$ (TKM), and subcellular particles were pelleted by centrifugation at 105,000 *g* for 60 min as previously described (9). Any radiolabeled Ig in the culture supernate is actively secreted, whereas the cell lysate contains only *de novo* synthesized labeled Ig. Both the culture supernate and the cell lysate were dialyzed against 0.01 M phosphate buffered saline, pH 7.5 (PBS), and TKM, respectively, to remove any free [^{3}H]leucine.

Preparation of antisera and serologic assay for Ig. Methodolologic details have been recently described (10), thus only major points will be summarized. New Zealand white rabbits were immunized by two separate intramuscular injections consisting either of 2.5 mg keyhole limpet hemocyanin (KLH; Schwartz/Mann, Orangeburg, N. Y.) or 0.5 mg human $F(ab')_2$ in 0.5 ml of PBS emulsified with 1.5 ml of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). This was followed by similar inoculations 6 wk after the first. 2 wk after the secondary immunization, the animals were bled and the sera heat-inactivated. All the sera were monospecific, and no cross-reactivity between the antigens under study was observed. To remove any materials which might nonspecifically interact with the rabbit antiserum-antigen matrix, a volume of neat rabbit anti-KLH serum capable of precipitating 50 μg of antigen was added to each 2-ml sample, followed by 50 μg of unlabeled KLH. The mixture was incubated at 37°C for 60 min and overnight at 4°C. The resulting precipitate was discarded, and the supernate was divided into two equal aliquots. One aliquot was incubated with 100 μl of rabbit anti-human $F(ab')_2$ serum and precipitated with 50 μg of human immune serum globulin, Cohn fraction II (E. R. Squibb & Sons, Princeton, N. J.). A nonspecific serologic blank consisted of rabbit anti-KLH plus carrier KLH, yielding a precipitate approximately equal to that of the specific serologic reaction. The precipitates were washed with PBS at 4°C, dissolved in 0.5 ml 0.2 M KOH, and added to 10 ml of scintillation fluid (Aquasol; Packard Instrument Co., Inc., Downers Grove, Ill.). Radioactivity was measured in a liquid scintillation counter. Net serospecific Ig was calculated by subtracting total nonspecific cpm from the specific counts. Percent inhibition was calculated according to the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{\text{Ig cpm}/5 \times 10^6 \text{ cells in co-culture}}{(0.5 \times \text{Ig cpm}/5 \times 10^6 \text{ donor cells} + 0.5 \times \text{Ig cpm}/5 \times 10^6 \text{ patient cells})}\right) \times 100$$

Note that when the observed count was higher than the predicted value, the enhancement above 100% is indicated by a "+" sign before the result.

Mitogen stimulation experiments. Isolation of cells and culture conditions have been previously described (11). Briefly, PBL from patients at a concentration of approximately 2–4 × 10^6 cells/ml were pretreated with mitomycin C (MC; Sigma Chemical Co., St. Louis, Mo.) diluted to 50 μg/ml for 30 min. A 0.1-ml aliquot containing 5 × 10^4 freshly prepared untreated responder lymphocytes from healthy donors was mixed with an equal volume and number of MC-pretreated allogeneic patient cells in microculture plates. Control cultures consisted of 5 × 10^4 untreated responder lymphocytes plus an equal number of MC-treated allogeneic cells from another normal donor. Various concentrations of either one of the mitogens, phytohemagglutinin-P (PHA, 2.5 μg/ml), concanavalin A (Con A, 60 μg/ml), or PWM (20 μg/ml) were added to designated cultures, and the cells were incubated for 5 days in a humidified 5% CO_2, 95% air incubator at 37°C. Extensive

studies have shown that these final concentrations of mitogens are within the optimal range for stimulation of lymphocyte transformation (12). During the last 16 h of incubation, 2 μCi of [^{3}H]thymidine was added to each well. The cells were collected by an automatic harvester and incorporation of [^{3}H]thymidine into DNA was measured by using a liquid scintillation counter. Percentage of mitogenic stimulation of responder cells influenced by the addition of patient's lymphocytes to co-cultures was calculated according to the following formula which acknowledges the contribution of allogeneic stimulation in the absence of mitogens:

$$\frac{(C_m^P - C^P)}{(C_m^N - C^N)} \times 100$$

= percentage response to mitogens influenced by

MC-treated patient's lymphocytes, where C_m^P is cpm of normal lymphocytes plus MC-treated patient's cells plus mitogen; C^P is cpm of normal lymphocytes plus MC-treated patient's cells; C_m^N is cpm of normal lymphocytes plus MC-treated allogeneic normal lymphocytes plus mitogen; C^N is cpm of normal lymphocytes plus MC-treated allogeneic normal donor cells.

