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Studies of Peripheral Blood T- and B-Lymphocytes in Acute Infections

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E-binding (T) and immunoglobulin-bearing (B) lymphocytes were determined in 22 patients with acute infections. The percentage of T-cells was depressed in most patients in the early stages of disease, whereas the B-cell proportion was usually elevated compared with convalescence and controls. The B-cell increase occurred earlier in a small group of viral or mycoplasmal infections than in bacterial infections. Four patients with unusually high numbers of B-cells had no corresponding increase in EAC3-binding lymphocytes. Almost all lymphocytes from pleural effusion in a patient with tuberculosis were found to be T-cells.

In recent years, an increasing body of evidence has accumulated for the presence of two main groups of lymphocytes in peripheral blood: (i) bone marrow-derived B-cells, which bear readily detectable surface immunoglobulin (Ig) receptors (7, 14), and (ii) T-cells or thymus-derived cells, which have no easily detectable surface Ig (2, 16). The T-lymphocytes can be determined by their ability to form nonimmune rosettes with sheep red blood cells (5, 9). Clinical and experimental evidence suggests that T-cells are largely responsible for cell-mediated immunity, whereas B-cells mediate humoral immunity. T-cells also appear to function as so-called helper cells in collaboration with B-cells in certain humoral immune responses (18).

In some infections, such as listeriosis, salmonellosis, brucellosis, tuberculosis, and tularemia, where the bacteria are mainly intracellular, cell-mediated reactions seem to play a crucial role in recovery and resistance to infection. Studies in *Listeria*-infected mice showed that the protective cells were indeed T-cells (10). In other infections, such as viral hepatitis and measles, humoral immunity appears to play an important role, since protection can be achieved by the passive transfer of antibodies, a B-cell product. Little is known, however, about the relative importance and behavior of the two compartments of immune response in most of the common acute infections. This investigation was designed to detect shifts in the ratio between the two types of lymphocytes in peripheral blood during the course of acute infections.

MATERIALS AND METHODS

Patients. Studies of T- and B-lymphocytes were performed in 23 patients admitted to Bernalillo County Medical Center or Veterans Administration Hospital in Albuquerque, N.M. The mean age was 51.8 years (range of 24 to 78). All patients but one were male. Nineteen patients had bacterial infections: 10 had pneumonia, three had urinary tract infection, two had staphylococcal abscesses, and one patient each had *Bacteroides* septicemia, tuberculous pleuritis, pleural empyema, and acute cholecystitis. Of the four remaining patients, two had undifferentiated respiratory disease of presumed viral etiology, and two had mycoplasmal pneumonia.

Lymphocyte separation. Lymphocytes were isolated from heparinized peripheral blood as described by Böyum (1) and were washed three times in Hanks balanced salt solution. Differential counting of Wright stained smears usually showed more than 90% mononuclear cells. In a few instances, however, considerable numbers of polymorphonuclear cells were present. These could easily be distinguished by their size and multinucleated appearance. Since it is very difficult to differentiate large lymphocytes from monocytes in unstained preparations, only small and medium-sized lymphocytes were counted in the studies described below. The percentage of large lymphocytes was small, ranging from 0 to 5%.

Surface Ig-bearing lymphocytes (B-cells). Antisera to human immunoglobulins were prepared and conjugated with FITC, and the lymphocytes were stained for surface IgG, IgA, and IgM as previously described (7, 12). The number of fluorescent small and medium-sized lymphocytes per 200 cells was determined. The percentage of B-cells was calculated from the sum of cells staining for IgG, IgA, and IgM, since previous work using anti-$F(ab)_2$ fluorescent reagents had indicated that most cells bear one major Ig class of surface immunoglobulins (6).

EAC3-binding lymphocytes (B-cells). B-cells were also enumerated in some patients by using the EAC3 method previously described (12). Sheep erythrocytes were sensitized with rabbit amboceptor, and the first four components of human complement (EAC1423). The percentage of lymphocytes capable

of binding EAC3 was determined, and results were compared with percentages of B-cells obtained by direct immunofluorescence.

E-binding lymphocytes (T-cells). T-cells were enumerated by the sheep cell rosette method as outlined by Frölund (5) and Jondal et al. (9). Two hundred lymphocytes were counted, and all cells binding at least three sheep red blood cells were considered positive.

