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Research Article

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ABSTRACT B- and T-cell populations in 32 patients with different forms of primary immunodeficiency disease were studied. The B-cells in peripheral blood were investigated with respect to surface immunoglobulins by means of immunofluorescence. The T-cell function was studied utilizing quantitation of proliferative response to phytochemagglutinin (PHA)[1] and delayed allergy to various antigens. In 10 patients lymph node lymphocytes were also evaluated. 11 male children with infantile x-linked agammaglobulinemia were divided into two subgroups. One did not show immunoglobulin spots on peripheral blood lymphocytes at all, the other contained a very low percentage of IgM- and occasionally IgA-bearing lymphocytes. Eight patients with common variable immunodeficiency had moderately decreased percentages of peripheral blood and lymph node lymphocytes with surface immunoglobulins, but these patients lacked immunoglobulin secreting cells. Four cases of isolated IgA deficiency had normal or high percentages, and two cases of ataxia-telangiectasia had high percentages of lymphocytes with IgA in so called receptor distribution in both peripheral blood and lymph nodes. In three patients with infantile combined immunodeficiency that had been corrected by marrow transplantation, the percentages of Ig-bearing lymphocytes increased to normal or high levels together with establishment of functional T-cell population and ultimate secretion of serum immunoglobulins. One case of Di George syndrome reconstituted by fetal thymus transplant showed gradual decrease of B lymphocytes in circulation parallel to restoration of T-cell population.

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[1] *Abbreviations used in this paper:* DNCB, dinitrochorbenzene; FCS, fetal calf serum; MEM, Dulbecco's Modified Eagle Medium; PBS, phosphate-buffered solution; PHA, phytochemagglutinin.

INTRODUCTION

The demonstration of plasma membrane-associated immunoglobulins on the B lymphocytes[2] provides an excellent method for the differentiation of this population of cells, both in humans and in other species (1–3). Recent investigations indicate that the immunoglobulin in the so-called receptor distribution are present on B lymphocytes only, at least in amounts detectable by immunofluorescence, and that they bind antigen in immune stimulation and subsequent humoral response (4–10). When B lymphocytes mature into immunoglobulin-secreting plasma cells, the number of the surface-associated immunoglobulin receptors markedly decrease (11, 12). Although the immunohistochemical composition of receptors seems to be identical with that of secreted immunoglobulins, evidence now indicates that the surface immunoglobulin produced, but not secreted by B-cells, differs in certain chemical characteristics from the secreted immunoglobulin. The heavy chain of the surface immunoglobulin is smaller in size and lacks galactose residues (13, 14). Further, there is already additional evidence that the production of secretory and plasma membrane-associated immunoglobulins may be separately controlled by the cells (15).

B lymphocytes in primary immunodeficiency disease in man have been studied by Siegal, Pernis, and Kunkel (16); Grey, Rabellino, and Pirofsky (1); Cooper, Lawton, and Bockman (17); Preud'homme and Seligmann (18), and Papamichail, Brown, and Holborow (19). Altogether 34 cases of primary immunodeficiency have been reported in which the number of B-cells has been estimated. The largest group comprises the 14 cases presented by Siegal et al. (16). Only a few of these cases have been evaluated for cellular as well as for humoral immunity. Most recently Lawton, Royal, Self, and

[2] B lymphocytes, bursa equivalent or bone-marrow derived; T lymphocytes, thymus-dependent of thymus processed.

Cooper (20) reported the study of IgA determinants in 13 patients with deficiency of circulating IgA.

We have been able to study 32 cases of primary immunodeficiency disease and to evaluate both B- and T-cell populations in the blood of these patients. Quantitation of the T-cell population utilized the method introduced by Park and Good (21). It involves short term blood cultures in the presence of PHA, and reflects the number of circulating T-cells. Moreover, in 10 of 32 cases, the lymph nodes were investigated for B-cells with membrane-bound immunoglobulins and B-cells containing stainable cytoplasmic immunoglobulins (plasma cells and their immediate precursors). We believe that our study of this large series of patients helps to explain some of the controversial opinions concerning B-cell populations and the nature of the immunologic defects in patients with different forms of primary immunodeficiency disease.

METHODS

Antisera. The antisera against major heavy-chain classes, γ and α, were produced in goats by immunization with normal IgG [Red Cross purified by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography] and IgA (as described by Litman and Good) (22). The antisera were rendered monospecific by affinity chromatography (23). After immunoadsorption, the IgG fractions of goat antisera prepared by DEAE-cellulose chromatography were conjugated with fluorescein isothiocyanate as described by Cebra and Goldstein (24) and further fractionated on DEAE-cellulose. The antiserum against human μ-heavy chain was purchased from Nordic Pharmaceuticals and Diagnostics, Tilburg, Holland (lot no. 7-171).