RESULTS

Specificity of the radioimmunoassay for Ig synthesis and secretion. A direct serologic precipitation assay for radiolabeled Ig, which was noted to trap considerably less nonspecific radiolabel than a previously employed indirect double antibody technique, was used in these studies. Table II presents representative data concerning synthesis and secretion of Ig by PBL from a normal individual, an agammaglobulinemic patient, and the co-culture of the two. The table shows that the PBL of the normal person synthesize and secrete Ig in relatively large amounts after stimulation with PWM, whereas the agammaglobulinemic cells show minimal synthesis and secretion of Ig. When cultured together, the normal and agammaglobulinemic lymphocytes synthesize and secrete amounts of Ig considerably less than would be predicted by the mean of these activities of the two individual cell populations. Note that the counts in the nonspecific serologic controls for the normal donor lymphocytes are less than 5% of the specific counts for both the protein-rich intracellular lysate and the culture supernate whose major human protein component is secreted Ig. By contrast, the total counts recovered in the serologic assays for the hypogammaglobulinemic patients were merely equivalent to background, reflecting little to no synthesis of Ig. The failure of our specific antisera to react with the products of the agammaglobulinemic lymphocyte cultures is a good biologic control for its specificity.

Pokeweed mitogen stimulation of Ig synthesis and secretion by cultured human lymphocytes. As shown in Fig. 1 A, analysis of 27 different PWM-stimulated normal donor lymphocyte cultures yields respective mean values of 19,604±2,358 (±SE) and 47,639±6,238 cpm for intracellular and secreted serospecific Ig per 2.5×10^6 lymphocytes. PWM

