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Surface Markers on Lymphocytes of Patients with Infectious Diseases

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Peripheral blood lymphocytes from normal subjects and patients with viral and bacterial infectious diseases were examined for the presence of three surface markers: (i) surface immunoglobulins, (ii) receptor for C3 complement component (EAC test), and (iii) spontaneous binding of sheep red blood cells (E rosette formation). The first two markers are used to detect bone marrow-derived lymphocytes (B cells); the E rosette formation is dependent on thymus-derived lymphocytes (T cells). We demonstrated these assumptions, as defined by others, by the fractionation of lymphocytes on bead columns coated with immunoglobulin plus anti-immunoglobulin. The peripheral blood lymphocytes of normal individuals consisted of 52% T cells, 23% B cells with EAC receptor, and 21% B cells with membrane immunoglobulin. There was no significant difference in these values from those obtained in viral or bacterial diseases. Only a few cases of infectious mononucleosis had an increase in T cells. These results give us a partial picture of the T- and B-cell frequency in normal subjects and in patients with infectious diseases.

Lymphocytes can be divided into two major groups according to their origin and function. Thymus-derived lymphocytes (T cells) derive their immunological maturity from the thymus (20, 3) and are concerned with cellular immunity (19). Bone marrow-derived lymphocytes (B cells) are derived from an unknown site, homologous to the bursa of Fabricius in birds, and are concerned with humoral antibody production (13, 37). T and B cells not only can be differentiated by origin and function (14), but also by specific anti-T lymphocyte serums in animals (27) and recently in man (2), thus permitting investigators to link certain functions to the B or T cells (13, 16, 22). Also, surface markers have been described on lymphoid cells which can be used to determine the course and stage of their differentiation (27, 32, 36). Animal and human B cells have high surface concentrations of immunoglobulins (15, 25, 35, 40), characteristic receptors for the C3 complement component (5, 10), and they bind certain antigen-antibody complexes (4). T cells have the ability to form rosettes directly with sheep red blood cells under experimental conditions (6, 17, 23). Most of these surface markers have been identified in animals; their recognition in man and their use in the study of normal and abnormal immune responses is just beginning. The present report is a comparative study on three specific surface markers on lymphocytes in normal subjects and patients with infectious diseases. The surface markers are: (i) surface immunoglobulins detected by immunofluorescence; (ii) receptors for the C3-activated component, identified by the capacity of the lymphocytes to form rosettes with sheep red blood cells sensitized with rabbit antibody and human complement (EAC cells); and (iii) spontaneous rosette-forming capacity for sheep red blood cells (SRBC; E cells).

MATERIAL AND METHODS

Patient population. Normal controls were laboratory personnel, medical students, and their respective relatives. All controls were examined according to accepted clinical and laboratory criteria including estimation of the serum immunoglobulin levels, and were found to be normal in every respect. Fifty-three cases of infectious diseases were tested of which there were six infectious hepatitis, four serum hepatitis (with hepatitis-associated antigen, HAA), nine measles (one complicated by encephalomyelitis), seven infectious mononucleosis, two chicken pox, four parotitis, three herpes zoster, five brucellosis, five typhoid fever, two rheumatic fever, three subacute endocarditis, and three sepsis. The diagnoses of the

infectious diseases were based on accepted clinical and laboratory criteria, including the Paul-Bunnell test and identification of a high percentage of atypical lymphocytes in the peripheral blood for patients with infectious mononucleosis. Measles, parotitis, and chicken pox were tested for hemagglutinin-inhibiting or complement-fixing antibodies; serum and infectious hepatitis were tested for HAA; and blood cultures and serodiagnosis were performed for the bacterial diseases.

Lymphocyte preparations. A 20-ml sample of defibrinated blood was left to spontaneous sedimentation at 4 C for 1 h. The serum, buffer coat, and upper red-cell layer was removed, gently stratified over a solution composed of 24 parts of 9% Ficoll (Pharmacia, Uppsala, Sweden) and 10 parts of 33% Isopaque (Nyegaard Co., Oslo, Norway), and centrifuged at 800 × *g* for 40 min at 10 C (34). The leukocytes at the interface were aspirated and washed twice with gelatin-Veronal buffer (GVB) and adjusted to a concentration of 2 × 10^6/ml in the same buffer or in Hanks solution. This cell suspension was used for all tests performed. In some experiments the cell suspension was filtered at 37 C through a nylon fiber column (0.5 by 15 cm; Pharmacia, Uppsala, Sweden). The lymphocyte preparations were used only if their purity was 95% or greater. The number of dead cells in the final preparation, assessed by the trypan blue test, was never more than 2 to 3%.

