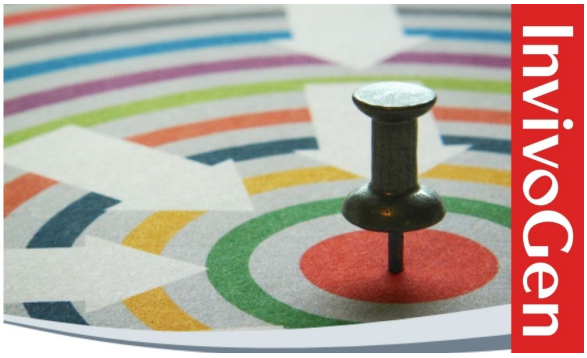

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S A Schwartz

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HEAVY CHAIN-SPECIFIC SUPPRESSION OF IMMUNOGLOBULIN SYNTHESIS AND SECRETION BY LYMPHOCYTES FROM PATIENTS WITH SELECTIVE IgA DEFICIENCY[1]

STANLEY A. SCHWARTZ

From the Department of Pediatrics and Communicable Diseases, The University of Michigan, Ann Arbor, Michigan 48109

A simple, solid-state immunofluorescent assay specific for the heavy chain of human immunoglobulins (Ig) was adapted for studying pokeweed mitogen- (PWM) stimulted Ig biosynthesis and secretion by peripheral blood lymphocytes (PBL) *in vitro*. PBL from patients with panhypogammaglobulinemia were noted to synthesize and secrete decreased amounts of all classes of immunoglobulin after polyclonal activation of B cells *in vitro* with PWM. PBL from 14 patients with selective deficiency of IgA were capable of synthesizing and secreting significant levels of IgG and IgM after culture with PWM; however, they did not produce appreciable amounts of IgA. Thus, B lymphocyte differentiation *in vitro* parallels the clinical status. Several different immunoregulatory phenomena were observed when PBL from patients with selective IgA deficiency were co-cultivated with cells from healthy donors. Most notable was the specific suppression of IgA synthesis and secretion seen in 8 of 14 patients studied, although other modulatory effects including enhancement and suppression of all Ig classes were also observed. These results suggest that the pathologic basis of selective IgA deficiency may be heterogeneous and support a model for the role of Ig class-specific suppressor cells in the pathogenesis of some cases of selective IgA deficiency.

Recent advances in our understanding of the immunoregulation of B lymphocyte differentiation and function have resulted in numerous investigations on B cell modulation in human disease states (1-9). These studies reveal a complex series of interactions between effector and regulatory cells and further support the concept of the "network theory" of the immune system proposed by Jerne (10). The role of suppressor T lymphocytes in the etiology of some forms of common variable immunodeficiency has been demonstrated by Waldmann and his co-workers (1); however, other mononuclear leukocyte subpopulations also appear to be capable of effectively suppressing B cell differentiation to immunoglobulin- (Ig) synthesizing and secreting plasma cells (2). In an earlier report, we have further demonstrated a functional heterogeneity of immunoregulatory mechanisms (5). To date, most of the clinical studies on the modulation of B lymphocyte functions have focused on patients with panhypogammaglobulinemia. However, the selective deficiency of IgA has been proposed to be the most common of all the immunodeficiency diseases (11, 12). Clinical expression of this disorder ranges widely from completely asymptomatic individuals to patients with recurrent infections usually of the upper respiratory tract and associated with an increased incidence of allergies, autoimmune phenomena, and gastrointestinal dysfunction (13, 14). This divergence of clinical symptomatology may also underlie a heterogeneity in the pathophysiologic mechanisms of the disease. Accordingly, we undertook the following investigations to determine if any immunoregulatory defects were associated with selective IgA deficiency and if so whether we could demonstrate specificity of their activities. Several abnormal immunoregulatory activities were observed *in vitro*, including the selective suppression of IgA synthesis and secretion by B lymphocytes undergoing polyclonal stimulation.

Simple and effective techniques for studying B cell functions have been limited. Immunofluorescent staining of intracytoplasmic immunoglobulin is subject to investigator interpretation and cannot analyze biochemical events or processes at the molecular level. Radioimmunoassay (RIA)[2] for the quantitative determination of Ig biosynthesis using isotope incorporation by cultured cells possesses the problems of preparation of the immunoreagents, disposal of radiolabeled compounds, and the high cost of these materials and required equipment. Furthermore, serologic precipitation assays for quantitating Ig production *in vitro* are often complicated by the nonspecific "trapping" of high background radioactivity (15). Also described herein is a technique for assaying polyclonal activation of B lymphocytes to Ig-synthesizing and secreting plasma cells and the regulatory effects, including "suppressor" and "helper" activities, upon cocultivation of peripheral blood lymphocytes (PBL) from patients with panhypogammaglobulinemia or selective IgA deficiency and normal donors. This technique employs a commercially available solid-state quantitative immunofluorescent assay specific for the heavy chain of human immunoglobulin.

MATERIALS AND METHODS

Patients and normal donors. All patients involved in these studies were apprised of the nature of this investigation and

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[2] Abbreviations used in this paper: GG, immune serum globulin; KLH, keyhole limpet hemocyanin; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline, pH 7.5; PWM, pokeweed mitogen; RIA, radioimmunoassay; ERFC, sheep erythrocyte rosette-forming cells; PV, polyvalent fluoresceinated anti-human immunoglobulin; SA, salivary IgA; S.C., secretory component.

heparinized (20 units/ml) blood samples were drawn from a peripheral vein after obtaining informed consent. Control blood was similarly obtained from healthy volunteer donors. Patients with panhypogammaglobulinemia had serum concentrations of IgG, IgA, and IgM considerably less than 2 standard deviations below the mean normal value for age. This group includes individuals with the diagnosis of common variable immunodeficiency and infantile X-linked agammaglobulinemia. Clinical and immunologic parameters of the patients with selective IgA deficiency are presented in Table I.