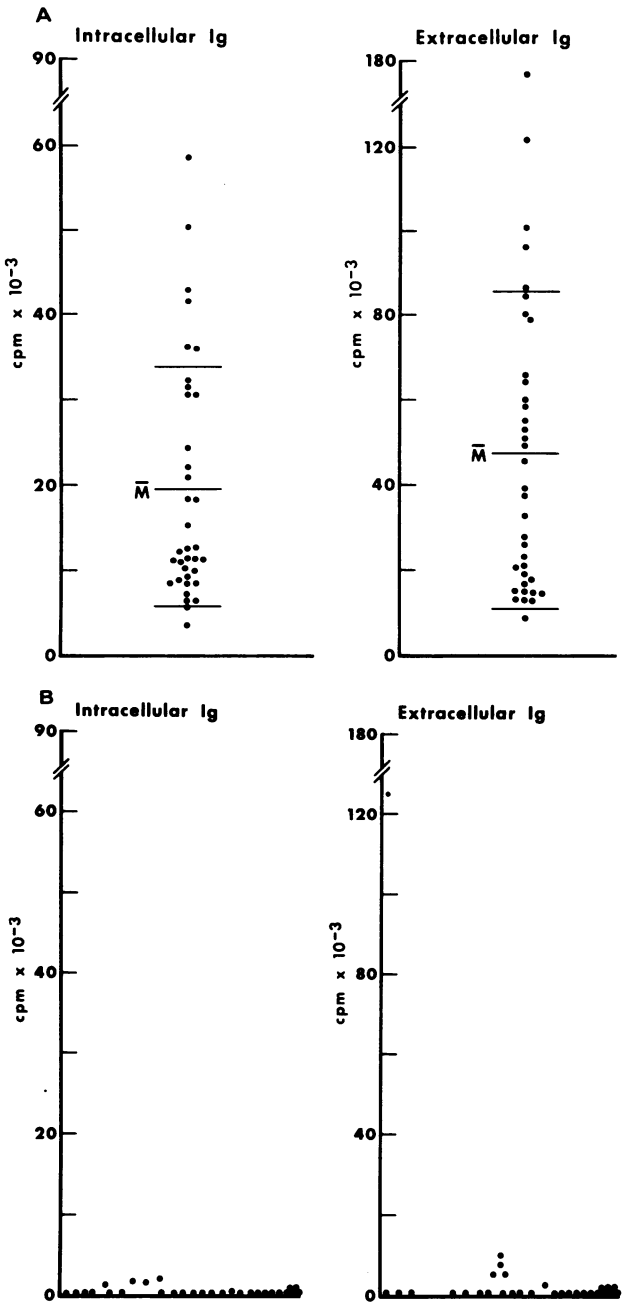

FIGURE 1 Ig synthesis and secretion by PWM-stimulated lymphocytes. 5×10^6 mononuclear cells were cultured in the presence of PWM for 7 days and allowed to incorporate [^{3}H]leucine into proteins during the last 4 h. Serologically precipitable Ig was measured as cpm in the cell lysate and the culture medium. (A) Normal donor cells. (B) Hypogammaglobulinemic patients' cells. Horizontal lines define mean±SD.

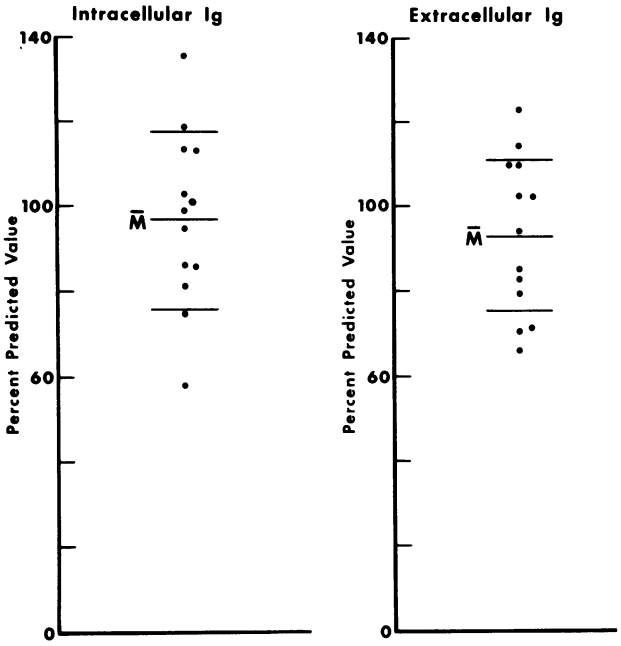

FIGURE 2 Effect of co-culture of PWM-stimulated normal lymphocytes on Ig synthesis and secretion. Ig production by allogeneic cultures of lymphocytes from each of two normal donors was compared to the sum of serologically precipitable Ig produced by individual cultures of equal numbers of autologous cells. Horizontal lines define mean±SD.

treatment of lymphocytes from hypogammaglobulinemic patients, however, fails to stimulate significant amounts of Ig synthesis or secretion (Fig. 1 B). With regard to these latter studies involving patients with a variety of primary immunodeficiencies manifested by hypogammaglobulinemia among other diagnostic criteria, mean values of 262±88 and 1,082±456 cpm for intracellular and secreted Ig, respectively, were obtained. Thus the extremely low serum Ig levels in these patients were also paralleled by a decreased in vitro Ig synthetic capacity of the lymphocytes cultured in the presence of PWM.

Effect of co-culture of normal lymphocytes on Ig synthesis and secretion. Because we co-cultured allogeneic cells, patient and normal, it was necessary to determine whether or not allogeneic stimulation affected PWM induction of Ig. To exclude the possibility of allogeneic effect, equal numbers of cells were incubated either alone or in co-culture. The data in Fig. 2 demonstrate that the admixture of equal numbers of cells from healthy volunteer donors does not appreciably affect the amount of Ig synthesized or secreted as predicted from the amounts recovered from individual autologous cultures. Thus, profound suppressor or enhancing activity is not readily manifested by the normal, healthy population at large. We have occasionally found significant deviations from the expected values in co-culture experiments with purportedly normal donors; however, a subsequent detailed clinical history has then regularly revealed underlying medical problems in one member of the pair. Although no attempt was made to HLA type these normal donors, their random selection precludes significant matching, eliminating the role of allogeneic stimulation on Ig synthesis in mixing experiments. Furthermore, sex differences did not seem to affect the co-cultures.