Antisera. Rabbit-immunoglobulin sera specific for the heavy chains of each immunoglobulin class (IgG, IgA, IgM) were produced in rabbits by immunization with isolated normal IgG or a pool of paraprotein IgA or a pool of paraprotein IgM. Purified antibodies were obtained by acid elution from the immunoabsorbent Sepharose 4B CNB_r-activated column containing the homologous immunoglobulins. After absorption, the IgG fraction of the rabbit sera prepared by diethylaminoethyl-cellulose chromatography was conjugated with fluorescein and filtered through Sephadex G-25 columns. The protein concentration of this IgG ranged from 1 to 1.5 mg/ml and was used in a dilution of 1:8. Additional antisera specific for the heavy chains of each immunoglobulins class (IgG, IgA, IgM) were obtained from Hyland Laboratories (Los Angeles, California), and classes IgD and IgE were obtained from Beheringwerke (Marburg-Lahn, Germany). The specificity of all conjugates was ascertained on bone marrow plasma cells from patients with myeloma or macroglobulinemia. No immunofluorescence was observed when these conjugates were applied to bone marrow specimens from patients with agammaglobulinemia. The antisera were also controlled by immunodiffusion in gel and by radial immunodiffusion against purified myeloma proteins (IgG, IgA, IgM, IgD) and whole human serum.

Immunofluorescent staining. Lymphocytes (2 × 10^6 in 0.1 ml of GVB) were incubated with 0.1 ml of fluorescein-conjugated antiserums (diluted 1:8) specific for the different immunoglobulin classes for 30 min at 4 C. Each vial was gently shaken three times during incubation. The cells were washed three times with GVB and examined with a Leitz ultraviolet microscope equipped with vertical illumination (26). The filter combination was 1.5 mm BG12, and the secondary filter was S-525. Two hundred lymphocytes were examined per vial. Total cell numbers and cell types were evaluated under a normal light microscope. Controls were conjugated rabbit antiserum specific for sheep gammaglobulins and blocking test with unlabeled goat antihuman heavy-chain sera. The results obtained with commercial antisera and with "homemade" antisera were similar in all studies.

Fractionation of lymphocytes on bead columns. Anti-immunoglobulin columns were obtained by incubating Degalan V26 beads (Degussa Wolfgang AG, Aanau am Main, Germany) with 0.5% of the respective immunoglobulins in 0.5 ml of phosphate-buffered saline at PH 7.4 (25). The beads were then poured into glass columns (1.5 by 30 cm) (Pharmacia, Uppsala, Sweden) and washed with 100 ml of phosphate buffer. The columns were then filled with the anti-immunoglobulin serum diluted 1:6 (anti-IgG, anti-IgA, anti-IgM) and left at 4 C for 3 h. The antibody concentration was in excess. The columns were washed with the same buffer and then were ready for the lymphocyte fractionation. This was performed by filling the columns with 10^7 lymphocytes and using a flow rate of 2 ml/min at 4 C. The passed cells were collected and then centrifuged and resuspended at 2 × 10^6/ml. Columns for controls with nonimmune sera were also prepared. The possibility of blocking membrane immunoglobulin by anti-immunoglobulin was excluded by controls performed with goat anti-rabbit immunoglobulin in previous unpublished experiments and by Wigzell et al. (39).

Test procedure for E rosettes. SRBC was stored at 4 C in Alsever solution and used for 1 week. Before use the cells were washed twice and adjusted to a 0.5% suspension in Hanks solution. The SRBC cell suspension (0.25 ml) was mixed with 0.25 ml of the lymphocyte suspension and incubated at 37 C for 15 min. The mixed cell suspension was spun at 200 × *g* for 5 min and then incubated overnight at 4 C. The supernatant fluid was removed, and the top layer of the pellet was gently resuspended by shaking. One drop of the cell suspension was mounted onto a glass slide, covered by a cover slip, and sealed. Two hundred lymphocytes were counted, and all of the lymphocytes binding more than three SRBC were considered positive (17).