All antisera were tested for specificity by immunoelectrophoresis, gel double diffusion, and absorption with appropriate antigens. They were absorbed further with Fab'_2, if necessary. The fluorescein protein ratios of the reagents used varied between 2:1 and 3:1. The labeled antisera were used in direct staining at the maximal dilution which gave effective staining (protein concentration 1.5–3 mg/ml).

Lymphocyte preparation. Peripheral blood lymphocytes were obtained by centrifugation on a Ficoll-Triosil gradient (25) (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), using Hypaque (sodium diatrizoate, Winthrop Laboratories, New York) instead of Triosil. The preparations obtained contained 85–95% lymphocytes that showed 97–100% viability when tested by Trypan blue exclusion. The cell suspensions of lymph nodes were made by gently mincing tissue in culture Dulbecco's Modified Eagle Medium [(MEM) with 10% fetal calf serum (FCS)] and aspiration with the syringe with diminishing gauge needles. The viability of cells was 95–98% as tested by Trypan blue exclusion, and the suspensions contained 80% or more small lymphocytes. Both peripheral blood and lymph node lymphocytes were washed three times in phosphate-buffered solution (PBS) with 5% bovine serum albumin (BSA), or PBS with 10% FCS in room temperature.

Immunofluorescent staining. The $1.5–2 \times 10^6$ cells in 0.1 ml of PBS with 5% BSA (or PBS with 10% FCS) were added to 0.1–0.15 ml of fluoresceinated antiserum, and the staining was carried out in the presence of 0.01% sodium azide at ice-bath temperature for 30–45 min. The cells were washed four times with PBS-BSA and 0.01% sodium azide in the cold, and examined immediately in suspensions under the cover glass sealed with nail polish. For the microscopy, a Zeiss ultraviolet microscope (Carl Zeiss, Inc., New York) equipped with an Osram (Germany) HBO 200 mercury lamp and an interference primary filter FITC (Carl Zeiss, Inc.) was used (transmitted light). Examination of each field was done after removing the primary filter. 500 lymphocytes were examined on every slide. A second sample of stained preparation was made into a permanent preparation using the cytocentrifuge (Shandon/Elliott, London, England), according to the method described by Pernis, Forni, and Amante (2). Cells stained with unrelated conjugated antisera (e.g. antihuman fibrin, antirabbit IgG), and stained with antisera absorbed with corresponding and different human heavy chains served as control.

Inguinal lymph nodes were removed 4 days after stimulation by intradermal injection of diphtheria-tetanus vaccine into the skin draining to the nodes. Part of each lymph node was processed for routine histology, another part for obtaining suspensions of living cells. The remaining tissue was rapidly frozen in isopentane (precooled in liquid nitrogen) for use in immunofluorescent staining. The cryostat sections were fixed for 10 min in acetone and stained directly with antisera against human IgG, IgM, IgA, IgE, beta 1C, and fibrin (the antisera against IgE, beta 1C, and fibrin were generously provided by Dr. Alfred Michael).

Immunoglobulin determination. Determination and quantitation of serum immunoglobulins was performed by immunoelectrophoresis and by single radial immunodiffusion according to the method described by Mancini, Carbonara, and Heremans (26).

T-cell evaluation. PHA and PHA-dose response was carried out as described previously (19). The skin tests used for evaluation of T lymphocytes were as follows: mumps, PPD, candida, histoplasmin, streptokinase-streptodornase, trichophyton, and sometimes dinitrochlorobenzene (DNCB) after appropriate stimulation.

RESULTS

Our series of primary immunodeficiency disorders under study here comprises 32 patients. 6 of 32 are adults, and the remainder infants, children, and youngsters under 19 yr of age.

The results of B- and T-cells evaluation are presented in Table I. Only microgranular, ring-like fluorescence was regarded as positive (Fig. 1). Since our cell suspensions always contained a small percentage of monocytes and an occasional granulocyte, only cells which showed morphological features of lymphocytes were counted. However, the monocyte precursors which cannot be distinguished from small lymphocytes on morphological bases, probably accounted for a small percentage of positive cells in preparations stained with anti-IgG reagent. This antiserum reacted very often with monocytes and granulocytes which most probably were covered with IgG cytophilic antibodies. We feel that these lymphocyte-like monocyte precursors accounted for the small percentage of IgG positive lymphocytes in some patients with established diagnosis of x-linked agammaglobulinemia. We did not attempt to remove all