Effect of co-cultures of lymphocytes from hypogammaglobulinemic patients and normal donors on Ig synthesis and secretion. Co-cultures of lymphocytes between normal donors and hypogammaglobulinemic patients with several different primary immunodeficiencies often resulted in significant deviations from the expected values of Ig synthesis and secretion based upon activities in the respective autologous cultures. Most notable was suppression of Ig synthesis and secretion as presented in Tables III–V. In all cases where suppression of Ig secretion was noted, this merely paralleled inhibition of intracellular biosynthesis. No separation between these two functions was observed, that is, suppression of secretion of Ig in the presence of continued intracellular synthesis. A concomitant analysis of viability using trypan blue dye exclusion did not reveal any differences between control and experimental cultures, decreasing the likelihood that cytotoxicity caused the suppression. In contrast to the above suppressor activity, other co-cultures resulted in a striking enhancement of Ig synthesis and secretion (Table VI). As presented, percent enhancement refers to the percent increase above the predicted value equivalent to 100%. Significant suppression of enhancement was only seen in lymphocyte co-cultures containing cells from patients with primary immunodeficiencies and was not observed in "normal" allogeneic mixtures except as noted above. As with the suppressor effect, enhancement occurred proportionately in both the intracellular and extracellular fractions. Enhancement or suppression of Ig synthesis was not limited to cells from patients with any particular immunodeficiency diagnosis as is evident from Tables III–VI. Finally, several hypogammaglobulinemic patients with different primary immune deficiency states including CVI did not show any enhancement or suppression of PWM-stimulated Ig synthesis (data not shown).

Variability of modulation effect with different normal donors. Because many patients involved in this study were lymphopenic and/or anemic, we were often unable to obtain sufficient numbers of cells for extensive analyses. Hence, the frequent use of only one normal donor in co-cultures was limited by the availability of patients' cells. On occasion we were able

to obtain enough patient cells for admixture with either of two different normal donors. The data in Table VII show that the expression of either enhancing or suppressor activity may vary, depending upon the normal donor used. Experiment I involves the use of two different normal donors with lymphocytes from a single patient, W. St., a 44-year-old man with a benign thymoma and associated hypogammaglobulinemia. When the patient's cells were co-cultured with lymphocytes from normal R, profound suppression of Ig synthesis and secretion occurred; however, admixture with cells from normal A failed to demonstrate any suppression. On another occasion lymphocytes from W. St., however, were suppressive for Ig synthesis and secretion for a different normal donor as well as normal A (Table V). It should be further noted that in additional experiments involving cells from other normal donors, as compared to lymphocytes from normal A, suppression of the latter by cells of immunodeficient patients was consistently less in simultaneous experiments than the other normals studied.

TABLE III

Effect of Co-culture of Normal and Hypogammaglobulinemic Patients' Lymphocytes on Suppression of Synthesis and Secretion of Ig

Subject	Intracellular Ig	Inhibition	Secreted Ig	Inhibition
	cpm	*%*	*cpm*	*%*
Common variable immunodeficiency				
Normal B	11,024	—	18,625	—
M. Ed.	0	—	21	—
M. Ed. + N_B	2,299 (5,512)	58.3	3,097 (9,323)	66.8
P. En.	59	—	151	—
P. En. + N_B	3,132 (5,542)	43.5	4,836 (9,888)	51.1
T. Sc.	0	—	23	—
T. Sc. + N_B	3,627 (5,512)	34.2	6,720 (9,324)	28.0
Normal C	18,224	—	177,972	—
B. McD.	0	—	722	—
B. McD. + N_C	103 (9,112)	98.9	3,495 (89,347)	96.1
Normal D	15,197	—	25,904	—
M. Ro.	6,807	—	12,393	—
M. Ro. + N_D	9,268 (11,002)	15.8	17,179 (19,149)	10.8
Normal E	7,714	—	64,281	—
M. D'A.	0	—	302	—
M. D'A. + N_E	29 (3,857)	99.2	2,409 (32,292)	92.5
Normal F	24,213	—	58,609	—
G. Sca.	14	—	11	—
G. Sca. + N_F	8,848 (12,114)	27.0	19,554 (29,310)	33.3

Numbers in parentheses refer to predicted values.