Test procedure for EAC rosettes. A 5% concentration of SRBC was washed twice in Hanks solution and then incubated at 37 C with the IgM fraction of rabbit anti-sheep red blood cells diluted 1:5,000. The cells were washed twice and resuspended to 5% in Hanks solution. A 10-ml sample of this cell suspension was mixed with an equal volume of human complement (fresh normal human serum) diluted 1:20, incubated at 37 C for 1 h, then washed twice and adjusted to 0.5%. The test procedure for EAC rosettes was performed as follows: 0.25 ml of 0.5% SRBC, treated as previously described, was mixed with 0.25 ml of a 2 × 10^6 lymphocyte suspension in VBS in 0.01 M ethylenediaminetetraacetic acid. The mixture was left at 37 C for 15 min, then centrifuged at 200 × *g* for 5 min. Most of the supernatant fluid was aspirated, and the cells were resuspended by vigorous mixing on

a whirl-mixer. Two hundred cells were counted under a sealed cover slip. Lymphocytes binding more than three SRBC were considered positive (17).

RESULTS

The characterization of the surface markers on normal human lymphocytes are reported in Table 1. The four experiments performed on bead columns coated with immunoglobulins and anti-immunoglobulins in excess showed a specific retention of lymphocytes with surface immunoglobulins (IgG, IgA, IgM). It should be noted that lymphocyte surface immunoglobulins were also slightly retained in columns coated with normal rabbit serum, indicating that the columns have nonspecific retention for cells with surface immunoglobulins. The column experiments demonstrated highly significant changes in the average cell populations tested for E and EAC rosettes. The E-cell population increased significantly in all passed columns coated with Ig and anti-Ig, whereas the percentages in the control columns were not appreciably modified. These experiments clearly demonstrate that the lymphocyte population consisted of two major cell populations, one of which (with surface immunoglobulin) was selectively retained by the anti-Ig Ig columns. The lymphocyte population forming EAC rosettes also decreased significantly in comparison with the population forming E rosettes; however they didn't completely disappear in columns coated with Ig and anti-Ig.

TABLE 1. *Changes in percentage of different markers on lymphocytes after filtration through anti-Ig columns*[a]

Experiments	E	EAC	Surface Ig[b]
1 C	57	24	22
1 PI	78	7	0
1 PN	59	18	13
2 C	51	28	18
2 PI	72	3	0, 5
2 PN	54	17	9
3 C	53	19	23
3 PI	70	5	1
3 PN	56	6	12
4 C	51	22	18
4 PI	75	6	0
4 PN	58	12	13

[a] Abbreviations: C, control lymphocytes before being passed through the columns; PI, passed through columns coated with rabbit antihuman immunoglobulin serum; PN, passed through columns coated with normal rabbit serum; E, lymphocytes which spontaneously form rosettes with sheep red blood cells; EAC, lymphocytes which form rosettes with sheep red blood cells coated with anti-sheep rabbit erythrocyte of class IgM plus human complement.

[b] Percentage of lymphocytes positive on immunofluorescence with polivalent antihuman immunoglobulin sera.

Lymphocytes from the peripheral blood of 18 healthy controls were examined for the presence of surface markers: (i) surface immunoglobulins detected by immunofluorescence; (ii) receptors for the C3-activated complement component (EAC cells); and (iii) spontaneous formation of rosettes with SRBC (E cells; see Table 2).