Cell culture conditions. Details of the method for the preparation and culture of PBL have been previously described (5). Briefly, PBL were isolated by density gradient centrifugation (16) at 400 × G for 30 min at 18°C by using a solution of sodium metrizoate and Ficoll (Lymphoprep, Nygaard and Co., Oslo, Norway). Washed PBL were suspended at a concentration of 1 × 10^6 cells/ml in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum (FCS), 80 μg of gentamicin (Schering Pharmaceutical, Kenilworth, N. J.) and 300 μg of fresh glutamine/ml. Cultures consisted of 5-ml aliquots in polypropylene tubes. Cell co-cultures were prepared by adding 2.5 ml of each cell suspension (5 ml total volume) to the plastic tubes. Each culture received 50 μg of pokeweed mitogen (Grand Island Biological Co., Grand Island, N. Y.) in 50 μl of water and was incubated for 7 days at 37°C in a humidified atmosphere of 5% CO_2 and air. On the last day of incubation replicate cultures were either washed and resuspended in 2 ml of Dulbecco's modified Eagle's medium (MEM) containing 5% FCS and 25 μCi of L-(4,5-^{3}H(N)) leucine (1.0 mCi/ml, New England Nuclear, Boston, Mass.) and incubated for an additional 4 hr or left in the RPMI 1640 medium for the final 4 hr of culture. Culture supernatants were obtained by sedimenting the cells at 850 × G.

Radioimmunoprecipitation assay for Ig. A direct precipitation assay for *in vitro* biosynthesis of radiolabeled human Ig has been extensively described (17). Monospecific rabbit antiserum to human F(ab')$_2$ fragments was titrated to determine the volume necessary to precipitate 50 μg of human immune serum globulin (GG), Cohn fraction II (E. R. Squibb & Sons, Princeton, N. J.) and twice that volume was used for all experiments. A nonspecific serologic control consisted of aliquots of rabbit anti-keyhole limpet hemocyanin (KLH) serum plus purified KLH (Schwarz/Mann, Orangeburg, N. Y.) capable of yielding the same amount of precipitate as the specific serologic precipitates. The supernatants of the radiolabeled MEM cultures above were dialyzed against 0.15 M NaCl and 0.01 M phosphate, pH 7.5 (PBS), and cleared of radiolabeled substances capable of nonspecifically interacting with the rabbit antiserum-antigen complex by a "clearing" serologic reaction consisting of rabbit anti-KLH serum and carrier KLH. These precipitates were discarded and the remaining supernatant was divided into two equal aliquots. A volume of rabbit antiserum was added to one aliquot followed by the unlabeled carrier GG. To the remaining aliquot a volume of rabbit anti-KLH serum and carrier KLH yielding a precipitate equivalent in amount to the specific serologic reaction was also added. The resulting precipitates were washed, dissolved in 0.5 ml of 0.2 M KOH, mixed with 10 ml of scintillation fluid (Aquasol, Packard Instrument Co., Downer's Grove, Ill.), and counted by scintillation spectroscopy. The net radioactivity of Ig was determined by subtracting nonspecific cpm from the specific counts. Percent suppression of Ig synthesis and secretion was calculated according to the following formula:

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

All results correspond to the net Ig from 2.5 × 10^6 cells since an aliquot representing one-half of the original starting volume was used for the specific serologic reaction. A "+" sign preceding any value refers to enhancement above 100%.

Immunofluorescent assay for Ig. Supernatants from the unlabeled cultures in RPMI 1640 medium above were used directly without any preparatory treatment. *De novo* synthesized Ig secreted into the culture medium was measured by a modification of a solid-state quantitative immunofluorescent assay (Immuno-Fluor, Bio-Rad Laboratories, Richmond, Calif.). Detailed descriptions of the preparation and specificity of similar solid-phase fluorescent assays have been previously published (18, 19). These reagents consist of derivatized polyacrylamide

TABLE I

Immunologic parameters of patients with selective IgA deficiency

Patient	Age	Sex	Immunoglobulins					S.C.	Lymphocytes						Mitogen Responses		
									No.	Surface markers[a]							
			G	A	M	E	S-A			ERFC	PV	IgM	IgG	IgA	PHA	Con A	PWM
	yr		*mg/dl*			*IU/dl*						%			*net cpm*[b]		
A.	7	M	1850	0	79	ND[c]	–	ND	1953	53.0	ND	ND	ND	ND	ND	ND	ND
B.	59	M	1760	0	100	25	–	+	1210	74.5	11.0	7.0	0.5	2.0	23,541	8,997	8,115
C.	7	M	1280	12	80	ND	ND	ND	960	67.0	ND	ND	ND	ND	ND	ND	ND
D.	10	F	2400	10	190	328	–	+	1925	85.5	28.5	17.5	ND	ND	ND	ND	ND
E.	6	F	1940	0	90	47	–	–	3160	86.0	13.0	4.0	0	0	20,996	18,803	7,435
F.	64	F	1050	0	41	ND	ND	ND	1760	ND	6.3	ND	ND	ND	ND	ND	ND
G.	4	M	1147	18	92	120	ND	ND	5883	74.0	11.0	8.0	1.0	1.0	19,570	13,951	10,849
H.	18	F	1500	0	172	210	–	ND	1728	78.0	15.0	10.0	2.0	2.0	19,537	ND	ND
I.	44	M	1318	0	131	120	ND	ND	2240	74.0	10.0	6.5	1.0	1.0	21,784	16,745	12,796
J.	5	F	1380	0	112	40	ND	ND	1500	71.0	ND	ND	ND	0.8	42,267	36,248	12,112
K.	12	F	1480	0	130	630	–	+	1848	80.0	10.0	6.0	1.5	0	20,315	13,091	6,753
L.	19	M	1134	0	100	90	–	+	915	87.5	16.5	10.5	ND	ND	25,465	10,710	9,608
M.	5	F	1590	0	110	110	–	ND	2384	85.0	10.5	10.4	2.0	0	26,209	26,222	8,248
N.	55	M	2300	0	220	170	ND	ND	1050	85.0	14.0	7.5	ND	ND	19,793	6,510	9,608

[a] Surface Ig were determined using heavy chain specific F(ab')$_2$ fragments of goat antisera.