Controls. E-binding and surface Ig-bearing lymphocytes were determined concurrent with the patient studies in 14 normal subjects. All of the controls and patients were studied by the same person. In addition to this, the normal values for E-binding and EAC3-binding lymphocytes as well as Ig-bearing lymphocytes have been determined in this laboratory on the basis of large numbers of observations in normal individuals. The results are summarized in Table 1. Two measures of standard deviation are shown. That designated as "between individuals" was calculated by using the mean of values when several observations were made in the same individual. "Within individuals" refers to the variability of values in single individuals over time.

RESULTS

The proportions of T- and B-cells were determined in 18 patients with acute bacterial infections and four patients with viral or mycoplasmal infections. Three or more serial determinations were done in 11 patients, and two were done in seven patients. Single observations were made in an additional four cases. The results are summarized in Fig. 1 and 2 and in Table 2. The data are separated into three groups according to the time from onset of disease to test day. The observations in the first group were all done within six days after onset, when the patients were still febrile. In cases where serial determinations were possible, the second test was usually done 4 to 5 days after the first. The second group (7 to 14 days) also includes the first determination in five patients with a history of fever for more than a week. The third group represents the convalescent stage; these latter determinations were done when the patients had been afebrile for at least nine days.

TABLE 1. *Normal values for peripheral blood lymphocyte markers*

Lymphocytes	No. of individuals	No. of observations	Mean %	One standard deviation	
				Between individuals	Within individuals
Ig-bearing	37	85	20.2	6.4	1.9
EAC3-binding	81	112	19.2	7.8	2.5
E-binding	45	74	63.1	15.8	4.9

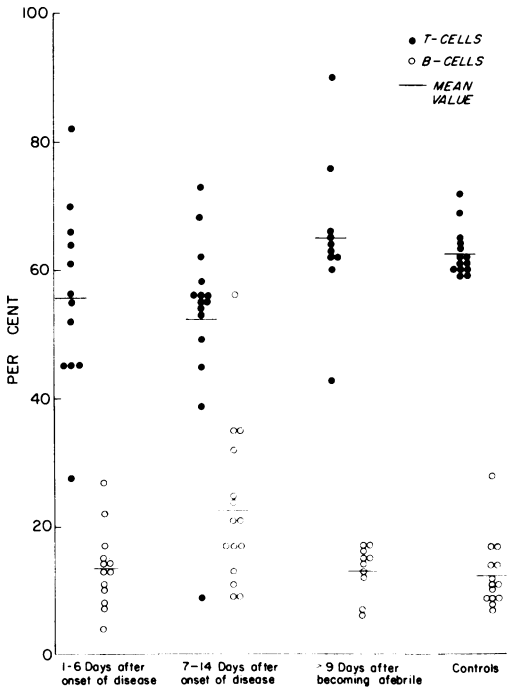

FIG. 1. *Percentages of T- and B-lymphocytes in 18 patients with acute bacterial infections and 14 normal individuals.*

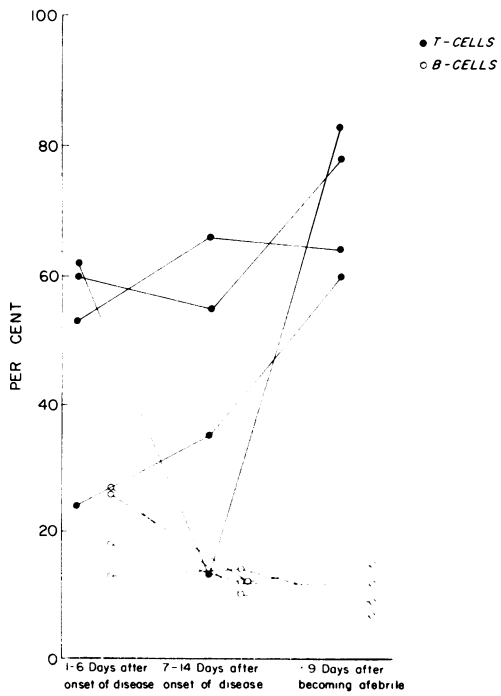

FIG. 2. *Percentages of T- and B-lymphocytes in four patients with viral or mycoplasmal infections.*