The positive cells observed by immunofluorescence showed a characteristic uneven or granular fluorescence on the greater part of the cell surface (Fig. 1A, B); negative cells showed no detectable fluorescence. Of the normal lymphocytes, a mean value of 7.16% (standard deviation [SD] of 3.16) reacted with the fluorescein-conjugated antiserum specific of the heavy chain of IgG; 4.27% (SD of 2.24) with anti-IgA; 8.90% (SD of 2.85) with anti-IgM; 0.88% (SD of 1.00) with anti-IgD; and 0.67% (SD of 1.00) with anti-IgE. We should also note that "Cap" pattern (a common phenomenon appearing when lymphocytes are incubated with fluorescent antisera at 37 C) were rarely observed, and the few cells noted with intracytoplasmic fluorescence were omitted from the counting. The control lymphocytes in blocking experiments (incubated with nonimmune fluorescenated antisera) were 0.5% positive in some cases and 1.0% positive in others. We considered the preparations with 0.5% positive cells as negative. All lymphocytes were examined with a conventional light microscope. The small and medium-size lymphocytes were mostly positive for immunoglobulins. The very large positive lymphocytes resembling monocytes were not counted. Before studying the surface markers, a control with May-Grunwald-Giemsa stain was performed. Lymphocyte preparations with more than 5% granulocytes or monocytes were not performed. Five cases in the control experiment with lymphocytes passed through nylon columns did not give significantly different results from those obtained with lymphocytes isolated only with Ficoll-Isopaque gradient. There was a decrease only in IgG positive lymphocytes on membrane immunofluorescence. No difference was obtained in staining patterns with the different antisera specific for the heavy-chain immunoglobulin classes.

Normal lymphocytes with membrane receptors for activated complement revealed by the EAC test (Table 2) had a mean value of 23.38% (SD of 5.74). Medium and small lymphocytes with more than three SRBC on the membrane

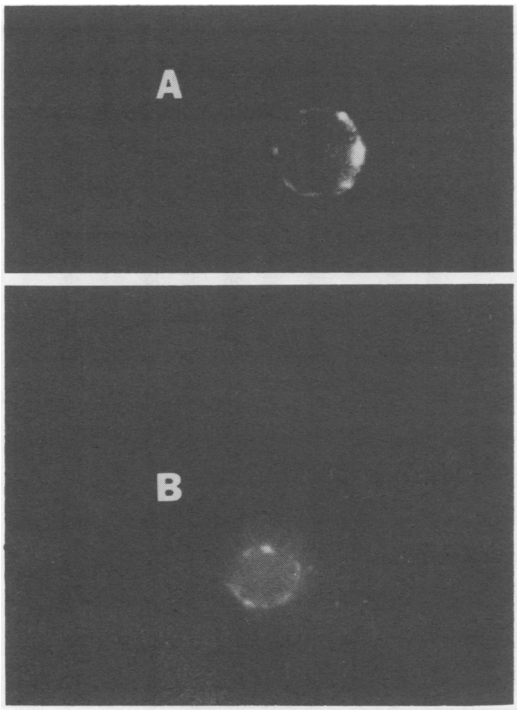

FIG. 1. *(A) Peripheral blood lymphocyte stained with fluorescein-conjugated anti-IgG. Note granular fluorescence around the cell surface. (B) Peripheral blood lymphocyte stained with fluorescein-conjugated anti-IgM. Note granular fluorescence over most of the cell surface.*

were considered positive (Fig. 2). Most of the positive lymphocytes had four or five SRBC on the cell membrane. Lymphocytes which were completely covered by SRBC were rarely observed in the EAC test, whereas they were observed more frequently in the E test.

The spontaneous rosette-forming capacity for SRBC (E tests) by peripheral blood lymphocytes in normal subjects (Table 2) had a mean value of 52.36% with an SD of 7.28 (Fig. 3). Some cases had increased E rosettes with a low number of EAC rosettes. In others the sum of lymphocytes identified by the three surface markers was greater than 100%. Because these findings are in the minority, no significant correlation can be assumed.

The results obtained for the viral diseases are presented in Table 3. The surface markers were positive in all cases. The patients with hepatitis and infectious mononucleosis showed an inconstant increase of lymphocytes positive for IgM. In three of these patients, IgM membrane immunofluorescent staining was very scarce and difficult to interpret in about 20% of the lymphocytes; these cells were not considered positive. In other patients, the staining patterns were similar to those observed on normal immunoglobulin-staining cells. In three cases of infectious mononucleosis, there was a decrease in EAC rosettes and an increase in E rosettes. In measles, only case 16 had a low number of E rosettes. It is interesting to note that this case was complicated by encephalomyelitis during the examination of the lymphocyte surface markers.