[b] A normal maximal response to PHA has been determined to be >17,000 cpm; normal maximal response to Con A and PWM are ≤PHA response.

[c] Not determined.

beads covalently coupled to rabbit anti-human heavy chain-specific antibody and a soluble fluorescein isothiocyanate conjugated monospecific rabbit anti-human heavy chain antiserum. Specificity of the reagents was determined by double diffusion immunoprecipitation in agar, by the inability of the fluorescent antibody to couple with immunoadsorbent-bound human Ig of a different class specificity and with purified myeloma proteins of known heavy chain class. Volumes of undiluted culture supernatant usually consisting of 250 or 500 μl were directly added to separate 12 x 75 mm borosilicate glass tubes (Kimble Glass, Toledo, Ohio). Known concentrations of Ig were prepared by diluting IgG, IgA, or IgM standards calibrated against WHO International Reference Preparation 67/95 supplied by the manufacturer and 250-μl aliquots of buffer blank, diluted standards or culture supernatants were added to borosilicate glass tubes followed by 1.0 ml of the reconstituted solid-state immunoadsorbent (Immunobead, Bio-Rad Laboratories). After vortexing, the tubes were incubated for 1.5 hr in a 37°C water bath. A 50-μl aliquot of the appropriate fluorescent antiserum was next added to each tube and the contents were mixed. The tubes were returned to the 37°C water bath for an additional 1-hr incubation. Centrifugation of each tube at 1700 × G for 8 min sedimented the insoluble reagents, which were washed with an additional 3 ml of PBS. The precipitate was resuspended in 2.0 ml of PBS and the fluorescence of each sample was determined in a Gilson Model 3302 Spectra/Glo filter fluorometer (Gilson Medical Electronics, Inc., Middleton, Wis.) by using filters at an excitation wavelength of 490 nm and an emission wavelength of 520 nm. The corrected relative fluorescence equals the value of the sample minus the reading of a reagent blank. Thus, the amount of bound fluorescent antibody is directly proportional to the amount of antigen absorbed by the solid-state reagent. Immunoglobulin concentrations of unknown samples were determined from the standard curve. Percent suppression was calculated as in the formula above substituting absolute values of Ig in nanograms for cpm. All results have been normalized to correspond to the net Ig produced by 1×10^6 PBL.

RESULTS

Sensitivity of immunofluorescent assay. Standard curves for the fluorescent immunoassay of various classes of human Ig were found to be linear over the concentration ranges indicated. Each point in Figure 1*a* represents the mean ± S.E.M. for 10 separate determinations. Although this assay was observed to be consistently linear with concentrations of antigenic Ig less than 1000 ng/ml, when concentrations of Ig were increased to levels resulting in antigen excess, deviations from linearity were observed as shown in Figure 1*b*.

Kinetics of PWM-stimulated Ig synthesis and secretion. As shown in Figure 2, synthesis and secretion of significant amounts of Ig in response to polyclonal B cell activation with PWM became readily detectable by day 5 and increased steadily to day 8. Thereafter, the concentration of Ig in the culture supernatants increased more slowly through day 14. Since this assay measures the total Ig secreted over a period of time, the values obtained on any particular day reflect the total amount accumulated. Concomitant measurements of cell viability by trypan blue dye exclusion revealed a steady loss of viable cells. Thus, by day 14 only approximately 25% of the cultured cells were still viable.

Immunoglobulin synthesis and secretion by PBL from hypogammaglobulinemic patients and normal donors. The data in Figure 3 reflect the total amounts of various classes of Ig

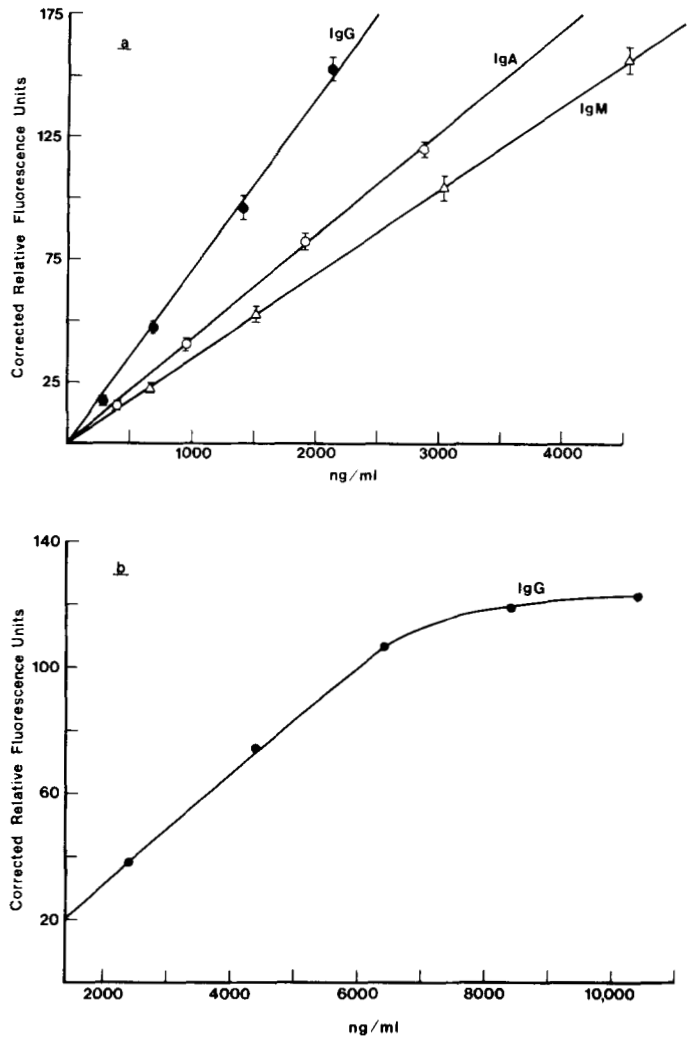

Figure 1. Standard curves for the immunofluorescent assay of human Ig. Known concentrations of Ig standards were assayed as described in *Materials and Methods* and the corrected relative fluorescence, the sample reading minus the reagent blank, was determined for each concentration. a, Routine concentration range; each point is the mean ± S.E.M. b, High concentration range (antigen excess), IgG only. IgG (●), IgA (○), IgM (△).