TABLE IV

Effect of Co-culture of Normal and Hypogammaglobulinemic Patients' Lymphocytes on Suppression of Synthesis and Secretion of Ig

Subject	Intracellular Ig	Inhibition	Secreted Ig	Inhibition
	cpm	*%*	*cpm*	*%*
Infantile sex-linked agammaglobulinemia				
Normal A	43,115	—	85,999	—
Normal G	50,743	—	122,110	—
L. Ha.	254	—	92	—
L. Ha. + N_A	8,890 (21,685)	59.0	11,990 (43,046)	72.2
L. Ha. + N_G	3,603 (25,499)	85.9	2,949 (61,101)	95.2
N_A + N_G	55,780 (46,929)	+18.9	127,720 (104,055)	+22.7
Normal H	36,138	—	59,784	—
R. Ar.	0	—	45	—
R. Ar. + N_H	6,526 (18,069)	63.9	7,088 (29,915)	76.4
Normal I	9,389	—	14,972	—
G. Be.	0	—	9	—
G. Be. + N_I	336 (4,695)	92.8	614 (7,491)	91.8

Numbers in parentheses refer to predicted values.

The paired experiments 2 and 3, as well as 4 and 5, in Table VII reflect the use of different normal lymphocytes in co-culture with the patient's cells on separate occasions. Here the difference is even of greater magnitude with suppression occurring at one time and enhancement at another.

Effect of co-culture of lymphocytes on mitogenic responses. To determine if cell populations from hypogammaglobulinemic patients with diverse primary immunodeficiencies were capable of inhibiting proliferative responses, cultures consisting of normal responder lymphocytes stimulated with added mitogens plus MC-treated mononuclear cells from patients were established. These results are presented in Fig. 3. No statistically significant difference in proliferative responses could be noted whether allogeneic MC-treated cells were obtained from healthy donors or hypogammaglobulinemic patients with primary immunodeficiencies. Furthermore, significant deviations from the range of normal values were not frequently seen in the experimental cultures containing patients' PBL. However, the most heterogeneous re-

sponses, including a number of co-cultures which resulted in less than the predicted amount of [^{3}H]-thymidine incorporation, were observed when the cultures had been stimulated with PWM, a lectin known to activate both T and B lymphocytes. A comparison of PBL from individual patients known to either suppress or enhance Ig synthesis and secretion failed to reveal any correlation with stimulatory or suppressive influences on proliferative responses to the lectins.

DISCUSSION

Ig deficiency syndromes may have a genetic basis, for example infantile sex-linked agammaglobulinemia, Bruton's disease (13). Some may be acquired as an idiopathic disorder or may be a secondary manifestation of other diseases, such as neoplasia or viral infection. These hypogammaglobulinemias can be further classified on the basis of the presence, as in CVI, of surface membrane Ig-bearing B lymphocytes

TABLE V

Effect of Co-culture of Normal and Hypogammaglobulinemic Patients' Lymphocytes on Suppression of Synthesis and Secretion of Ig

Subject	Diagnosis	Intracellular Ig	Inhibition	Secreted Ig	Inhibition
		cpm	%	*cpm*	%
Other immunodeficiencies					
Normal A	—	24,306	—	45,510	—
Normal J	—	30,519	—	52,828	—
M. Ru.	SCID*	46	—	158	—
M. Ru. + N_A	—	10,155 (12,176)	16.6	16,443 (15,488)	+6.2
M. Ru. + N_J	—	4,113 (15,282)	73.1	5,542 (26,493)	79.1
N_A + N_J	—	27,346 (27,412)	0	50,213 (49,169)	+2.1
Normal K	—	37,584	—	55,333	—
Normal L	—	6,555	—	8,504	—
K. Wh.	♀ Congenital agammaglobulinemia	342	—	16	—
K. Wh. + N_K	—	3,078 (18,963)	83.8	1,839 (27,674)	93.4
K. Wh. + N_L	—	1,384 (3,449)	60.0	301 (4,260)	92.9
R. Wh.	♀ Congenital agammaglobulinemia	458	—	14	—
R. Wh. + N_K	—	6,396 (19,021)	66.4	4,173 (27,673)	84.9
R. Wh. + N_L	—	1,012 (3,507)	71.1	189 (4,259)	95.6
N_K + N_L	—	16,659 (22,070)	24.5	22,332 (31,918)	30.0
Normal M	—	8,348	—	79,801	—
C. Di.	IgG + A deficient	0	—	9,504	—
C. Di. + N_M	—	1,599 (4,174)	61.7	3,465 (44,653)	78.8
Normal A	—	24,306	—	45,510	—
Normal N	—	30,519	—	52,828	—
W. St.	Thymoma + agammaglobulinemia	29	—	71	—
W. St. + N_A	—	7,008 (12,168)	42.4	10,636 (22,790)	53.3
W. St. + N_N	—	2,399 (15,274)	84.3	3,241 (26,449)	87.7
N_A + N_N	—	27,346 (27,412)	+2.1	50,213 (49,169)	0

Numbers in parentheses refer to predicted values.
* Severe combined immunodeficiency disease.