TABLE I

B and T Cells Evaluation in Patients with Primary Immunodeficiency Disease

Patient	Surface Ig on peripheral blood lymphocytes				Circulating lympho-cytes/mm³	Serum Ig			PHA and dose PHA response‡	Skin tests
	G	A	M	Total		G*	A	M		
	%					mg/100 ml				
12 normal adults (mean)	16.7	5.8	7.8	30	1950					
1. D. M.	0.1	0.0	0.5	<1	4560	10	0	0	N	+
2. J. S.	0.2	0.0	0.0	<1	2184	200*	0	0	N	+
3. D. R.	0.6	0.0	0.2	<1	2116	124*	0	0	N	+
4. T. S.	3.2	0.0	0.2	3	7964	52*	0	0	N	+
5. W. T.	1.3	0.1	0.1	<2	3702	92*	0	0	L	+
6. A. R.	1.0	0.6	2.4	3	2556	340*	0	0	N	+
7. G. R.	5.4	0.6	1.5	7	3000	320*	0	0	N	+
8. C. P.	4.6	1.8	2.2	9	4850	100*	0	0	N	+
9. M. R.	2.2	0.4	1.0	3	6750	144*	0	5	N	+
10. D. P.	1.4	1.4	2.4	5	3520	230*	0	9	LN	+
11. D. P.	2.2	0.4	1.2	4	4255	100*	0	0	N	+
12. M. A.	0.8	0.4	0.6	<2	1822	336	10	7	nd	+
13. D. S.	3.5	4.1	6.3	14	4200	220	46	30	LN	variable
14. B. K.	8.2	4.8	6.7	20	3904	20	0	0	LN	+
15. D. H.	15.2	2.0	6.2	23	1200	164*	0	0	L	+
16. N. H.	6.0	0.1	3.0	9	510	46	0	3	L	+
17. A. S.	9.3	2.6	3.8	16	1700	400*	3	3	N	+
18. L. L.	4.5	4.6	5.4	14	840	320*	5	5	LN	+
19. L. H.	5.5	3.2	2.4	11	1225	276*	0	0	N	+
20. S. E.	4.4	1.0	2.4	8	3960	144*	0	0	Neg	variable
21. C. W.	11.2	5.8	5.8	23	4400	1437	0	175	nd	+
22. J. P.	4.8	9.3	6.0	20	1794	1720	0	50	N	+
23. B. S.	14.5	10.5	9.7	35	4063	1840	0	145	N	+
24. A. P.	8.4	5.2	5.6	19	4505	1410	0	75	nd	+
25. S. B.	18.7	6.3	7.4	37	2250	810	500	11	N	−
26. D. L.	17.0	5.4	10.6	33	4200	940	265	30	N	−
27. K. H.	8.4	14.6	10.4	33	780	775	30	160	L	−
28. L. D.	10.0	9.0	10.0	29	1170	320	5	215	L	−
29. A. E.	32.9	23.7	26.6	84	3300	410	20	14	Neg	−

* Low levels of IgG are due to gamma globulin therapy.

‡ N, normal; LN, low normal; L, low; Neg, negative; nd, not done.

monocytes by nylon column filtration because we feel that populations other than monocyte populations are partially eliminated by this manipulation. The percentage of stained cells below 1% is regarded as negative because in control preparations (stained with antisera against determinants other than those on human immunoglobulins) occasional lymphocytes were also positively stained.

The percentage of Ig-bearing lymphocytes is a reliable indication of the level of circulating B lymphocytes only when the total number of circulating lymphocytes falls within normal limits. Therefore, the number of lymphocytes per cubic millimeter is included in Table I. Indeed, in some cases these figures are very low (cases numbered 16, 18, 27, 28). Cases 27 and 28, both children 4 yr of age with ataxia-telangiectasia, show normal per-

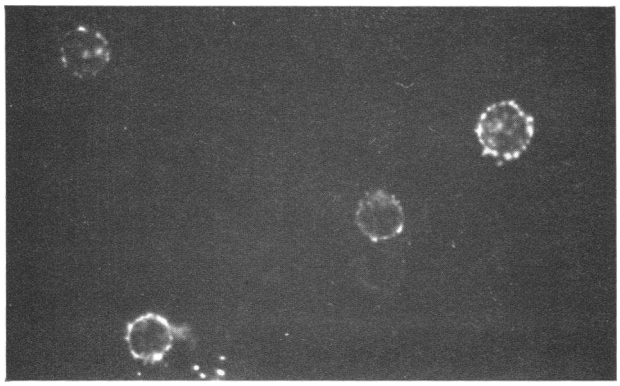

FIGURE 1 Immunofluorescent appearance of Ig-bearing lymphocytes. Peripheral blood lymphocytes stained with fluoresceinated anti-IgM × 800.

