Characterization of Two Populations of Human Lymphocytes Bearing Easily Detectable Surface Immunoglobulin

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ABSTRACT Two separate lymphocyte populations, each bearing easily detectable surface immunoglobulin, have been detected in human peripheral blood. The first, B cells, has surface determinants that are stable at 37°C, but are removed by pronase and regenerate in culture. The cells are nylon adherent and have a receptor for C3, and studies with unit gravity sedimentation indicate they are mostly small lymphocytes. B cells comprise 9.5% of the total lymphocytes, with the normal range from 3–16%.

As many or more lymphocytes lack membrane-incorporated Ig determinants but have an Fc receptor that binds IgG_1 and IgG_3 in normal serum maximally at 4°C. This receptor for cytophilic IgG is removed by pronase but not by trypsin. The second population has been named L lymphocytes because of membrane-*labile* IgG determinants. L cells do not adhere to nylon, do not form rosettes with sheep erythrocytes sensitized with antibody and mouse complement, and are larger than small lymphocytes. These lymphocytes with cold-reactive Fc receptors for serum IgG do not form E-rosettes or respond to phytohemagglutinin. Since L cells do not have surface markers of T and B lymphocytes, it is likely that they comprise a separate population.

INTRODUCTION

Human lymphocytes with easily detectable surface immunoglobulin can be divided into two populations by direct immunofluorescence: those with "membrane-stable" Ig determinants and an even larger number with "membrane-labile" IgG determinants. When lymphocytes were preincubated and washed at 37°C before staining, the number of IgG-bearing cells found was significantly less than when cells were prepared at 25° or 4°C (1). These studies suggested two separate populations of Ig-bearing lymphocytes: B cells and others with cold-reactive Fc receptors for cytophilic IgG.

In 1960 Boyden and Sorkin first observed that serum antibodies fixed to the surface of normal spleen cells without first combining with antigen, and created the term "cytophilic" antibodies (2). In 1966 Berken and Benacerraf found that Ig cytophilic for mouse macrophages was restricted to certain subclasses and that the binding site was for the Fc fragment (3). Subsequently others have reported the attachment of IgG to Fc receptors on human monocytes (4–6), neutrophils (6, 7), lymphocytes (6, 8), and other cells (9).

This report focuses on the newly described lymphocyte population with membrane-labile IgG-determinants. We will examine some properties of cytophilic IgG taken up by these cells. Second, we will examine the surface properties of lymphocytes with cold-reactive Fc receptors for IgG and compare them with T and B lymphocytes as defined by a recent World Health Organization Workshop. These criteria state that B lymphocytes should have "readily demonstrable surface IgG (Sm Ig) [1] . . . a product synthesized by the cell which carries it, and . . . distinguished from external IgG which has become attached to the membrane." T lymphocytes in contrast lack Sm Ig and most form rosettes with sheep erythrocytes (10).

METHODS

Subjects. Healthy adults, 22–55 yr old, were used in this study. One of the authors (P. L.) was found previously to have an increased number of immunoglobulin (Ig)-

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[1] *Abbreviations used in this paper:* EAC, sensitized erythrocytes; E-RFC, E-rosette-forming cells; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; L cell, lymphocyte with membrane-labile IgG, MEM, minimal essential medium; NHS, normal human serum; PBL, peripheral blood lymphocyte; PHA, phytohemagglutinin; Sm Ig, membrane-stable Ig; SRBC, sheep red blood cells; TRITC, trimethylrhodamine-conjugated.

bearing lymphocytes (1), and his cells were again used in some of these experiments. This individual was of Indian origin, had lived in East Africa for 25 yr, and had malaria as a child.