TABLE VI

Stimulation of Synthesis and Secretion of Ig by Co-culture of Normal and Immunodeficient Patients' Lymphocytes

Subject	Diagnosis	Intracellular Ig	Enhancement	Secreted Ig	Enhancement
		cpm	%	*cpm*	%
Normal O	—	12,819	—	21,239	—
L. Ar.	CVI	168	—	245	—
L. Ar. + N_O	—	14,071 (6,494)	+116.7	23,429 (10,742)	+118.1
Normal P	—	6,908	—	13,178	—
F. Ro.	CVI + rheumatoid arthritis	8,167	—	13,140	—
F. Ro. + N_P	—	9,366 (7,538)	+24.3	17,287 (13,159)	+31.4
Normal C	—	18,224	—	177,972	—
V. Ka.	CVI	1,026	—	7,570	—
V. Ka. + N_C	—	13,088 (9,625)	+36.0	136,911 (92,771)	+47.6
Normal M	—	24,213	—	58,609	—
F. Ka.	Malignant thymoma + agammaglobulinemia	40	—	15	—
F. Ka. + N_M	—	15,410 (12,127)	+27.1	34,438 (29,312)	+17.5
Normal Q	—	32,110	—	50,813	—
H. Wa.	IgA deficient	45,641	—	53,941	—
H. Wa. + N_Q	—	72,682 (38,876)	+87.0	129,792 (52,377)	+147.8
M. Ri.	X-linked agammaglobulinemia	0	—	37	—
M. Ri. + N_Q	—	28,152 (16,055)	+75.3	50,423 (25,425)	+98.3

Numbers in parentheses refer to predicted values.

or their absence, as in X-linked agammaglobulinemia (14, 15). Thus, in the latter case lymphocyte developmental arrest may occur at a more primordial level, whereas in CVI, terminal B-cell differentiation appears to be inhibited. Accordingly, we undertook the present investigation to extend the analysis of the role of suppressor cell mechanisms in several primary immunodeficiencies including both acquired and congenital diseases with different apparent stages of developmental arrest.

Previous assays of in vitro Ig production by lymphocytes in co-culture have either measured Ig secretion (2) or have estimated development of intracellular Ig by immunofluorescent staining (16). The latter method, although permitting precise quantitation of the cells involved, is not susceptible to a rigorous analysis of the molecular events or the kinetics of the processes involved at the subcellular level. We have thus combined the concepts of these two techniques and examined both the intracellular Ig pool to measure *de novo* synthesis and also assayed Ig secretion.

The results obtained upon culture of lymphocytes from hypogammaglobulinemic individuals parallel the clinical status of the patients. In no instance did we note Ig biosynthetic and secretory capacity by patients' lymphocytes comparable to that obtained with cells from normal donors, after the addition of PWM. In our experience, addition of PWM to cultures of hypogammaglobulinemic cells did not stimulate Ig production contrasting with previous observations of Wu et al. (17) that PWM could increase secreted Ig. Heterogeneity of the patients studied as well as methodologic differences could account for the dissimilar observations. Although lymphocytes from many agammaglobulinemic patients are capable of blast transformation in response to PWM (see Table I),

TABLE VII

Dependence of Modulator Activity on Normal Indicator Lymphocytes

Experiment	Subject	Diagnosis	Intracellular Ig	Inhibition	Secreted Ig	Inhibition
			cpm	%	*cpm*	%
1	Normal R	—	32,998	—	96,063	—
	Normal A	—	58,924	—	100,859	—
	W. St.	Benign thymoma + agammaglobulinemia	0	—	23	—
	W. St. + N_R	—	6,853 (16,499)	58.5	10,300 (48,043)	78.6
	W. St. + N_A	—	30,108 (29,462)	+2.2	41,993 (50,441)	16.7
	N_A + N_R	—	47,388 (45,961)	+3.1	84,803 (98,461)	13.9
2	Normal D	—	15,197	—	25,904	—
	R. Ar.	X-linked agammaglobulinemia	156	—	4	—
	R. Ar. + N_D	—	19,747 (7,677)	+157.2	28,725 (12,954)	+121.7
3	Normal H	—	36,138	—	59,784	—
	R. Ar.	X-linked agammaglobulinemia	0	—	45	—
	R. Ar. + N_H	—	6,526 (18,069)	63.9	7,088 (29,915)	76.4
4	Normal B	—	11,024	—	18,625	—
	T. Sc.	X-linked agammaglobulinemia	0	—	23	—
	T. Sc. + N_B	—	3,627 (5,512)	34.2	6,720 (9,324)	28.0
5	Normal S	—	20,804	—	38,312	—
	T. Sc.	X-linked agammaglobulinemia	0	—	50	—
	T. Sc. + N_S	—	17,118 (10,402)	+64.6	39,416 (19,181)	+105.5