TABLE II

Summary of Clinical and Laboratory Data

Patient	Age	Sex	Type of deficiency	Lymphocytes positive for Ig	Serum Ig	Cellular immunity	Clinical summary
				%			
1. D. M.	16 mo	M	Infantile x-linked	<1	Absent	Intact	Two maternal uncles died in childhood. Recurrent otitis and sinusitis since age 1, pneumonia once.
2. J. S.	18 yr	M	Infantile x-linked	<1	Absent	Intact	Maternal uncle and brother died in infancy of agammaglobulinemia. Recurrent pneumonia and otitis since age 2.
3. D. R.	18 yr	M	Infantile x-linked	<1	Absent	Intact	Two maternal uncles died in infancy of agammaglobulinemia. Recurrent pneumonia and urinary tract infections since age 5 wk. Rheumatoid arthritis at age 2.
4. T. S.	2 yr	M	Infantile x-linked	3	Absent	Intact	Recurrent pneumonia, otitis and conjunctivitis since age 8 mo.
5. W. T.	10 yr	M	Infantile x-linked	<2	Absent	Intact	Recurrent respiratory infections since age 4, recently chronic obstructive pulmonary disease.
6. A. R.	10 yr	M	Infantile x-linked (?)	3	Absent	Intact	Brother of G. R. One male sibling died in infancy of pneumonia. Chronic bronchitis since age 3 mo, subsequently recurrent pneumonia, enteritis and conjunctivitis.
7. G. R.	14 yr	M	Infantile x-linked (?)	7	Absent	Intact	Brother of A. R. Recurrent bronchopneumonia, otitis, and coniunctivitis, chronic bronchitis since age 4 mo.
8. C. P.	15 mo	M	Infantile x-linked (?)	9	Absent	Intact	Recurrent pneumonia and otitis since age 6 mo.
9. M. R.	5 yr	M	Infantile x-linked (?)	3	Absent	Intact	Recurrent pneumonia and otitis, chronic sinusitis since age 5 mo.
10. D. P.	16 yr	M	Infantile x-linked (?)	5	Absent	Intact	Recurrent pneumonia and otitis since age 5, chronic sinusitis.
11. D. P.	7 yr	M	Infantile x-linked (?)	4	Absent	Intact	Recurrent otitis and chronic pneumonia since age 4, presently chronic obstructive pulmonary disease. Sister has IgA defic. (A. P.)
12. M. A.	18 yr	M	Variable immunodefic. (?)	<2	Low	Intact	Recurrent bronchitis and urinary infections since age 12. One male sibling has agammaglobulinemia.
13. D. S.	1 yr	M	Variable immunodefic.	14	IgG low	Markedly impaired	Severe pneumocystis pneumonia at age 6 mo. He rejected several bone marrow transplants, recently (10 mo. later) improved, PHA normal, low IgG.
14. B. K.	7 yr	M	Variable immunodefic.	20	Absent	Present	Severe malabsorption since age 9 mo, giardiasis, minimal infections.
15. D. H.	45 yr	F	Variable immunodefic.	23	IgM, IgA absent, IgG low	Present (low PHA)	Pneumonia, otitis, recurrent skin infections since age 10.
16. N. H.	32 yr	F	Variable immunodefic.	9	IgM, IgA absent, IgG low	Present (low PHA)	Recurrent otitis, sinusitis, and pneumonia since age 20.
17. A. S.	42 yr	F	Variable immunodefic.	16	IgM, IgA absent, IgG low	Intact	Recurrent pneumonia since age 10.
18. L. L.	39 yr	M	Variable immunodefic.	14	All Ig low	Intact	Recurrent pulmonary infections and sinusitis since age 23.
19. L. H.	57 yr	M	Variable immunodefic.	11	IgM, IgA absent, IgG low	Intact	Recurrent pulmonary infections, sinusitis, otitis, and conjunctivitis since age 13. Identical twin brother died of agammaglobulineamia in infancy.
20. S. E.	26 yr	M	Variable immunodefic.	8	IgM, IgA absent, IgG low	Markedly impaired	Recurrent otitis, sinusitis and pneumonia since age 6, presently severe obstructive pulmonary disease.
21. C. W.	5 yr	F	IgA deficiency	23	IgA absent	Intact	Recurrent bronchitis and bronchopneumonia since age 9 mo.

TABLE II—(*Continued*)

Patient	Age	Sex	Type of deficiency	Lymphocytes positive for Ig	Serum Ig.	Cellular immunity	Clinical summary
22. J. P.	13 yr	F	IgA deficiency	20	IgA absent	Intact	Chronic otitis starting at age 4.
23. B. S.	4 yr	M	IgA deficiency	35	IgA absent	Intact	Recurrent bronchitis and otitis since age 1.
24. A. P.	2 yr	F	IgA deficiency	19	IgA absent	Intact	Sister of D. P. No clinical symptoms of immunological deficiency.
25. S. B.	7 yr	M	Wiskott-Aldrich Synd.	37	IgM low IgA increased	Impaired	Chronic eczema, septic arthritis, bronchitis, and pneumonia since age 10 mo.
26. D. L.	3 yr	M	Wiskott-Aldrich Synd.	33	IgA increased	Impaired	Chronic eczema, sinusitis, recurrent otitis, and bronchitis since age 9 mo, meningitis once.
27. K. H.	4 yr	F	Ataxia-telangiectasia	33	IgA low	Markedly impaired	Ataxia since age 11 mo, conjunctival telangiectasia.
28. L. D.	4 yr	F	Ataxia-telangiectasia	29	IgA, IgG low	Markedly impaired	Ataxia, recurrent pneumonia, and otitis, chronic sinusitis since age 16 mo.
29. A. E.*	5 mo	M	Di George syndrome	84	Normal	Absent	Severe hypocalcemia, respiratory tract infections. After transplant with fetal thymus cellular immunity restored, per cent of Ig-bearing lymphocytes decreased to normal level.