synthesized and secreted into the culture supernatant by PWM-stimulated PBL from normal donors and panhypogammaglobulinemic patients over a 7-day period. Ig was measured by the solid-state immunofluorescent technique described in *Materials and Methods.* The mean values for the total amounts of PWM-stimulated IgG, IgA, and IgM synthesized and secreted by PBL from 16 different normal donors over 7 days were 1092 ng, 445 ng, and 2018 ng/1×10^6 PBL, respectively. These results compare favorably with those determined by Waldmann *et al.* (20) who, using a double antibody RIA, reported geometric means of 813 ng for IgG, 635 ng for IgA, and 2455 ng for IgM per million lymphocytes after 7 days of culture with PWM.

Although Ig synthesis and secretion by PWM-stimulated normal donor lymphocytes varied over a wide range, Ig production by PBL from panhypogammaglobulinemic patients was greatly depressed in comparison to the normals. Reproducibility of results was evidenced by the observations that repeat assays of supernatants, using different batches of fluorescent reagents, stored for over 1 year at 4°C gave values almost identical to the original. Like the radioimmunoprecipitation technique previously described (5), this *in vitro* assay also correlated with the

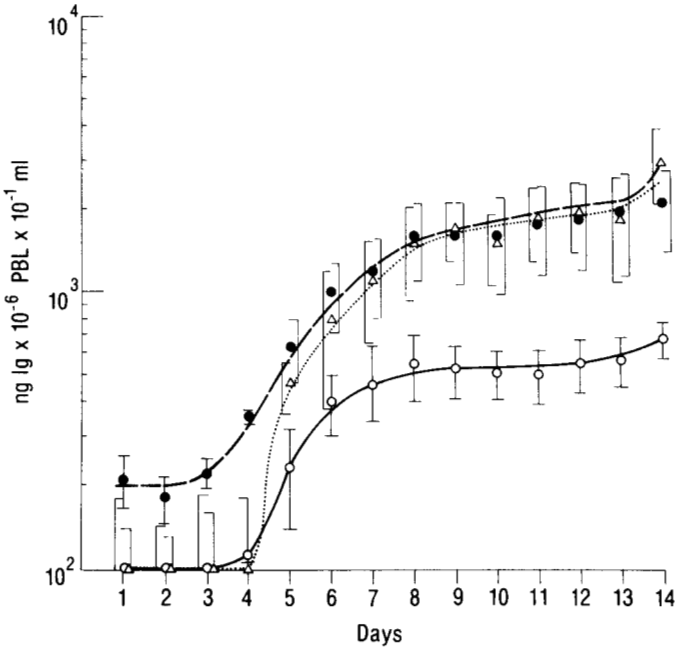

Figure 2. Kinetics of Ig biosynthesis and secretion by PWM-stimulated PBL from healthy donors. The supernatants of cell suspensions, 10^6 PBL/ml, were assayed for Ig by the immunofluorescent technique described in *Materials and Methods* at the indicated times after the initiation of culture containing 10 μg PWM/ml. The data represent the mean ± S.E.M. for five individual subject donors. IgG (●), IgA (○), IgM (△).

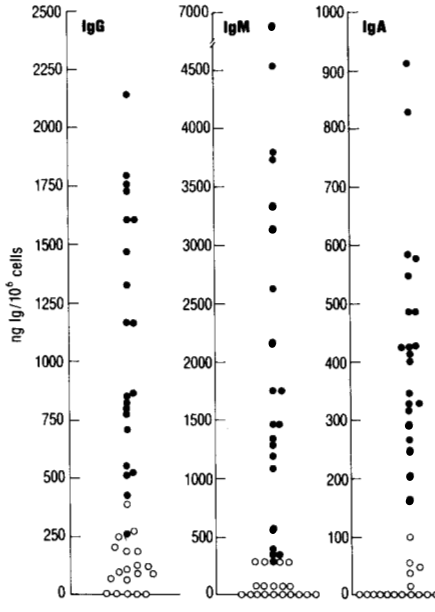

Figure 3. Ig biosynthesis and secretion by PBL from normal donors and hypogammaglobulinemic patients. PBL were cultured with PWM (10 μg/ml) for 7 days and the levels of Ig in the supernatants were determined by the fluorescent immunoassay described in *Materials and Methods.* Normal donors (●); hypogammaglobulinemic patients (○).

depressed Ig levels in the patients' serum. A similar correlation between the clinical findings and the capacity to synthesize and secrete Ig *in vitro* was observed when PBL from patients with selective IgA deficiency were compared with lymphocytes from normal donors. As noted in Figure 4, PBL from these patients were unable to synthesize and secrete significant amounts of IgA after 7 days culture with PWM. Although some patients also showed depression of other Ig classes, IgA production was most profoundly and consistently diminished.

Comparison of fluorescent and radioimmunoprecipitation assays for demonstrating modulation of Ig synthesis and secretion. A variety of immunoregulatory phenomena including both "helper" and "suppressor" effects were manifested when PBL from patients with various primary and secondary immunodeficiencies were co-cultivated with lymphocytes from normal donors in the presence of PWM. Typical examples of these phenomena are presented in Table II. As noted in experiment 1, PBL from the Patient X with a diagnosis of infantile X-linked agammaglobulinemia were unable to synthesize or secrete significant amounts of Ig in response to stimulation with PWM, as measured by either the radioimmunoprecipitation assay or the heavy chain-specific immunofluorescent method when compared to PBL from the normal donor. Co-cultivation of donor and patient's PBL resulted in marked suppression of the total amount of synthesized and secreted Ig. The degree of suppression was comparable regardless of the assay system. Experiment 2 demonstrates that enhancing activities may also be observed. PWM-stimulated PBL from another hypogammaglobulinemic patient again synthesized and secreted less Ig than similarly treated normal donor cells; however, co-culture of lymphocytes resulted in greater recoveries of Ig than predicted from the autologous cultures. Finally, experiment 3 is an example of the absence of significant immunoregulatory effects upon co-cultivation of the designated PBL. It should be emphasized that in all cases, cell recovery and viability of autologous and co-cultured PBL was comparable as demonstrated by trypan blue dye exclusion. Co-cultivation of PBL from random pairs of different normal healthy donors, however, usually did not produce significant deviations in the amounts of synthesized and secreted Ig as compared to individual autologous cultures (1, 3, 5).