Numbers in parentheses refer to predicted values.

plasma cell precursors may be selectively unable to proliferate and/or undergo terminal differentiation to Ig synthesizing the secreting cells.

In every instance of suppression of Ig secretion, a concomitant inhibition of intracellular Ig synthesis was observed. This supports the interpretation that the suppressor activity observed is a manifestation of inhibition of differentiation of B cells. An isolated block in secretion of *de novo* synthesized Ig was not seen after PWM stimulation. Choi et al. (18) have previously shown that freshly isolated lymphocytes from patients with CVI, when cultured for 4 h in the absence of PWM, could synthesize but not secrete Ig, whereas cells from patients with infantile sex-linked agammaglobulinemia did not appear to be able even to synthesize Ig. Additional studies by Geha and his colleagues (19) demonstrated that CVI is a heterogeneous group of diseases with blocks at various stages of B-cell development. These include patients whose B lymphocytes could synthesize but not secrete Ig. Our present data do not rule out a potential for Ig synthesis by B lymphocytes from individuals with CVI, but rather demonstrate support for the heterogeneity of CVI. Although subgroups of patients whose B lymphocytes may be capable of differentiating to cells containing but not secreting intracytoplasmic Ig have been demonstrated, differences in both the patient populations studied and the techniques employed may have precluded similar findings. Moreover, the suppressor activity manifested by lymphocytes from our patients with hypogammaglobulinemia of divergent etiologies seems to have a common mechanism, namely, inhibition of B-cell differentiation.

Our data (Table III) confirm the original observa-

tions by Waldmann and co-workers (2) that some patients with CVI manifest significant Ig suppressor activity in co-culture experiments; however, a significant proportion (data not shown) of CVI patients in our studies did not exhibit any deviation from predicted levels of Ig synthesis in co-cultures. These data support the conclusions that CVI encompasses defects in a spectrum of mechanisms affecting terminal B-cell differentiation (19) and thus several subgroups of patients with and without Ig suppressor activity may be observed.

Among the other patients with demonstrable suppressor effects, we also find a substantial number with the diagnosis of infantile sex-linked agammaglobulinemia. These results are, in general, consonant with the findings of Siegal et al. (16), who, by using PWM for the induction of intracellular Ig-staining plasmacytoid cells, found that mixing experiments between lymphocytes from patients with Bruton's agammaglobulinemia and normal individuals could demonstrate suppression of the development of cells with cytoplasm stained by fluorescent anti-Ig reagents. These findings raise the issue addressed by Blaese and co-workers (20), who found that suppressor T lymphocytes can develop in neonatally bursectomized birds and be transferred to sublethally irradiated chickens, causing an "infectious" or transmitted agammaglobulinemia (21). Although the initial pathogenic mechanism for certain hypogammaglobulinemias need not involve suppressor cell activity, the latter may develop as a secondary manifestation. The finding of suppressor cells associated with the few immunodeficiency syndromes described herein does not distinguish whether they are a primary or secondary phenomenon. The difficulty in establishing functional humoral immunity after lymphoid cell, bone marrow, or fetal tissue transplantation in several cases of primary immunodeficiencies (22–25) may be especially relevant as secondary suppressor cells may arise and inhibit therapeutic intervention. Further investigations on the developmental origin of suppressor cells may resolve this critical issue.