* The case history of this infant reported in detail in ref. 27.

centages of lymphocytes possessing surface-associated immunoglobulins. However, the absolute numbers of lymphocytes are low and a relatively high percentage of B lymphocytes is probably attributable to the decreased number of T lymphocytes. Both children have markedly impaired cellular immunity. In adult cases the number of circulating lymphocytes per cubic millimeter is always lower than that of the infants and children. However, in cases 16 and 18, both classified as variable immunodeficiency according to World Health Organization classification (27) the numbers of circulating lymphocytes are very low. These patients show also low PHA response in the presence of positive skin tests. B-cells are present, but their relatively high percentage in case 18 is certainly misleading without consideration of the absolute values.

The serum immunoglobulins determination shows in most cases the presence of low levels of IgG. However, the great majority of the first 20 patients in the table were being given gamma globulin treatment weekly, biweekly, or monthly.

The summary of clinical and laboratory data is presented in Table II. Our criteria for diagnosis of infantile x-linked agammaglobulinemia are as follows: (*a*) positive family history involving male infants, (*b*) clinical course disclosing recurrent pyogenic infections which start early in infancy and childhood, (*c*) absence of serum immunoglobulins (even IgG is at levels below 25 mg/100 ml), (*d*) absence of antibody responses to potent antigens, (*e*) intact cellular immunity, (*f*) characteristic histological patterns of lymph nodes and/or other lymphoid tissues showing absence of primary and secondary follicles, absence of plasma cells, and depleted "bursa"-dependent B-cell areas of lymph nodes and other lymphoid organs in the presence of abundant cellularity in the thymus-dependent regions, e.g., in the deep cortical areas of the lymph nodes.

The first 11 cases recorded in Table II are males, infants, or children. All show the absence of serum immunoglobulins and intact cellular immunity. In all of them recurrent pyogenic infections started in infancy or early childhood. However, only five cases (nos. 1–5) qualify as unquestionable infantile x-linked agammaglobulinemia (Bruton's type). These showed the absence of immunoglobulin receptors on circulating lymphocytes. Cases no. 4 and 5 have negative family history, but by other criteria represent examples of the Bruton's type agammaglobulinemia. Case no. 5 had a low PHA response but this evaluation was done only once without dose response analysis and the child did have positive skin tests. Since the family history was negative and the PHA aberrant for this disease, it is possible that this case represents another disorder. However, because the other characteristics fit well with the diagnosis of Bruton's type, and because in classical Bruton cases isolated PHA determinations may give low values, especially during viral infections, we consider this case as belonging to the Bruton category. In two cases lymph nodes were examined also by means of immunofluorescence (Table III). It is of interest that in D. M. 2% of IgM-bearing lymphocytes were found in the suspension

TABLE III

B-Cells in Lymph Nodes of Patients with Primary Immunodeficiency Disease

Patient	Type of deficiency	Surface Ig on lymphocytes				Secretory Ig and complement in cryostat sections
		G	A	M	Total	
		%				
10 control subjects (mean)		21.1	6.1	11.3	38	IgG-plasma cells always present, IgM- and IgA-plasma cells variable. Germinal centers contain IgG, IgM, and beta 1C globulin.
D. M. (no. 1)	Infantile x-linked	0.4	0.0	2.0	<3	Small lymph node, no plasma cells nor germinal centers.
T. S. (no. 4)	Infantile x-linked	0.2	0.0	0.1	<1	Small lymph node, no plasma cells nor germinal centers.
C. P. (no. 8)	Infantile x-linked (?)	0.8	0.4	2.4	<4	Small lymph node, no plasma cells nor germinal centers.
M. A. (no. 12)	Variable immunodef. (?)	0.8	0.2	0.6	<2	Large lymph node, no plasma cells (IgG, IgM, IgA, IgE), nor germinal centers.
B. K. (no. 14)	Variable immunodef.	8.3	3.2	8.4	20	Medium size lymph node, no plasma cells but germinal centers contain IgG, IgM, and beta 1C globulin.
N. H. (no. 16)	Variable immunodef.	2.6	2.6	11.2	16	Medium size lymph node, no plasma cells but germinal centers contain IgG, IgM, and beta IC globulin.
A. S. (no. 17)	Variable immunodef.	5.0	2.2	1.8	9	Not done.
B. S. (no. 23)	IgA deficiency	12.8	9.8	7.8	31	IgA-plasma cells absent, otherwise within normal limits.
K. H. (no. 27)	Ataxia-telangiectasia	9.0	24.0	13.8	37	IgA-plasma cells absent, IgM- and IgG-plasma cells present, germinal centers contain IgG, IgM, and beta 1C glob.
A. E. (no. 29)	Di George syndrome	24.3	10.9	17.8	53	Many IgG-, IgM-, and IgA-plasma cells, germinal centers large, numerous, contain IgG, IgM, and beta 1C globulin.

of cells from stimulated lymph node, but not in peripheral blood.