Reagents. The following sera or serum fractions were used: autologous or pooled AB human serum; human IgG (Cohn fraction II, ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio); purified human IgM and IgA kindly supplied by Dr. W. F. Hymes (Meloy Laboratories Inc., Springfield, Va.); and guinea pig IgG (Cappel Laboratories, Inc., Downington, Pa.). Antisera used were: fluorescein-conjugated (FITC) polyvalent goat anti-human immunoglobulins (IgG, IgM, IgA), heavy chain-specific (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.), and trimethylrhodamine-conjugated (TRITC) IgG fraction of rabbit anti-guinea pig IgG (gamma-chain specific) from Cappel Laboratories. FITC-conjugated antisera specific for human IgG subclasses (IgG_1, IgG_2, IgG_3, and IgG_4) was obtained as a gift from Dr. Peter Schur, Robert B. Brigham Hospital, Boston, Mass. Sodium Hypaque was obtained from Winthrop Laboratories, Div. of Sterling Drug, Inc., New York, courtesy of Dr. F. Nachod. Enzyme preparations used were trypsin (type XI), 7,500 *N*-benzoyl-L-arginine ethyl ester (BAEE) U/mg powder (Sigma Chemical Co., St. Louis, Mo.) and grade V, 3,000 BAEE U/mg (Miles Laboratories, Inc., Miles Research Div., Elkhart, Ind.). Pronase (type IV) and deoxyribonuclease (type 1) were obtained from Sigma Chemical Co.

For the studies to determine the C3 receptor, 19S rabbit antibody to sheep red blood cells and lyophilized guinea pig complement were obtained from the Cordis Laboratories, Miami, Fla.

Lymphocyte separation procedure. Lymphocytes were obtained by density gradient centrifugation of heparinized blood as described previously (1) (yield 60–85%). To remove adsorbed serum proteins, the isolated cells were routinely incubated at 37°C for 30 min and washed twice (centrifugation 200 *g* for 10 min) in warm minimal essential medium (MEM, Grand Island Biological Co., Grand Island, N. Y.). The cells were divided into three portions: two were used for membrane immunofluorescence and one for quantitation of E-rosette-forming lymphocytes (E-RFC).

Membrane immunofluorescence. Procedures for immunofluorescent staining have been described previously (1, 11). In brief, polyvalent FITC antiserum to human immunoglobulins was added to one portion of cells routinely pretreated at 37°C to remove external surface protein, and incubated at 4°C for 30 min. The cells were washed at 4°C, and the cell suspension was kept on ice until read. Ig-positive cells observed by this procedure were considered to have membrane-stable Ig determinants (Sm Ig). Normal human serum (NHS) or IgG was added to a second portion of cells and incubated at 4°C for 30 min. These cells were washed twice with cold medium and then stained at 4°C. This procedure yielded the maximum number of Ig-positive cells. Lymphocytes with temperature-labile Ig determinants were calculated by subtracting cells with Sm Ig from the maximum number observed. All sera used were examined for antilymphocyte antibodies with a sensitive two-step microcytotoxicity procedure (12) and none were detected. All sera were centrifuged at 140,000 *g* for 2 h to remove large aggregates. Conjugated antisera were diluted 1:5 and the top third of the centrifuged material was used to stain lymphocytes.

Lymphocytes counted had scanty cytoplasm and a diameter no more than two thirds that of typical monocytes, which were easily recognized by phase contrast microscopy. In freshly prepared suspensions, cells defined by these criteria did not phagocytose latex particles and were considered to be small or medium lymphocytes. Mononuclear cell preparations studied contained 18% (range 10–26%) monocytes that were excluded from cell counts.

Preparations of aggregated and monomeric IgG. Aggregated and monomeric human IgG used in immunofluorescence studies were prepared as follows: 30 mg of human gamma globulin (Cohn fraction II) was dissolved in 1 ml of phosphate-buffered saline, pH 7.3, and heated to 62°C for 15 min. An equal amount of unheated IgG was added to this and the mixture was chromatographed on Bio-gel A-5m agarose (Bio-Rad Laboratories, Richmond, Cal.). Fractions obtained from the first peak and descending portion of the second peak were selected for study. Fractions were examined for aggregates by complement consumption studies (13).

Separation of lymphocyte populations with nylon-fiber columns. Lymphocytes were separated into adherent and nonadherent populations with the procedure of Trizio and Cudkowicz (14). Briefly, a 12-ml plastic syringe was packed with 0.6 g of nylon fibers (E. I. Dupont de Nemours & Co., Inc., Wilmington, Del.). After the columns were rinsed with medium containing 5% fetal calf serum and warmed to 37°C, 10^8 mononuclear cells in warm medium containing heat-inactivated fetal calf serum or autologous serum were applied to the columns. The syringe was further incubated at 37°C for 45 min. Nonadherent cells were eluted with warm MEM (yield 50%). Adherent cells were obtained by compressing the nylon with sterile forceps and elution with warm MEM-containing serum (yield 18%, total yield 68%).