Effect of co-cultivation of PBL from patients with selective IgA deficiency and normal donors on Ig synthesis and secretion. This laboratory and others have shown that PBL from patients with panhypogammaglobulinemia can manifest suppressor activity directed against synthesis and secretion of all

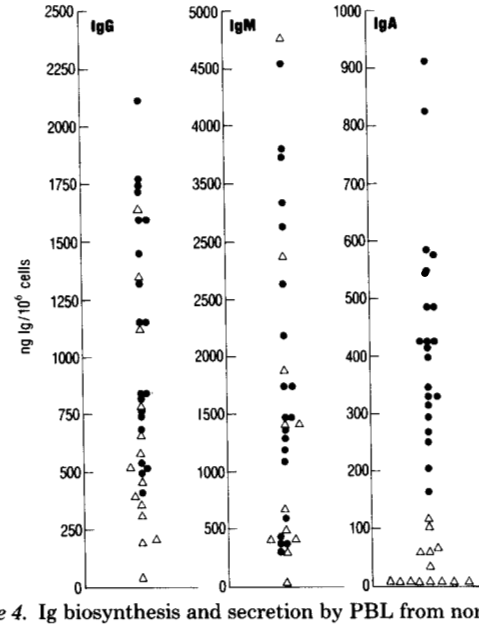

Figure 4. Ig biosynthesis and secretion by PBL from normal donors and patients with selective IgA deficiency. Culture and assay conditions were the same as in legend for Figure 3. Normal donors (●); patients (△).

TABLE II

Comparison of radioimmunoprecipitation and immunofluorescence assays for determining modulatory effects on the synthesis and secretion of Ig

Expt.	Subject	Diagnosis	RIA		Heavy Chain Specific Immunofluorescent Assay					
			Net Ig[a]	Inhibition	IgG[b]	Inhibition	IgA[b]	Inhibition	IgM[b]	Inhibition
			cpm	%		%		%		%
1	Normal 1		30,578		42.0		13.0		57.0	
	Patient X	XLA[c]	0		7.0		0		0	
	$P_X + N_1$		7,743	49.4	12.0	51.0	3.0	53.8	15.0	47.4
			(15,289)[d]		(24.5)		(6.5)		(28.5)	
2	Normal 2		60,316		51.0		5.0		97.0	
	Patient Y	CVI[e]	4,356		2.0		0		0	
	$P_Y + N_2$		67,079	+107.4	52.0	+96.0	6.5	+160.00	94.5	+95.0
			(32,336)		(26.5)		(2.5)		(48.5)	
3	Normal 3		184,346		67.0		48.0		236.0	
	Patient Z	CVI[e]	93,429		53.0		14.0		98.0	
	$P_Z + N_3$		175,081	+26.1	78.0	+30.0	9.7	+9.7	163.0	2.4
			(138,888)		(60.0)		(31.0)		(167.0)	
4	Normal 4		37,252		24.5		98.0		15.0	
	Patient K	SIgAD[f]	22,093		18.5		18.0		23.5	
	$P_K + N_4$		22,876	22.9	19.5	9.3	19.5	66.4	23.0	+19.2
			(29,673)		(21.5)		(58.0)		(19.3)	

[a] Specific serologic reaction minus nonspecific serologic control.
[b] Corrected relative fluorescence.
[c] X-linked agammaglobulinemia.
[d] Numbers in parentheses refer to predicted values equal to the mean value of Ig from individual cultures of normal donor and patient PBL.
[e] Common variable immunodeficiency.
[f] Selective IgA deficiency.

Ig classes (1, 3-6). Patients with a deficiency of a single class of Ig, however, may fail to demonstrate any *in vitro* abnormalities regarding total Ig synthesis and secretion or modulation of B cell differentiation when a polyvalent assay for *in vitro* Ig production is used, as seen in experiment 4 of Table II. By contrast, the heavy chain-specific immunofluorescent assay clearly shows that PBL from the IgA-deficient patient made considerably less IgA than concomitantly studied PBL from a normal donor and, moreover, co-cultivation of these cells resulted in specific suppression of only IgA synthesis and secretion. When PBL from other patients deficient in IgA only were co-cultivated with lymphocytes from healthy allogeneic donors and stimulated for polyclonal B cell activation with PWM, several different immunoregulatory phenomena were observed. Most notable was the specific suppression of IgA synthesis and secretion further demonstrated in Table III. As noted above, PBL from these patients failed to produce significant amounts of IgA when stimulated with PWM, compared to levels of IgA similarly obtained with normal donor cells. Moreover, synthesis and secretion of IgG or IgM by patients' cells did not differ appreciably from volunteer donors. Co-cultivation of equal numbers of PBL from these patients and normal donors, however, resulted in suppression of IgA synthesis and secretion without similar inhibition of IgG or IgM production. Specific suppression of IgA by PBL from IgA-deficient patients was not the only immunoregulatory dysfunction observed. As seen in Table IV, immunoregulatory activities ranged from suppression to enhancement of all Ig classes. These phenomena clearly differ from the absence of suppressor or helper effects observed when control PBL from allogeneic healthy volunteer donors are co-cultured. Furthermore, in no instance did we observe selective suppression or enhancement of any other Ig class.