We have also observed that co-culture of lymphocytes from various hypogammaglobulinemic patients may result in greater than the anticipated amount of Ig synthesis. The immunodeficiency syndromes associated with enhancing phenomena in this study do not appreciably differ from those where suppressor activity was shown. Such deviations from predicted responses were noted only with admixtures containing patients' cells and not among co-cultures of normal cells. On one occasion, a child with severe combined immunodeficiency disease previously noted to suppress Ig synthesis in co-culture, was subsequently found to have profound enhancing activity (26). In this

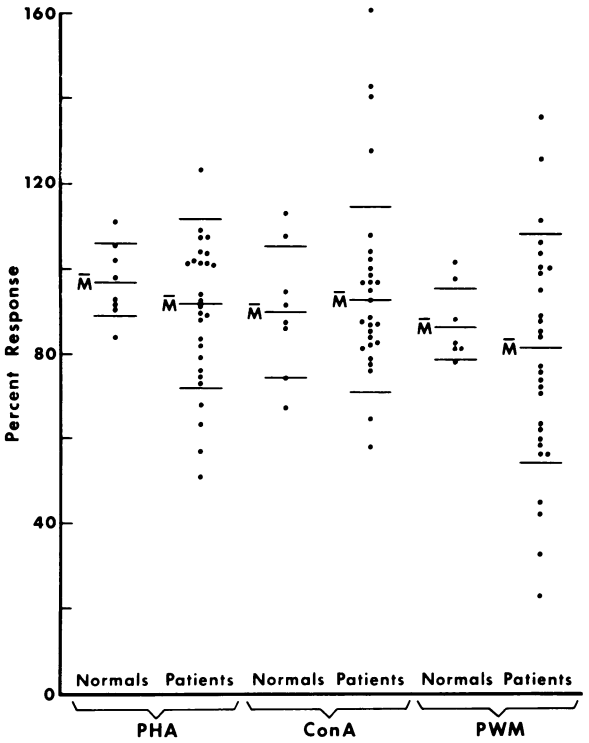

FIGURE 3 Effect of co-culture of mononuclear cells on the mitogen response by normal donor lymphocytes. MC-treated allogeneic normal or hypogammaglobulinemic patients' mononuclear cells, as indicated, were added to untreated normal responder lymphocytes plus the designated mitogen, and assayed for incorporation of [^{3}H]thymidine. Percent response to mitogens by normal lymphocytes influenced by MC-treated allogeneic cells was calculated as described in Methods. Horizontal lines define mean±SD.

case, the patient had undergone previous immunotherapy and may thus have had major changes in functional lymphocyte subpopulations.

Eardley and Gershon (27) have proposed that helper activity is the modulating effect elicited by a weak immunological reaction, such as occurs upon challenge with a low dose of antigen, whereas suppression would be the subsequent control mechanism associated with a vigorous immunologic response. Our observation that the same patient may have grossly different immunoregulatory responses at various times and/or with different normal donors is consistent with the hypothesis that regulatory cell effects vary inversely with the activity of the cells they control (28). This is especially significant when considered relative to the findings that changes in the assay system or the responder population may affect the regulatory activity observed (29). These data cannot distinguish between the possibility that the potential suppressor or helper activity of the patient or normal donor can vary, with the net outcome reflecting a balance of immunologic capacities, negative and posi-

tive, of both individuals. That the potential for suppressor activity may exist in the normal, healthy population has recently been reported by Shou and co-workers (11). Thus, any individual's modulator capacity may vary as a consequence of the day-to-day immunologic changes associated with diet, environmental factors, infections, etc. Variation of suppressor activity as a function of the normal donor used was also recently reported by Broom et al. (30). It is interesting to note that in our studies, cells from one specific donor, normal A, were consistently less suppressed than those of other volunteers when cocultured with cells from a number of hypogammaglobulinemic individuals and coincidentally, PWM-stimulated Ig synthesis and secretion by normal A was always most vigorous. This individual may be an example of someone whose balance between helper and suppressor cell activity may be weighted in favor of the former. Support for such a model also comes from our observations that a single normal donor can vary at different times with regard to PWM-stimulated Ig synthesis response in vitro. Further analysis of the mechanisms involved in these apparently complex relations may derive from correlative studies of the T_μ and T_γ subpopulations of lymphocytes recently described by Moretta et al. and others (31, 32).

A subclassification of immunoregulatory cell populations in man may be made on the basis of differential modulations of B or T cell-associated activities as our data suggest. Thus, patients with clinically defined humoral immune deficiencies can manifest in vitro perturbations of B-cell differentiation and function without a concomitant effect on such T-lymphocyte properties as mitogen-stimulated DNA synthesis. We are currently investigating whether the reciprocal situation also exists, that is, whether patients with isolated cell-mediated immune defects can manifest selective in vitro regulation of mitogen responses in the absence of effects on Ig metabolism.

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