Quantitation of E-rosette and EAC RFC. The procedure used to detect E-RFC was described previously (1). Tubes containing a sheep red blood cell (SRBC)-to-lymphocyte ratio of 50 to 1 were read after incubation overnight. Lymphocytes with three or more adherent SRBC were considered positive.

The method of Shevach et al. (15) was used to quantitate lymphocytes with the receptor for C3. Sheep erythrocytes were sensitized with an optimal concentration of rabbit IgM antibody to SRBC (usually 1:2,000). After exposure to fresh mouse serum, the sensitized erythrocytes (EAC) were mixed with lymphocytes in a ratio of 25 to 1 and incubated at 37°C for 30 min. Wet mounts with cover slips compressed tightly were examined by phase contrast optics with a 100 × objective, and three or more EAC adhering to a lymphocyte were considered positive. Mononuclear cells in these studies had been exposed to latex particles, and all phagocytic cells were excluded from counts.

Exposure of lymphocytes to proteolytic enzymes. Lymphocytes were suspended in Hank's balance salt solution (HBSS) containing 0.1% pronase and incubated at 37° for 30 min. After three washes, they were examined for Sm Ig and Fc receptors. Other portions of washed, pronase-treated cells were incubated overnight in MEM-containing fetal calf serum, and then examined to detect regeneration of surface determinants. Other cells were treated with trypsin (0.025–0.25%) in HBSS and examined for surface markers.

Response of lymphocyte populations to mitogens. T cells were separated from Ig-bearing populations by density centrifugation. E-RFC were prepared with procedures described above, and the suspension was layered on Ficoll-Hypaque and centrifuged at 500 *g*. Lymphocytes harvested in the interface were again mixed with sheep erythrocytes and

the procedure was repeated to deplete further the T cells in this fraction. SRBC in the pellet were removed by hypotonic lysis. 7×10^6 lymphocytes from partially purified T cells (the pellet) and Ig-bearing cells (the interface) were stimulated with phytohemagglutinin M (PHA) (2.5 μl/cm^3) (Difco Laboratories, Detroit, Mich.), by methods described previously (16). [^{3}H]thymidine incorporation was measured after a 3-day culture period.

Fractionation of lymphocyte populations by velocity sedimentation at unit gravity. Lymphocytes were separated primarily on the basis of size with a unit gravity velocity sedimentation apparatus obtained from Johns Scientific, Toronto, Canada. The procedure of Miller and Phillips (17) was followed. 10^8 mononuclear cells were loaded into the small chamber (SP-180) and the cells were allowed to sediment for 4 h in 100 ml of nonlinear 0.3% bovine serum albumin-phosphate-buffered saline gradient at 24°C. 15-ml fractions were collected through the bottom at this time. Cells in each fraction were washed three times, counted, and tested for surface markers.

RESULTS

Characterization of a cold-reactive Fc surface receptor for IgG. The first series of experiments confirmed and extended previous observations indicating two populations of Ig-bearing lymphocytes. The mean number of lymphocytes with Sm Ig from 50 normal donors was 9.5% (range, 3-16%, ±2 SD). An additional 5–33% of lymphocytes had Fc receptors. Fig. 1 indicates a bimodal frequency distribution with one peak at 10–15% lymphocytes and another at 20–25%. All 50 subjects studied worked in the medical center. In general, those with high values were hospital personnel and those with low values clerical personnel. For convenience, lymphocytes with membrane-stable determinants will be defined as B cells and those with membrane-*labile* IgG as L cells. A correlation between these two cell populations was not observed, ($r = 0.07$).

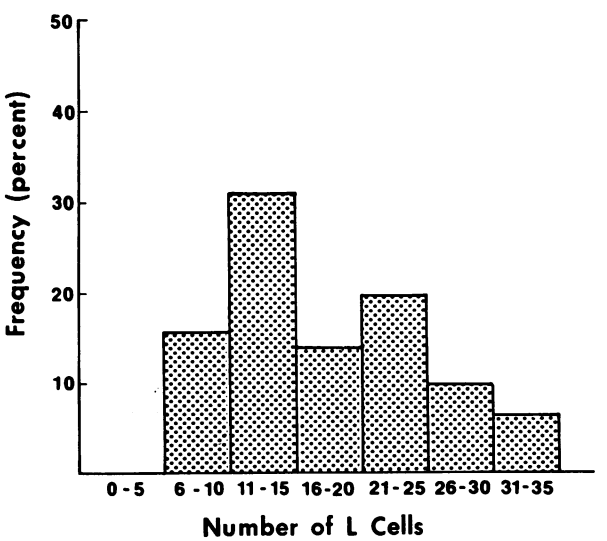

FIGURE 1 Frequency distribution of lymphocytes with membrane-labile IgG determinants (L cells) in the blood of 50 normal donors. All subjects had normal numbers of B cells (mean 9.5%, range 3–16%).