In six cases, the diagnosis of x-linked agammaglobulinemia is probable but each of these cases lacks some of the important components of the rigid criteria we have used in the classification of the prototype cases of this group. All of them have low percentage of circulating lymphocytes possessing immunoglobulins on the surface. What seems to be of most importance to us is that they show small numbers of cells with IgM and IgA receptors and not only the possibly confusing IgG receptors. Therefore, they cannot be explained by false-positive results attributable to the small monocytes (monocyte precursors) covered with cytophilic antibodies (usually IgG). The lymph node of C. P. (case no. 8) shows small accumulations of cells in clusters in the far cortical regions. They are remindful of early stages of germinal center development, a finding not seen in the classical x-linked infantile agammaglobulinemia. The patient D. P. (no. 11) has a sister with clinically asymptomatic isolated IgA deficiency (A. P., no. 24). All cases of this first group of 11 patients had normal or high normal levels of circulating lymphocytes. Further, all showed normal responses to PHA and normal numbers of PHA-responding cells, as well as positive skin tests.

Patient M. A. (no. 12) is difficult to classify. He has a brother with agammaglobulinemia. However, the stimulated lymph node that was removed was large and although it did not show primary and secondary lymphoid follicles, few plasma cells were encountered on histological examination. The immunohistochemical staining with antisera against γ, μ, α, and ϵ heavy chains were negative but the plasma cells encountered in routine histology may represent the immunoglobulin secretors that account for the low levels of serum IgG, IgM, and IgA.

TABLE IV
Di George Syndrome

Patient	Age	Before treatment: Surface Ig on peripheral blood lymphocytes G	A	M	Total	Circulating lymphocytes/mm³	Cellular immunity	After fetal thymus transplantation: Surface Ig on peripheral blood lymphocytes G	A	M	Total	Circulating lymphocytes/mm³	Cellular immunity
		%						%					
A. E.	5 mo	32.9	23.7	26.6	84	3,300	Absent	16.0	6.4	14.0	36	3,145	Normal

Further, his recurrent infections started late in life, at age 12 yr.

The next large group of eight cases comprises clear examples of the variable immunodeficiency (common) according to World Health Organization (WHO) classification (27). The first two cases are children, the remainder adults. Patient D. S. presented with markedly impaired cellular immunity associated with low levels of serum IgG and surprisingly low number of IgG-bearing lymphocytes together with normal percentages of lymphocytes possessing IgM and IgA surface immunoglobulins. Presently his cellular immunity is normal but his serum IgG levels remain low. Patient B. K. possesses low normal per cent of circulating immunoglobulin-bearing lymphocytes but virtual absence of serum immunoglobulins. His lymphocytes show also low normal PHA responses. Severe malabsorption and giardiasis are his main clinical complaints.

The common feature of six adult cases is the presence of low or nearly normal percentages of immunoglobulin-bearing lymphocytes and small amounts of serum IgG (before treatment with immunoglobulins) but no detectable serum IgM and IgA. These cases also show much lower responses to PHA stimulation, most often associated with a lower than normal number of circulating lymphocytes. Sometimes, however, in spite of high numbers of circulating lymphocytes complete lack of PHA responses was observed (patient S. E., no. 20). Three cases in which lymph node biopsy was done show the presence of lymphocytes possessing surface immunoglobulins, usually in lower percentages than in controls. They regularly lack or have a gross deficiency of differentiated immunoglobulin secretors (Table III). By contrast to lymph nodes of patients with infantile x-linked agammaglobulinemia, lymph nodes of patients with variable (acquired) immunodeficiency have more normal structure with germinal centers containing IgG, IgM, and beta 1C globulin, as revealed by immunofluorescent staining (Table III).

Four cases of isolated IgA deficiency (nos. 21–24) show very consistent laboratory findings. All have normal numbers of circulating lymphocytes having plasma membrane-associated immunoglobulins. By contrast to the absence of serum IgA, and IgA-secreting plasma cells, the per cent of IgA-bearing lymphocytes is normal or high. The number of circulating lymphocytes and the cellular immunity of these patients is normal.

The evaluation of B-cells of two cases of Wiskott-Aldrich syndrome (nos. 25 and 26) reveal high normal values of all B-cell classes. The PHA response in these patients was normal but skin tests were negative.

Two cases of ataxia-telangiectasia present markedly impaired cellular immunity (low PHA response and negative skin tests). That the per cent of peripheral and lymph nodes B lymphocytes is high is probably attributable to a depletion of T lymphocytes reflected in the lower than normal numbers of circulating lymphocytes and the very low PHA responses. It is very interesting that both children show extremely high percentages of IgA-bearing lymphocytes, both in peripheral blood and in lymph node populations. Serum IgA, however, is low, and IgA-producing plasma cells are absent.