DISCUSSION

Polyclonal activation of B lymphocyte differentiation to Ig-synthesizing and secreting plasma cells with PWM has provided a model *in vitro* system for analyzing modulation of the humoral immune response in man (1). We have previously demonstrated that PBL from patients with a variety of primary immunodeficiencies manifested by the clinical criterion of panhypogammaglobulinemia fail to synthesize *de novo* intracellular Ig when stimulated with PWM (5). This contrasts with high levels of Ig biosynthesis by similarly treated PBL from healthy volunteers. These initial efforts, however, utilized a polyvalent direct radioimmunoprecipitation assay and could not distinguish any differences in amounts of various heavy chain-specific Ig classes. The present investigations employ an immunofluorescent assay specific for either IgG, IgA, or IgM. Thus, when PBL from patients with panhypogammaglobulinemia were cultured with PWM and assayed for Ig production, synthesis of all Ig classes was observed to be uniformly depressed, also paralleling the clinical situation.

Regulation of B cell activation may involve a variety of cellular and molecular mechanisms; however, the role of suppressor cells in the differentiation of functionally active plasma cells is now widely accepted. We have previously demonstrated that immunoregulatory activities could be divided into functional subpopulations (5). Suppressor cells specifically inhibiting B lymphocyte activities without affecting T cell functions have been found in panhypogammaglobulinemic patients possessing normal cell-mediated immunity. Conversely, we have also noted that mitomycin C-treated or irradiated PBL from some patients with deficient cell-mediated immune functions could suppress the mitogenic responses of lymphocytes from healthy donors. That both of these potential suppressor activities may coexist in PBL from normal donors has also been demonstrated (21, 22).

The present investigation provides data suggesting that the pathophysiologic mechanisms underlying the syndrome of selective IgA deficiency may be heterogeneous and further supports a model wherein, in certain cases, IgA synthesis and secretion may be under the influence of selective suppressor cell activity. Thus, just as there is evidence for the selective

TABLE III

Specific suppression of IgA synthesis and secretion by PBL from patients with selective IgA deficiency

Subject	IgA	Inhibition	IgG	Inhibition	IgM	Inhibition
	ng	%	*ng*	%	*ng*	%
Normal A	511		873		1591	
Patient G	0		353		1442	
$P_G + N_A$	0	100	543	11	2096	+38
	(256)[a]		(613)		(1517)	
Normal B	348		517		1203	
Patient K	60		383		1892	
$P_K + N_B$	66	68	405	10	1851	+20
	(204)		(450)		(1548)	
Normal C	360		722		1155	
Patient J	42		233		668	
$P_J + N_C$	56	72	353	26	1438	+58
	(201)		(478)		(912)	
Normal D	832		1755		3818	
Patient B	0		1336		1426	
$P_B + N_D$	183	56	1558	+1	2804	+7
	(416)		(1546)		(2622)	
Normal E	272		732		ND[b]	
Patient M	3		469		ND	
$P_M + N_E$	59	57	506	16	ND	
	(138)		(600)			
Normal F	525		876		ND	
Patient E	60		784		ND	
P_E and N_F	60	80	753	6	ND	
	(293)		(830)			
Normal G	114		223		260	
Patient A	0		282		262	
$P_A + N_G$	0	100	281	5	423	+62
	(57)		(268)		(261)	
Normal H	446		757		850	
Patient N	0		514		280	
$P_N + N_H$	23	90	372	41	486	14
	(222)		(635)		(565)	

[a] Numbers in parenthesis refer to predicted values.
[b] ND, not determined.

suppression of either T or B lymphocyte functions, among the latter category of immunoregulatory cells may be subpopulations specifically capable of class-specific suppression of Ig synthesis and secretion.

Kishimoto and his co-workers (23) have presented evidence for IgE class-specific suppressor T cells capable of the selective suppression of IgE antibody response in mice. Selective suppression of *in vitro* IgA synthesis and secretion by human PBL has recently been reported (24, 25). It is significant to note that in these latter studies only a proportion (21 to 50%) of the patients examined demonstrated selective IgA suppressor cell activity in the peripheral blood. Atwater and Tomasi (25) found a single patient with selective IgA deficiency whose cultured PBL, although unable to synthesize and secrete IgA by themselves, could make sufficient quantities of IgA when co-cultured with a T cell-enriched population from a healthy allogeneic donor. This was interpreted as being consistent with the absence of a specific "helper" cell activity. Waldmann and his coworkers (24) observed that some patients with selective IgA deficiency had PBL that could be stained for intracytoplasmic IgA with fluorescent antisera after 7 days culture with PWM; however, no significant amounts of IgA were secreted into the culture supernatant. Co-culture of these cells with lymphocytes from normal allogeneic donors did not result in any deviations in the expected recoveries of IgA. PBL from another group of IgA-deficient patients were unable to develop intracytoplasmic IgA after polyclonal stimulation with PWM. These patients, however, demonstrated selective suppression of IgA synthesis and secretion in co-culture experiments when using PBL from healthy volunteers. By contrast a recent study by Cassidy *et al.* (26) failed to demonstrate any Ig class-specific suppressor cell activity with PBL from their series of patients with selective IgA deficiency. They concluded that the results were consistent with an intrinsic B cell defect resulting in failure of terminal functional differentiation to IgA synthesizing and secreting plasma cells. Thus, the findings herein complement these other observations and further provide an explanation for the apparent discrepancies between them by demonstrating a heterogeneity of pathologic mechanisms underlying the syndrome of selective IgA deficiency. Such a conclusion is not without precedent since Buckley and her colleagues (27) have proposed a similar functional and cellular heterogeneity underlying the clinical syndrome of severe combined immunodeficiency.