Table I summarizes studies from five normal lymphocyte donors. Lymphocytes were first incubated and washed at 37°C to remove surface-labile serum proteins and were stained with a polyvalent conjugate. All subjects had normal numbers of lymphocytes with membrane-stable Ig determinants (6-15%) (step 1, Table I). When separate aliquots were incubated in fresh or heat-inactivated NHS at 4°C, an additional 9-31% became Ig-positive (step 2, Table I). As before (1), studies with class-specific antisera indicated binding of serum IgG. The subject (R. G.) with the 31% increase in cells with the cold-reactive receptor for IgG was a 28-yr-old white male nephrologist-in-training who had been in close contact with severely ill patients.

Examination of lymphocytes after staining at 4°C with anti-IgG indicated a uniform punctate staining pattern. When the cells were warmed to 37°C for 5-10 min, however, most of the membrane fluorescence was observed at one pole of the cell. After 30 min in the warm, the number of IgG-positive cells dropped sharply (step 3*a*, Table I). This decrease was not prevented by sodium azide. Sodium azide, however, blocked the elution of membrane-bound IgG-anti-IgG complexes at 37°C. This agent is an inhibitor of aerobic energy production and blocks the capping and shedding, or endocytosis, of ligand-receptor complexes (18). The sharp decrease in IgG-positive cells at 37°C in medium containing azide cannot then be explained by shedding. A more likely explanation is that the binding of IgG to the surface is much stronger in the cold, a known characteristic of cytophilic Ig (2).

To characterize the cold-reactive receptor for IgG further, the following studies were first conducted with lymphocytes from P. L., a subject previously found to have abnormally high numbers of these cells (1). Since the values obtained might not be representative of the normal population, all studies with P. L.'s cells were repeated with lymphocytes from other subjects with normal values of Ig-bearing cells. The results, however, were similar in all studies.

Lymphocytes were routinely pretreated at 37°C, and various concentrations of purified IgG, IgM, and IgA were added to the cell suspensions. The tubes were incubated at 4°C for 30 min and washed, and the cells stained at 4°C with class-specific antisera. Neither IgM nor IgA fixed to the cell membrane (Table II). The cold-reactive receptor specifically bound IgG. The binding threshold of IgG was 10 μg/ml, with a plateau effect noted at higher concentrations. Guinea pig IgG also bound to the receptor, although the threshold concentration was 10-fold higher than for human IgG. Heating

TABLE I

The Effects of Temperature and Sodium Azide on Lymphocytes Bearing Easily Detectable Surface Immunoglobulin

Step number		Procedure	Azide Present	Azide Absent
			% stained with FITC anti-human Ig	
1	Routine pretreatment	Incubate cells in media at 37°C for 30 min and wash twice with warm media	9.8 (6–15)	9.3 (6–15)
2	Binding of serum IgG at 4°C	Step 1 followed by adding NHS at 4°C for 30 min and washing twice at 4°C	29.8 (16–43)	30.5 (15–44)
3a	Elution of serum IgG at 37°C	Step 2 followed by a second incubation at 37°C for 30 min and washing twice with warm medium	11.0 (7–14)	10.1 (6–17)
3b	Binding of IgG antiIgG complexes at 37°C	Step 2 followed by adding FITC anti-IgG at 4°C for 30 min, washing twice with cold medium, a second incubation at 37°C for 30 min and finally washing twice with warm media	30.6 (17–48)	11.0 (6–20)

Values indicate the mean and range of six experiments with cells from five normal donors. Parallel studies were performed in tubes containing 0.02% Na azide and others without azide. Each procedure was conducted with a separate sample of cells. All cells were stained at 4°C.

IgG to 100°C for 30 min destroyed its binding properties. The addition of 0.02M EDTA did not affect the binding of IgG.