Table 4 summarizes the results for patients with bacterial diseases. The surface immunoglobulin, EAC rosettes, and E rosettes for all bacterial diseases tested were within normal range.

DISCUSSION

Peripheral blood lymphocytes are a heterogeneous population with respect to their origin and function. Unfortunately, these cells cannot be morphologically recognized through the conventional microscope. However, due to their innate possession of membrane surface markers and the use of specific antisera, it has been possible to recognize in animals and, to a degree, in man at least two classes of lymphocytes, T and B lymphocytes. By utilizing some of the specific membrane surface markers for the two populations of lymphocytes, it was possible to examine their frequency in normal subjects, healthy carriers of the HAA, and subjects with infectious diseases.

The bead columns coated with immunoglobulins and anti-immunoglobulins specifically retains B lymphocytes, in agreement with the results of others (39). E rosette-forming lymphocytes were not removed by the column, which would explain their increased percentage after the passage. With specific antiserum for human T lymphocytes, the population of lymphocytes forming spontaneous E rosettes was demonstrated, and upon addition of complement were completely killed (2). The T cells are not retained by this column either because they do not have surface immunoglobulins or because their immunoglobulins are embedded within the membrane, and the fixing complement (Fc) fragment is not exposed as postulated by Marchalonis (18). The formation of the E rosette-forming lymphocytes appears in the population of the T lymphocytes, which is in accord with Papamichail et al. (23). Fröland recently demonstrated that the phenomenon of rosette formation is probably dependent on thymus-derived lymphocytes (11). Jondahl et al. (17) have demonstrated that the T cells formed 100% E rosettes. In all normal subjects, the

TABLE 2. *Surface markers on normal peripheral blood lymphocytes*

Subjects	Surface immunoglobulins[a]					EAC[b] rosettes	E[b] rosettes
	G	A	M	D	E		
V.A.	7	3	8	0	0	24	46
S.C.	6	2	7	0	0	34	69
M.A.	12	4	16	1	4	33	50
D.A.R.	10	6	6	0	0	30	50
D.A.C.	8	6	6	0	0	18	54
C.A.	ND	ND	ND	ND	ND	18	65
L.E.	4	6	10	2	1	30	47
F.A.	8	8	12	1	2	ND[c]	ND
L.E.	2	4	8	1	0.5	21	61
M.O.	12	8	10	3	0	22	40
D.A.	8	2	4	5	2	22	43
S.A.	3	4	8	1	0	ND	50
C.M.V.	6	2	8	0	ND	21	57
V.M.	5	4	5	0.5	0.5	25	50
L.G.	12	4	12	0	0	13	57
S.E.	7	1	10	0	0	23	50
G.S.	12	4	12	0.5	0.5	23	53
P.F.F.	4	8	10	0	0	26	43
F.L.	ND	ND	ND	ND	ND	15	58
L.V.	3	1	9	1	1	23	52
Mean	7.16	4.27	8.90	0.88	0.67	23.38	52.36
Range	2–12	1–8	4–16	0–5	0–4	13–34	40–69
SD[d]	3.27	2.29	1.17	1.00	1.00	5.74	7.28

[a] Values are expressed as percentage of cells positive for membrane immunoglobulins.
[b] Abbreviations are same as in footnote to Table 1.
[c] ND, not done.
[d] Standard deviation.