The case history of Di George syndrome is reported in detail elsewhere (28). This infant was successfully treated by a fetal thymus transplant, and the extremely high level of B lymphocytes noted before treatment decreased to normal values in parallel with restoration of T-cell population as was revealed by appearance and progressive increase in numbers of PHA responding cells after thymic transplantation (Table IV).

Three cases of severe combined immunodeficiency were examined (Table V). Children T. T. and S. K. were studied after initial efforts at marrow transplantation and showed low percentage of B-lymphocytes and trace amounts of immunoglobulins but no clinical evidence of T-cell function (negative PHA response, negative skin tests). Later all three children were successfully transplanted with bone marrow cells and presently show restored cellular immunity and a very high percentage of circulating B lymphocytes. The case histories of these infants have been and will be reported in detail elsewhere (29, 30).[3,4]

[3] Biggar, W. D., B. H. Park, B. Dupont, K. Gajl-Peczalska, and R. A. Good. Submitted for publication.

[4] Park, B. H., W. D. Biggar, K. J. Gajl-Peczalska, and R. A. Good. Manuscript in preparation.

TABLE V

Combined Infantile Immunodeficiency

Patient	Age	Sex	Soon after transplantation: Surface Ig on peripheral blood lymphocytes G	A	M	Total	Serum Ig* G	A	M	Cellular immunity	Presently: Surface Ig on peripheral blood lymphocytes G	A	M	Total	Serum Ig G	A	M	Cellular immunity
			%				*mg/100 ml*				%				*mg/100 ml*			
1. T. T.	8 mo	F	5.0	0.8	2.6	8	216	5	20	Absent	19.8	2.8	4.2	27	140	23	43	normal
2. S. K.	5 mo	F	not done				300	5	4	Absent	22.2	10.2	17.2	49	1,000	30	31	normal
3. D. C.	7 mo	M	not done				not done			Absent	17.6	20.8	11.2	49	320	35	56	normal

* The values obtained after blood transfusions.

DISCUSSION

The classification of primary immunodeficiency diseases introduced by WHO committee indicates that new parameters are needed to make the classification more meaningful and precise. A helpful new parameter may be the method of quantitation of B lymphocytes by detection of the surface immunoglobulin (2, 3).

Absence of immunoglobulin secreting plasma cells in all cases of agamma and severe hypogammaglobulinemia has been described long ago. It was, however, impossible to establish whether the defect involves differentiation of the entire B-cell line, or only impairment of the maturation of B lymphocytes into the immunoglobulin secreting cells. During the past year the surface immunoglobulins on lymphocytes from patients with primary immunodeficiency disease have been intensively studied (1, 16–20). However, the number of such patients remains relatively small and therefore the results published on the basis of one or a few cases, although important, may be confusing.

The most uniform opinion concerns the infantile x-linked agammaglobulinemia. 10 of 13 well-defined cases are said to be lacking not only in plasma cells and thus immunoglobulin-secreting cells, but also the lymphocytes bearing immunoglobulin at their surface. In other words, the majority of such patients lack an identifiable B-cell population. Such a condition may result from a lack of the differentiation of hematopoietic stem cells along the B lymphoid pathway (17); alternatively, B lymphocytes of these patients could fail to incorporate Ig into their membranes (16). However, the three of six cases classified by Siegal and co-workers (16) as infantile x-linked agammaglobulinemia were shown to have high percentages of lymphocytes possessing Ig receptors (9–13%). These findings conflict with those of Grey et al. (1), Cooper et al. (17), as well as, to a degree, with our findings. Siegal et al. (16) studied B lymphocytes only by means of polyvalent antiserum, directed against whole human Cohn fraction II, therefore they could detect the cytophilic antibodies on premonocytes. It is possible, however, that these investigators studied different forms of x-linked agammaglobulinemia from those we studied. Aiuti, Lacava, and Fiorilli (31) reported recently a case of x-linked agammaglobulinemia which showed membrane-bound IgM on peripheral lymphocytes after in vivo bacteria stimulation but not before. Our series of 11 cases of unquestionable (5 cases) and possible (6 cases) x-linked agammaglobulinemia may be divided into two subgroups. One contains five cases which do not possess IgM and IgM receptors at all on peripheral lymphocytes. The other six cases show very low percentage (0.6–2.4%) of lymphocytes with IgM receptors, and in three cases also a very low percentage of IgA receptors (0.6–1.8%). The monocyte precursors are probably accountable for the very low percentage of IgG-bearing cells found in some cases of both groups. The same interpretation was made by Lischner, Valdes-Dapena, Biggin, Hann, and Amoni (32) in short term lymphocyte cultures of a patient with Bruton's agammaglobulinemia. Interestingly, in the stimulated lymph node of the patient belonging to the first group, 2% of IgM-bearing lymphocytes was found. Also, Aiuti et al. (31) found 6% of IgM-bearing lymphocytes in the lymph node of their patient who did not possess Ig-bearing circulating lymphocytes until he was stimulated in vivo by bacteria. We feel that until the molecular basis for the defect in x-linked agammaglobulinemia is determined, it will remain impossible to decide whether these cases represent two different varieties of the same defect, or are separate entities.