Aggregated but not monomeric preparations of IgG attached to the lymphocyte surface (Tables II and III). IgG was fractionated by agarose chromatography. Monomeric IgG isolated in the descending portion of the second peak did not fix complement, and did not bind to lymphocytes (Table III). Monomeric IgG, however, was not stable. When this material was kept at 37°C or 4°C for 90 min, spontaneous aggregation was detected by complement consumption studies and this material now bound to the lymphocyte surface.

Effect of proteolytic enzymes on membrane-Ig determinants. Treatment of lymphocytes with pronase removed Sm Ig (Table IV). When enzyme-treated cells were examined after overnight incubation at 37°C, the surface membrane-stable determinants had regenerated. Treatment of lymphocytes with pronase also resulted in the removal of the cold-reactive Fc receptor, and this receptor also regenerated in culture. When enzyme-treated cells were cultured for 18 h, and exposed to NHS at 4°C, the number of Ig-positive cells with membrane-labile IgG determinants returned to base-line values (Table IV).

In contrast to pronase, various concentrations of trypsin had no detectable effect on Fc receptors. Nor did trypsin significantly reduce the number of lymphocytes with Sm Ig detected by a polyvalent conjugate (Table IV).

TABLE II

Specificity of the Lymphocyte Cold-Reactive Fc Receptor for IgG

Immunoglobulin added	Concentration of IgG added (μg/ml) 0	1	10	100	1,000	Conjugated antiserum used
	% stained					
Human						
Monomeric IgG	5	—	—	—	5	FITC anti-human IgG
IgG	5	5	39	39	39	FITC anti-human IgG
IgM	10	—	—	—	10	FITC anti-human IgM
IgA	2	—	—	—	2	FITC anti-human IgA
Guinea pig						
IgG	0	0	0	29	30	TRITC anti-guinea pig IgG*

* This antiserum was absorbed with human lymphocytes and cross-reacting antibody to human IgG had been removed by passage of material through a solid-phase immunoabsorbent containing human IgG. Source of lymphocytes was subject P. L.

TABLE III

Binding of Heat-Aggregated, Monomeric, and Spontaneous Aggregates of IgG to Lymphocytes

Material tested	IgG-positive lymphocytes	Hemolytic complement consumption
	%	%
Medium	12	Nil
Peak I (heat-aggregated)	26	100
Peak II (monomeric IgG)		
Used immediately	15	Nil
Used after incubation at 37°C for 90 min	26	31
Used after incubation at 4°C for 90 min	23	21

Human Ig was fractionated on agarose A-5m, and 100 μg from peak I and the descending portion of peak II was incubated with lymphocytes or added to 0.2 ml of fresh serum containing 40 U of hemolytic complement (13).

TABLE IV

Percent Small Lymphocytes with Membrane Ig Determinants after Treatment with Pronase or Trypsin

	Subject P. L.		Subject P. G.	
	Sm Ig*	Fc receptor†	Sm Ig	Fc receptor
	%			
Untreated	24	44	12	12
Pronase-treated				
Before culture	1	0	0	0
After 37°C for 18 h	26	44	10	12
Trypsin-treated (before culture)	22	40	12	12

Cells were stained with a polyvalent FITC-antihuman Ig.

* After routine pretreatment at 37°C.

† Other cells were pretreated at 37°C. Then NHS was added and the tubes were incubated at 4°C for 30 min. The cells were washed and stained at 4°C. Values for Fc cells were calculated by subtracting cells with Sm-Ig from the total number of Ig-positive cells observed after treatment with NHS at 4°C.

Separation of Ig-bearing lymphocyte populations by nylon-fiber columns. 10 healthy subjects with normal numbers of B lymphocytes and subject P. L. were selected for these studies. Passage of mononuclear cells through a nylon-fiber column separated Ig-bearing lymphocytes into two populations. B lymphocytes were markedly depleted in column eluates. These cells adhered to nylon fibers. In contrast, most L cells did not adhere to nylon and were recovered in the eluate (Table V). These cells passed through the column whether suspended in autologous or fetal calf serum. T lymphocytes were also found in the eluate.

To characterize further the two populations of Ig-bearing lymphocytes, we quantitated lymphocytes forming EAC rosettes, thereby detecting receptors for C3b and C3d (36). The nylon column filtration studies indicated that adherent cells with Sm Ig also formed EAC rosettes but nonadherent cells with Fc receptors did not form EAC rosettes. 94% of cells forming EAC rosettes also had Sm Ig. These results clearly differentiate B cells from L cells.