In this paper we also described a simplified immunofluorescence assay for analyzing regulation of Ig synthesis and secretion by human PBL, which may be used as a routine laboratory parameter in the evaluation of immunodeficiency diseases. Caution is urged, however, in quantitatively determining the amount of Ig in highly concentrated samples. Although the

TABLE IV

Other regulatory activities of PBL from patients with selective IgA deficiency

Subject	IgA	Inhibition	IgG	Inhibition	IgM	Inhibition
	ng	%	*ng*	%	*ng*	%
Normal I	832		1755		3818	
Patient H	0		191		271	
$P_H + N_I$	47	89	376	61	552	73
	(412)[a]		(973)		(2045)	
Normal J	747		1028		2191	
Patient C	121		312		0	
$P_C + N_J$	202	53	297	56	0	100
	(434)		(670)		(1096)	
Normal K	915		1172		6910	
Patient I	72		1121		4774	
$P_I + N_K$	1183	+139	1737	+51	8196	+40
	(494)		(1147)		(5842)	
Normal L	301		556		ND[b]	
Patient L	127		29		ND	
$P_L + N_L$	274	+28	349	+19	ND	
	(214)		(293)			
Normal M	327		341		423	
Patient D	0		657		224	
$P_D + N_M$	120	27	575	+15	257	21
	(164)		(499)		(324)	
Normal N	2572		2676		1387	
Patient F	0		1623		625	
$P_F + N_N$	1291	0	1853	14	1601	+59
	(1286)		(2150)		(1006)	

[a] Numbers in parenthesis refer to predicted values.
[b] ND, not determined.

standard curves were found to be linear through the recommended concentrations of reference Ig, concentrations greater than 3 times the highest standard, a range in antigen excess, deviated from linearity. Presently available techniques for investigating B lymphocyte functions are either semi-quantitative and subject to investigator interpretation or are costly and require specially prepared antisera and reagents not readily applicable to clinical use. We have recently demonstrated that a direct serologic precipitation assay (17) could be used in the diagnosis of immunodeficiency diseases (5). Furthermore, Polmar and Chase (28) have shown that a solid-state radioimmunoassay for the *in vitro* biosynthesis and secretion of Ig may be particularly applicable to the diagnosis of humoral immunodeficiencies during infancy, a time when serum IgG is primarily of maternal origin. However, both of these assays employ complex reagents that are expensive and not readily available from commercial sources.

As compared with the direct serologic precipitation method also used for studying biosynthesis and secretion of Ig by cultured human PBL (5, 17), the present fluorescent technique differs in two significant ways. First, the RIA utilized a polyvalent antisera directed against the $F(ab')_2$ fragment of Ig, whereas the immunofluorescent assay is specific for the heavy chain of either IgG, IgA, or IgM. Secondly, the RIA detects Ig synthesized and secreted after a 4-hr pulse with labeled amino acid whereas the fluorescent technique measures the total amount of Ig synthesized and secreted at the time of assay. Thus, both techniques are complementary to each other, yielding different data.

Although the two methods differ, they nevertheless yield comparable results with regard to modulatory effects on polyclonal B cell activation. As previously described (5), the failure of Ig-specific serologic reagents to react with culture supernatants from PWM-stimulated PBL from agammaglobulinemic patients is a good biologic control for serospecificity. The quantitative degree of regulatory activity as expressed by percent suppression (or enhancement) was usually comparable with either assay. Occasional discrepancies between the methods may be due to the selective suppression or enhancement of only one Ig class that might not be evident when compared to a technique such as the radioimmunoprecipitation assay, which does not discriminate between these classes.

The kinetics of Ig synthesis and secretion as determined by the immunofluorescent assay showed a continued increase in the total Ig of all classes over a 14-day period (Fig. 2). This occurred in spite of a steady loss of viable cells. Selection pressures may favor the viability of a few plasma cells that are known to secrete most of the extracellular Ig (29–31). Alternatively, plasma cells that have been shown to have extractable intracellular pools of Ig (32) may die and lyse, releasing their intracellular Ig to the supernatant.

Thus, a simple immunofluorescent assay was adapted for analyzing modulation of B cell differentiation *in vitro* and by means of this technique selective IgA deficiency was shown to be a heterogeneous syndrome with regard to mechanisms of pathogenesis. As demonstrated, Ig class-specific suppressor cells may be responsible for the pathogenesis of IgA deficiency in some patients and studies are under way to define the active regulatory cell populations by using surface marker techniques. Special attention is being focused on the role of T lymphocytes bearing surface receptors for the Fc portion of IgA, as previously described (33, 34). Suppressor cells are sensitive to a variety of agents including corticosteroids (1) and irradiation (35). We are examining whether treatment of cell cultures *in vitro* with these agents may restore, in certain cases, suppressed immunologic functions. These are the initial steps in the ultimate application of such techniques to correct immunologic deficiencies in affected patients.