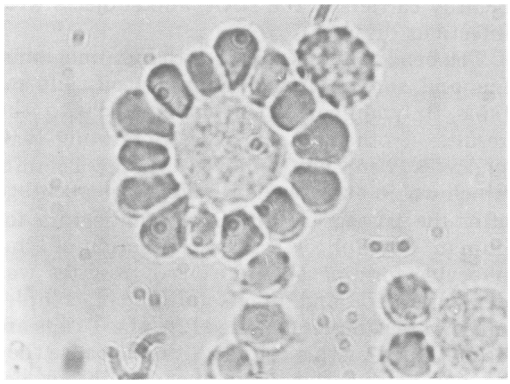

FIG. 2. *Typical rosette image obtained by E test.*

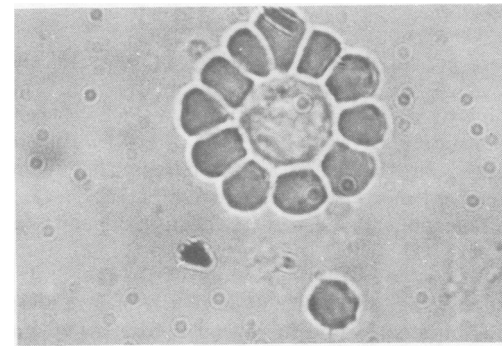

FIG. 3. *Rosette image with EAC test.*

values for peripheral blood E rosette tests were very similar; however, they were slightly below the values obtained by Stjernwald et al. (31). This can be related to different techniques. EAC forming rosettes made up a small number of population of B lymphocytes. Our results were lower than those of Stjernwald and higher than those of other authors (6). The EAC rosettes formation by B cells probably was slightly contaminated by monocytes that were not blocked in spite of the presence of ethylenediaminetetraacetic acid (28). From the column experiments and data obtained in normal subjects, we can conclude that lymphocytes with surface membrane immunoglobulins also possess receptors for the C3 complement, and postulate the existence of a subpopulation of B lymphocytes with receptors only for surface immunoglobulins and a population of lymphocytes with receptors only for C3. Our results in patients with primary immunodeficiencies (Aiuti et al., manuscript in press) and lymphoproliferative disorders (Aiuti et al., manuscript in

preparation) confirm this hypothesis.

The use of membrane surface markers in normal subjects reveals a clear picture of the principles of cellular immunity. Our immunofluorescent results were in accord with other authors (9, 12, 15, 29, 40). We demonstrated that the percentage of lymphocytes with membrane immunoglobulin were predominantly IgM followed by IgG and IgA, and a deficiency of lymphocytes with surface immunoglobulins is not present in normal subjects. In patients with infectious diseases, the behavior of lymphocyte surface markers did not vary significantly in percentage from those of normal subjects. Nevertheless, it should be noted that in viral infections with leukocytosis (relative lymphocytosis) there was an absolute increase in the positive cells for membrane immunoglobulins. This increase is especially evident for infectious mononucleosis. An intense proliferation induced by viruses can be invoked for the high absolute levels of B cells in peripheral blood of patients with viral infections. It is also possible to postulate an increase in membrane immunoglobulins at the single cell level. These interpretations are not supported by our data nor by the findings of Ada et al. (1), who did not show an increase of membrane receptors after immunization in mouse lymphocytes. Wigzell and Anderson (38) demonstrated indirectly that after immunization there appeared a similar concentration of surface-receptor molecules on antibody-forming and memory cells. We can conclude that there is probably no

TABLE 3. *Surface markers on lymphocytes of patients with viral diseases*

Case no.	Diagnosis	Surface immunoglobulins[a]					EAC[b] rosettes	E[b] rosettes
		G	A	M	D	E		
1	Infectious hepatitis	5	3	6	1	0	18	51
2	Infectious hepatitis	11	2	2	0	0	12	58
3	Infectious hepatitis	8	2	16	ND	ND	11	64
4	Infectious hepatitis	16	12	8	2	3	15	60
5	Infectious hepatitis	6	4	6	2	1	18	62
6	Infectious hepatitis	7	5	9	1	0	17	ND[c]
7	Serum hepatitis	6	4	8	0	0	24	58
8	Serum hepatitis	5	3	12	0	0	23	ND
9	Serum hepatitis	6	8	22	3	0	22	50
10	Serum hepatitis	6	2	11	2	0	20	ND
11	Measles	4	6	10	0	0	16	32
12	Measles	3	2	12	1	0	24	64
13	Measles	4	3	14	2	0	26	ND
14	Measles	4	2	12	2	0	17	58
15	Measles	3	1	7	0	0	15	ND
16	Measles	6	4	6	0	8	8	49
17	Measles	5	1	5	0	0	17	ND
18	Measles	3	1	11	0	2	12	64
19	Measles	7	2	8	0	0	20	ND
20	Infectious mononucleosis	2	14	6	ND	ND	8	64
21	Infectious mononucleosis	6	3	9	0	0	13	68
22	Infectious mononucleosis	10	2	24	ND	ND	ND	ND
23	Infectious mononucleosis	4	6	8	0	0	10	86
24	Infectious mononucleosis	1	4	10	0	0	25	60
25	Infectious mononucleosis	1	4	8	0	0	18	77
26	Infectious mononucleosis	6	3	13	1	0	22	65
27	Chicken-pox	14	8	12	0	0	14	51
28	Chicken-pox	8	5	10	0	0	ND	ND
29	Parotitis	4	4	8	ND	ND	ND	ND
30	Parotitis	5	2	8	0	0	23	56
31	Parotitis	6	3	13	0	1	18	48
32	Parotitis	5	2	10	1	0	23	53
33	Herpes zoster	6	6	8	0	0	18	54
34	Herpes zoster	10	14	2	0	1	15	51
35	Herpes zoster	2	2	4	0	0	21	68