Separation of lymphocytes with Fc receptors from T lymphocytes. Since both T lymphocytes and L lymphocytes were nonadherent cells (Tables V) the possibility that some T cells had the cold-reactive receptors was considered. Lymphocytes from five donors were incubated at 4°C with NHS, washed, and then mixed with SRBC for 18 h at 4°C. Only 2.7% of E-RFC from these donors also had detectable surface immunoglobulin. To exclude the possibility that the T cell receptor for SRBC was hidden by cytophilic IgG, lymphocytes were depleted of E-RFC and stimulated with PHA. Table VI indicates that purified Ig-bearing lymphocytes did not respond to PHA. In contrast, E-RFC-rich fractions responded strongly to PHA. The absence of a response by Ig-bearing lymphocytes to PHA, a mitogen that stimulates primarily T cells (20), provides additional evidence that most lymphocytes with Fc receptors are not T cells.

Fractionation of lymphocytes populations by unit-gravity velocity sedimentation. Mononuclear leukocytes

TABLE V

Effect of Nylon Column Filtration on Various Lymphocyte Subpopulations

	B Cells			L Cells			EAC-RFC			E-RFC		
Subject	Unsep*	Ad	Nonad	Unsep	Ad	Nonad	Unsep	Ad	Nonad	Unsep	Ad	Nonad
	%									%		
E. H.	6	60	2	6	6	15	5	38	3	84	31	63
M. L.	8	90	3	6	1	13	9	52	3	84	15	89
S. T.	10	73	2	12	5	17	6	27	1	67	23	80
K. J.	12	83	3	22	2	28		ND			ND	
L. B.	13	82	3	23	10	40		ND			ND	
P. C.	13	65	1	13	6	19	12	62	1	73	21	70
R. C.	14	86	1	5	4	10	17	56	1	71	8	84
S. G.	14	80	1	7	10	11	18	65	2	77	7	65
D. H.	15	80	4	10	3	28	15	57	4	69	3	55
M. J.	16	73	3	20	ND	23	17	55	1	66	10	76
‡P. L.	25	79	2	34	9	36	23	58	4	45	ND	56
Mean	12.2	77.2	2.2	12.4	5.6	20.4	12.3	51.5	2.0	73.8	14.7	70.8

* Abbreviations: Unsep, unseparated; Ad, adherent; Nonad, nonadherent; ND, not done.

‡ Omitted from calculations of mean value because of abnormally high numbers of Ig-bearing lymphocytes.

TABLE VI

Fractionation of Lymphocyte Subpopulations by Density Centrifugation of E-RFC

	Positive cells			[³H]Thymidine incorporation	
	Sm-Ig	Fc	E-RFC	Unstimulated	PHA
	%			*cpm*	
Cells from subject P. L.*					
Before fractionation	18	35	47	195	23,256
After fractionation					
Pellet	5	23	76	468	26,603
Interface	36	58	2	ND	345
Cells from subject K. J.					
Before fractionation	11	20	65	627	15,472
After fractionation					
Pellet	1	3	95	414	14,621
Interface	36	60	5	ND	2,207

* Values indicate the mean of two separate experiments.

were fractionated by unit-gravity sedimentation, a procedure that separate cells chiefly according to size (17). Fig. 2 indicates the sedimentation profile of cells from donor P. L., a subject known to have increased numbers of both types of Ig-bearing cells. Lymphocytes with Fc receptors sedimented before E-RFC. Secondly, the sedimentation profile of lymphocytes with Sm Ig did not correlate with cells that had cold-reactive receptors for IgG. The sedimentation profile of lymphocytes from seven other subjects with normal numbers of Ig-bearing lymphocytes also indicated that cells with Fc receptors are larger than those with Sm Ig or E-RFC.

DISCUSSION

These studies provide further evidence for two populations of human peripheral blood lymphocytes (PBL) bearing easily detectable surface Ig: B cells, and an even more numerous population that binds cytophilic IgG in NHS. Until recently, it was not appreciated that a significant number of human lymphocytes will passively take up serum IgG at 4°C (1). Investigators using immunofluorescent techniques to quantitate Ig-bearing lymphocytes inadvertantly scored these as typical B cells. We reported that 10% of PBL had Sm Ig. Another 20% lacked Sm Ig, but had Fc receptors. The remaining 70% were T cells; that is, they formed rosettes with SRBC (1). The present experiments have established that lymphocytes with membrane-stable determinants were B cells. Studies with pronase indicated that these determinants were synthesized by the lymphocytes and secreted into the membrane.