REFERENCES

1. Waldmann, T. A., M. Durm, S. Broder, M. Blackman, R. M. Blaese, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. Lancet II:609.
2. Broder, S., R. Humphrey, M. Durm, M. Blackman, B. Meade, C. Goldman, W. Strober, and T. Waldmann. 1975. Impaired synthesis of polyclonal (nonparaprotein) immunoglobulins by circulating lymphocytes from patients with multiple myeloma. N. Engl. J. Med. 293:887.
3. Siegal, F. P., M. Siegal, and R. A. Good. 1976. Suppression of B cell differentiation by leukocytes from hypogammaglobulinemic patients. J. Clin. Invest. 58:109.
4. Witmeyer, S. B., A. D. Bankhurst, and R. C. Williams, Jr. 1976. Studies on the suppression of normal B cell maturation by peripheral blood cells from patients with acquired hypogammaglobulinemia and from normal neonates. Clin. Immunol. Immunopathol. 6: 312.
5. Schwartz, S. A., Y. S. Choi, L. Shou, and R. A. Good. 1977. Modulatory effects on immunoglobulin synthesis and secretion by lymphocytes from immunodeficient patients. J. Clin. Invest. 59: 1176.
6. Broom, B. C., E. G. DeLaConcha, A. D. B. Webster, G. J. Janossy, and G. L. Asherson. 1977. Intracellular immunoglobulin production *in vitro* by lymphocytes from patients with hypogammaglobulinemia and their effect on normal lymphocytes. Clin. Exp. Immunol. 23:73.
7. Litwin, S. D., and E. D. Zanjani. 1977. Lymphocytes suppressing both immunoglobulin production and erythroid differentiation in hypogammaglobulinemia. Nature 266:57.
8. Dosch, H.-M., M. E. Percy, and E. W. Gelfand. 1977. Functional differentiation of B lymphocytes in congenital agammaglobulinemia. I. Generation of hemolytic plaque-forming cells. J. Immunol. 119:1959.
9. Percy, M. E., H.-M. Dosch, and E. W. Gelfand. 1977. Functional differentiation of B lymphocytes in congenital agammaglobulinemia. II. Immunochemical analysis of the *in vitro* primary immune response. J. Immunol. 119:1965.
10. Jerne, N. K. 1976. The immune system: a web of V-Domains. The Harvey Lectures. Academic Press, New York. Pp. 93–110.
11. Bachmann, R. 1965. Studies on the serum αA-globulin level. III. The frequency of A-αA-globulinemia. Scand. J. Clin. Lab. Invest. 17:316.
12. Collins-Williams, C., H. L. Kokubu, C. Lamenza, R. Mizami, A. W. Chiu, C. Lewis-McKinley, T. A. Comerford, and E. A. Varga. 1972. Incidence of isolated deficiency of IgA in the serum of Canadian children. Ann. Allergy 30:11.
13. Horowitz, S., and R. Hong. 1975. Selective IgA deficiency—some perspectives. Natl. Found. Birth Defects, Orig. Art. Ser. XI (1):129.
14. Buckley, R. 1975. Clinical and immunologic features of selective IgA deficiency. Natl. Found. Birth Defects, Orig. Art XI (1):134–142.
15. Melchers, F., and J. Andersson. 1973. Synthesis, surface deposition and secretion of immunoglobulin M in bone marrow-derived lymphocytes before and after mitogenic stimulation. Transplant. Rev. 14:76.
16. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21(Suppl. 97):77.
17. Choi, Y. S. 1977. Serological precipitation method for studying biosynthesis and secretion of immunoglobulins by human peripheral blood lymphocytes. J. Immunol. Methods 14:37.
18. Burgett, M. W., S. J. Fairfield, and J. F. Monthony. 1977. A solid phase fluorescent immunoassay for the quantitation of the C4 component of human complement. J. Immunol. Methods 16:211.
19. Burgett, M. W., S. J. Fairfield, and J. F. Monthony. 1977. A solid

phase fluorescent immunoassay for the quantitation of the C3 component of human complement. Clin. Chim. Acta 78:277.
20. Waldmann, T. A., S. Broder, M. Durm, B. Meade, R. Krakauer, M. Blackman, and C. Goldman. 1976. T cell suppression of pokeweed mitogen induced immunoglobulin production. *In* Mitogens in Immunology. Edited by J. J. Oppenheim, and D. L. Rosenstreich. Academic Press, Inc., New York. Pp. 509–521.
21. Shou, L., S. A. Schwartz, and R. A. Good. 1976. Suppressor cell activity after Con A treatment of lymphocytes from normal donors. J. Exp. Med. 143:1100.
22. Schwartz, S. A., L. Shou, R. A. Good, and Y. S. Choi. 1977. Suppression of immunoglobulin synthesis and secretion by peripheral blood lymphocytes from normal donors. Proc. Natl. Acad. Sci. 74:2099.
23. Kishimoto, T., Y. Hirai, M. Suemura, and Y. Yamamura. 1976. Regulation of antibody response in different immunoglobulin classes. I. Selective suppression of anti-DNP antibody response by preadministration of DNP-coupled Mycobacterium. J. Immunol. 117:396.
24. Waldmann, T. A., S. Broder, R. Krakauer, M. Durm, B. Meade, and C. Goldman. 1977. Defect in IgA secretion and in IgA specific suppressor cells in patients with selective IgA deficiency. Trans. Assoc. Am. Physicians. 89:215.
25. Atwater, J. S., and T. B. Tomasi, Jr. 1978. Suppressor cells and IgA deficiency. Clin. Immunol. Immunopathol. 9:379.
26. Cassidy, J. T., G. Oldham, and T. A. E. Platts-Mills. 1979. Functional assessment of a B cell defect in patients with selective IgA deficiency. Clin. Exp. Immunol. 35:296.
27. Buckley, R. H., R. B. Gilbertson, R. I. Schiff, E. Ferreira, S. O. Sanal, and T. A. Waldmann. 1976. Heterogeneity of lymphocyte subpopulations in severe combined immunodeficiency. Evidence against a stem cell defect. J. Clin. Invest. 58:130.
28. Polmar, S. H., and P. A. Chase. 1975. Quantitation of the biosynthesis of immunoglobulin in peripheral blood lymphocytes of normal and immunodeficient patients. J. Pediatr. 87:545.
29. Andersson, J., L. Lafleur, and F. Melchers. 1974. IgM in bone marrow derived lymphocytes. Synthesis, surface deposition, turnover and carbohydrate composition in unstimulated mouse B cells. Eur. J. Immunol. 4:170.
30. Melchers, F., R. E. Cone, H. von Boehmer, and J. Sprent. 1975. Immunoglobulin turnover in B lymphocyte subpopulations. Eur. J. Immunol. 5:382.
31. Lifter, J., P. W. Kincade, and Y. S. Choi. 1976. Subpopulations of chicken B lymphcoytes. J. Immunol. 117:2220.
32. Choi, Y. S., W. D. Biggar, and R. A. Good. 1972. Biosynthesis and secretion of immunoglobulins by peripheral blood lymphocytes in severe hypogammaglobulinemia. Lancet I:1149.
33. Lum, L. G., A. V. Muchmore, D. Keren, J. Decker, I. Korki. W. Strober, and R. M. Blaese. 1979. A receptor for IgA on human T lymphocytes. J. Immunol. 122:65.
34. Gupta, S., C. D. Platsoucas, R. S. Schulof, and R. A. Good. 1979. Receptors for IgA on a subpopulation of human T and B lymphocytes. Cell. Immunol. 45:464.
35. Siegal, F. P., and M. Siegal. 1977. Enhancement by irradiated T cells of human plasma cell production: dissection of helper and suppressor functions *in vitro*. J. Immunol. 118:642.