[a] Percentage of cells positive on membrane immunofluorescence.
[b] Percentage of cells forming EAC and E rosettes.
[c] ND, not done.

TABLE 4. *Surface markers on lymphocytes of patients with bacterial diseases*

Case no.	Diagnosis	Surface immunoglobulins[a]					EAC[b] rosettes	E[b] rosettes
		G	A	M	D	E		
1	Brucellosis	16	10	3	0	0	18	62
2	Brucellosis	4	8	6	2	0	19	60
3	Brucellosis	12	8	8	1	2	24	57
4	Brucellosis	7	5	10	0	0	22	54
5	Brucellosis	4	6	2	0	0	16	66
6	Typhoid fever	1	0	12	0	0	23	45
7	Typhoid fever	4	6	8	1	0	24	65
8	Typhoid fever	2	0	4	0	0	37	68
9	Typhoid fever	6	4	9	0	0	21	53
10	Typhoid fever	5	3	7	2	0	16	57
11	Rheumatic fever	4	1	8	0	0	24	53
12	Rheumatic fever	3	2	9	0	0	21	57
13	Subacute endocarditis	4	2	9	0	0	23	51
14	Subacute endocarditis	6	4	6	0	0	20	50
15	Subacute endocarditis	5	1	7	0	1	16	48
16	Sepsis	6	2	8	0	0	19	49
17	Sepsis	4	ND[c]	5	0	0	27	58
18	Sepsis	4	ND	7	0	0	24	60

[a] Percentage of cells positive on membrane immunofluorescence.
[b] Percentage of cells forming EAC and E rosettes.
[c] ND, not done.

difference on the concentration of surface immunoglobulins between virgin and immune cells.

The percentage of T cells did not differ significantly from normal in the E test (spontaneous rosettes formation), whereas it was modified moderately in some cases of infectious mononucleosis. This increase in T cells forming E rosettes is in agreement with Thomas (33), who found an elevated number of T cells in peripheral blood lymphocytes of patients with infectious mononucleosis. We should mention that, contrary to other investigations (6), there seems to be a correlation between the high heterophilic antibody titer and increase T cells forming E rosettes.

We did not observe in measles an increased number of T cells. This is not in agreement with the Burnet hypothesis (7), which states that the T cells in the skin, derived from the blood, are responsible for the delayed hypersensitivity of the skin in measles. Contrary to what would be expected, there is a failure of the tuberculin reaction during measles (8). It is believed that the T cells which normally react with tuberculin are either engaged in the measle response (21) or are suppressed by the measles virus (30).

In conclusion, these surface markers provide information as to the number of T and B lymphocytes in peripheral blood; however, they do not reveal the function and activity of these cells. With the simultaneous use of other tests, e.g., mitogen response or lymphotoxic effect (24), a complete analysis of the frequency and function of T and B cells can be obtained.

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ADDENDUM IN PROOF

In three additional cases of infectious mononucleosis, there was an increase only in IgM membrane-bound lymphocytes and a slight increase in IgG and IgA cells. In addition, lymphoid cells in continuous cultures derived from patients with infectious mononucleosis showed almost 100% membrane Ig-positive lymphocytes and 50% EAC rosettes. The E test (spontaneous rosettes) was negative. These data would suggest a B lymphocyte origin of the Epstein-Barr virus-carrying lines (Aiuti & Rocchi, manuscript in preparation.)

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