In this study of 50 normal donors we also confirmed our previous value for B cells and found these values were normally distributed. In contrast, we found a bimodal distribution of lymphocytes with membrane-labile IgG determinant (L cells). Medical students, physicians, and laboratory personnel often had twice as many L cells as clerical personnel. Furthermore, there was no correlation between numbers of B cells and L cells in the groups studied. Further studies are in order to pursue this intriguing finding.

We next examined the properties of IgG bound by L cells. Although it is well known that heat-aggregated IgG binds to a receptor for the Fc fragment (8, 22), the physical properties of cytophilic IgG are less well understood. It is not clear whether cytophilic IgG in NHS is monomeric or spontaneously forms aggregates. Lawrence et al. demonstrated with a highly sensitive autoradiographic method that deaggregated IgG_1 and IgG_3 myeloma proteins did attach to human PBL, whereas IgG_2 and IgG_4 attached only in the heat-aggregated state (6). Using a less sensitive immunofluorescent method, we observed that IgG_1 and IgG_3 in NHS were the only subclasses that bound. Secondly, we confirmed the findings of others (21) who reported that column-purified mono-

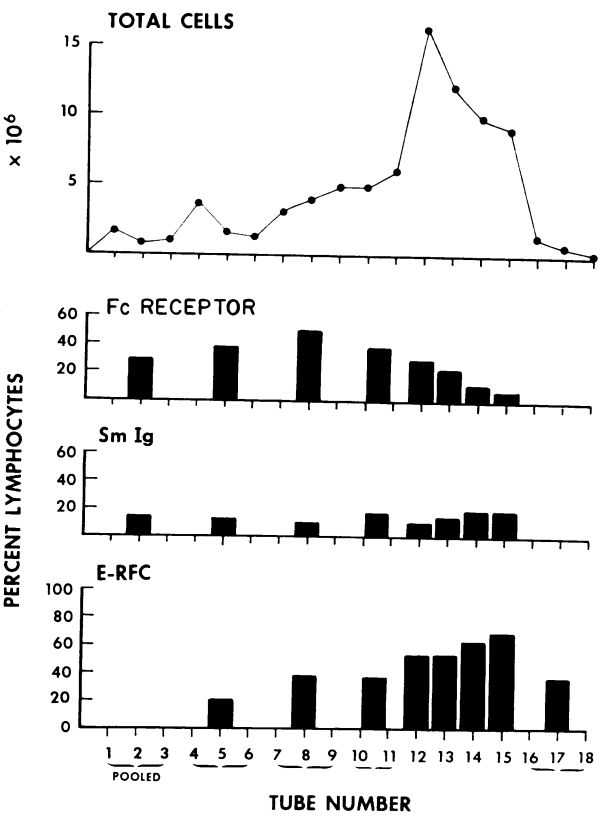

FIGURE 2 Fractionation of lymphocyte subpopulations by unit-gravity velocity sedimentation. Lymphocyte donor was subject P. L. Monocytes were found in tubes 1 through 6 and rarely thereafter.

meric IgG did not bind. We did observe, however, that these monomers were highly unstable and rapidly formed spontaneous aggregates that now bound. These studies suggest, though they do not prove, that similar spontaneous aggregates of IgG_1 and IgG_3 form in serum and are passively fixed to the lymphocyte surface membrane. This suggestion is supported by Capra and Kunkel, who reported that IgG_3 in human serum forms spontaneous aggregates (23).

Our next series of experiments indicated that the cold-reactive receptor is probably the Fc receptor for aggregated IgG. It has the following properties of the Fc receptor: (*a*) IgG was the only immunoglobulin that attached to the receptor (21, 22). (*b*) The observed low binding threshold of IgG and the plateau effect found with increasing concentrations were consistent with previous reports (21, 8). (*c*) The bond between IgG and the surface membrane was stabilized by the formation of an IgG-antiIgG complex (24). (*d*) With the formation of an IgG-anti-IgG complex on the cell membrane, capping occurred after incubation at 37°C (22). (*e*) The receptor was resistant to trypsin (22, 25), but was removed by pronase (22, 26).