The most controversial and thus largely unclassified group of patients with primary immunodeficiency is that of the common variable immunodeficiency. It seems almost certain that this group is not uniform but consists of several different entities. This interpretation could account for somewhat contradictory results published by several different investigators. Three cases of Siegal et al. (16), one case of Grey et al. (1), and one case of Papamichail et al. (19) show the total number of Ig-bearing lymphocytes to be below 4%, four cases of

Preud'homme and Seligman (18) were found to have peripheral B lymphocytes below 2%.[5]

However, the additional two cases described by Preud'homme and Seligmann (18) presented increased numbers of circulating B lymphocytes, associated with hypogammaglobulinemia. Two cases of Cooper et al. (17) and three cases of Siegal et al. (16) showed only slightly decreased percentages of B lymphocytes. All our eight cases of common variable immunodeficiency show the presence of immunoglobulin-bearing lymphocytes in moderately and slightly decreased numbers as compared to normal. The presence of B lymphocytes was also found in the lymph nodes of three patients of this series (one child and two adults). Lymph nodes generally contained secondary follicles with germinal centers, but did not show the hyperplasia of lymphoid follicles that has been described in similar cases by many investigators most recently by Cooper et al., (17) and Preud'homme and Seligmann (18) in their patients. Our present group comprises two children and six adults and in both instances impairment of cellular immunity was also noted. We feel that repeated evaluation of B- and T-cell populations in these patients may help in further differentiation of this possibly very diverse group. However, as mentioned repeatedly before, major variabilities of immunoglobulin levels and of lymphoid tissue, from time to time in the same patient, and from patient to patient in the same family, suggest that the manifestations of a fundamental underlying disorder may account for much of the variability observed. Choi and Good (33) have shown that at least some of these cases with common variable immunodeficiency can be sharply differentiated from patients with the classical x-linked infantile disease by virtue of the fact that the former have cells that produce immunoglobulins quite well but they cannot secrete immunoglobulins in normal amounts, while the latter can neither produce nor secrete immunoglobulins. It is possible to speculate that in some of the patients from this group, there is temporary or permanent arrest of maturation of surface immunoglobulin-bearing lymphocytes into immunoglobulin-secreting cells.

Similar to three cases of isolated IgA deficiency described by Grey et al. (1), and 10 cases of Lawton et al. (20), our four cases of IgA deficiency showed normal or high percentage of IgA-bearing lymphocytes in peripheral blood, and in one case also in the lymph node that was studied. In this condition the defect seems to involve the maturation of IgA B lymphocytes into IgA-producing plasma cells. The molecular basis of this defect is not yet known. The suggestion that a similar condition may be present in ataxia-telangiectasia is contained in the data presented herein. In two cases the percentages of IgA-bearing lymphocytes in peripheral blood, and in one case also in lymph node, were extremely high. The serum IgA was decreased and the IgA plasma cells absent from the lymph node in one case. One of two other cases of ataxia-telangiectasia described by Siegal et al. (16) was only evaluated by polyvalent fluoresceinated antiserum, but the case of Lawton et al. (20) possessed the normal percentage of IgA-bearing lymphocytes. This findings must be investigated further.

Three of our patients with combined immunodeficiency after immunologic reconstitution by marrow transplantation showed normal to increased numbers of circulating B-cells as is reflected in Table V. In only one of these were B-cells partially evaluated before transplantation (S. K.). In this infant 1.1% of IgG-bearing lymphocytes was found. In another case, the B-cells were evaluated recently after marrow transplantation and that case showed a distinct increase in numbers of B-cells with the establishment of immunologic capacity. Interestingly, in two of these patients, the percentage of IgA-bearing lymphocytes is extremely high.

The influence of fetal thymus transplantation on B-cell population in Di George syndrome is of interest and has not been previously reported. In our case a functional T-cell population was successfully restored as demonstrated by appearance and increase in PHA response and appearance of positive skin tests. A concomitant progressive fall in the absolute number of B-cells was noted in the 5 mo after restoration.

The observations presented indicate that the introduction of immunofluorescent evaluation of B-cells in primary immunodeficiency disease have provided a new and useful parameter which may be of great value in diagnosis and classification of these conditions.

[5] By contrast to the values for peripheral B lymphocytes ranging from 29 to 31% obtained as a mean by Grey et al. (1), Cooper et al. (17), Siegal et al. (16), and our group, Preud'homme and Seligmann (18) obtained somewhat lower value for B-cells (19%). The major difference lies in the percentages of IgG-bearing lymphocytes. The reason for these differences is not yet clear. It seems to us that high values could be explained by monocytes or even B lymphocytes accepting IgG immunoglobulin on their surface receptors rather than producing this immunoglobulin. On the other hand, some cell fraction techniques, like nylon wool column, may very well remove "sticky" B lymphocytes along with monocytes and their precursors.

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