Although IgG_1 and IgG_3 can also attach to the lymphocytes' surface by a receptor for activated C3, cells with membrane-labile IgG determinants did not have this receptor. After treatment with trypsin, a proteolytic enzyme known to remove the C3 receptor (27, 28), the number of cells binding IgG did not decrease. In addition, they did not form rosettes with SRBC coated with activated C3.

The relationship of L cells to T cells and B cells is not clear. Although several groups have reported that activated T lymphocytes develop an Fc receptor for IgG (29-31), most T cells in the blood of healthy subjects do not have cold-reactive receptors for IgG. Firstly, less than 3% of E-RFC had Ig determinants. Secondly, Ig-bearing cells depleted of E-RFC did not respond to PHA. Thirdly, the sedimentation profile of E-RFC differed from that of L cells.

L cells differed from B cells in surface characteristics and size. Firstly, lymphocytes with Sm Ig adhered to nylon fibers, whereas others with membrane-labile Ig determinants were not adherent cells. Other investigators have also reported that B cells adhered to nylon fibers (32-35). Dickler and Kunkel (8) and Dickler (22), however, used nylon columns to isolate B cells and reported that these were not depleted. It is likely that the isolation procedure used by these workers selectively depleted lymphocytes with Sm Ig (B cells) and both L cells and T cells were left in the column eluate. Other subtle methodological differences could perhaps explain these discrepant results. Secondly, we found that only B cells had a receptor for activated C3 detectable with an opsonic adherence method. Others have noted that the number of PBL forming EAC-rosettes is significantly less than the total number of IgG-bearing cells (19, 36). The observation that the population of lymphocytes that bind cytophilic IgG does not form EAC-rosettes may account for this finding. Thirdly, separation of PBL by unit-gravity sedimentation indicated that L cells were larger than B cells. These results clearly differentiate L cells from B cells.

It is possible that lymphocytes with Fc receptors for cytophilic IgG in NHS are neither T nor B cells. Frøland and Natvig have also postulated a third population of lymphocytes using different methods. These workers described "lymphocyte-like" cells with Fc receptors that formed rosettes with human erythrocytes coated with an IgG Rh antibody (EA), and found that EA rosette-forming cells were clearly separate from E-RFC and cells with Sm Ig (33). These investigators did not find Fc receptors on B cells (37), an observation that conflicts with other reports (8, 22). Since lymphocytes binding cytophilic IgG have previously been confused with B cells, further studies on carefully separated Ig-bearing populations are needed to resolve this issue. The possibility that the population with Fc receptors only is a subclass of B lymphocytes has not been excluded. Several investigators have described subjects with various disorders who have numerous lymphocytes with Fc receptors, but not Sm Ig (8, 33, 38).

Greenberg and co-workers have described large mononuclear mouse cells with a receptor for the Fc fragment of IgG, and suggested that these cells are nonphagocytic monocytes, because the two cell types have similar size, adherence properties, and binding avidity for immunoglobulin subclasses (39). The lymphocytes with membrane-labile Ig determinants described in the present experiments share certain characteristics of the mouse cells described by Greenberg et al. Both cell types are larger than most lymphocytes and lack T and B cell markers. The essential difference is that our human cells were nonadherent, whereas mouse cells were adherent. This may be a species-specific characteristic of cells with similar function, since previous studies from this laboratory indicated that human promonocytes were nonadherent (40). A second possible difference is that monocytes have a receptor for C3, whereas the cells with membrane-labile Ig determinants do not have this receptor. Greenberg did not examine cells for this receptor.

A function of L cells can be suggested. The mononuclear cells with Fc receptors described by the Norwegian (33, 41, 42) and British investigators (39) are effector cells in antibody-mediated lymphocyte cytotoxicity. It is likely that the lymphocytes described in the present report are similar effector cells.

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ADDENDUM

Since this manuscript was submitted, R. J. Winchester, S. M. Fu, T. Hoffman, and H. G. Kunkel have independently described a lymphocyte population with characteristics identical to L cells. These workers used pepsin-digested $F(ab')_2$ rhodamine-conjugated antisera to quantitate B cells, and found a special population of cells with Fc receptors but without membrane-incorporated Ig. (*J. Immunol.* **114**: 1210–1212, 1975).

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