TABLE 2. *Percentage of Ig-bearing and E-binding lymphocytes in 18 patients with acute bacterial infections*

Patient	Ig-bearing lymphocytes (%)[a]												E-binding lymphocytes (%)[a]		
	IgG			IgA			IgM			Σ-Ig					
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
J.G.	6	5		0	2		2	2		8	9		45	55	
R.F.	5	20	3	0	1	1	6	4	2	11	25	6	64	53	62
G.W.		15			1			5			21			56	
R.A.		11	7		3	1		3	4		17	12		45	60
P.C.	7	10	8	2	2	1	4	5	6	13	17	15	56	68	65
R.C.	8			2			4			14			61		
A.G.	3	7	3	2	1	2	2	3	2	7	11	7	52	55	90
R.G.		50			2			4			56		56	54	
J.R.	7			1			2			10					
K.P.	11			2			2			15			66		
P.A.	15	7		3	2		4	4		22	13			9	
M.C.	2	5	7	2	3	2	0	1	4	4	9	13	45	62	43
R.N.	4	10	9	2	1	1	8	6	1	14	17	17	28	49	63
S.M.	10	13	8	1	4	1	2	7	5	13	24	14	45	58	66
C.D.	16	8	10	1	4	2	10	20	5	27	32	17	70	39	62
J.C.	6	21	10	1	2	1	10	12	4	17	35	15	82	73	76
J.M.		14	9		2	2		5	5		21	16		56	64
C.A.		13			2			20			35			56	
Mean	7.7	13.9	7.4	1.5	2.1	1.4	4.3	6.7	4.4	13.5	22.8	13.2	55.8	52.5	65.1
SD										6.1	12.6	3.9	14.2	14.6	11.9

[a] I, 1 to 6 days after onset of disease; II, 7 to 14 days after onset of disease; III, more than 9 days after becoming afebrile.

T-lymphocytes. The percentage of T-cells was depressed in the acute stages of both bacterial and viral or mycoplasmal infections. The mean T-cell value in the group with bacterial infections was 55.7 ± standard deviation 14.2% in the first stage and 52.5 ± 14.6% in the second determination, which was followed by a rise to 65.1 ± 11.9% in convalescence. The depression in the second stage was significant in relation to both convalescence ($P < 0.02$) and normal controls ($P < 0.01$) (Wilcoxon rank sum test). Looking at serial determinations in individual patients, eight of 14 had significantly ($P < 0.05$) lower T-cell levels in one or both of the acute stages compared with convalescent values. This calculation of significance was based on the fact that these values were greater than 2.77 times the standard deviation in serial determinations performed in normal controls (Table 1). No patients had significantly lower values in convalescence. Very low recordings of T-cells were observed in four patients who had no apparent common clinical characteristics. The lowest value for T-cells (9%) occurred in a severely ill patient with subphrenic abscess and *Bacteroides* septicemia, which developed after resection of a carcinoma of the colon. Unusually low values were also recorded in one patient each with multiple sclerosis and undifferentiated respiratory disease, mycoplasmal pneumonia, pleural empyema, and staphylococcal abscesses.

B-lymphocytes. Both patient groups had increased levels of B-cells during some stage of the infection. Three of four patients with viral or mycoplasmal infections showed the highest recordings in the first stage. On the other hand, nine of 11 patients with bacterial infections where serial determinations were possible showed the highest B-cell proportions in the 7- to 14-day observation period. The mean value in the second determination (22.8 ± 12.6) was significantly ($P < 0.05$) elevated compared with convalescence (13.2 ± 3.9) and the 14 normal controls (12.8 ± 5.9). The difference in B-cell response between bacterial and viral or mycoplasmal infections was still more striking when the lymphocytes were separated into three groups according to the class of surface immunoglobulin (Fig. 3 and 4, Tables 2 and 3). The most striking changes occurred in the percentage of IgG-bearing cells, whereas IgA- and IgM-bearing cells remained fairly constant. The number of IgG lymphocytes was greater than the number of IgA or IgM in all but five patients and in all controls. Only one patient with

staphylococcal abscesses showed a higher count of IgM lymphocytes than IgG in more than one determination. Three other patients had higher numbers of IgM-bearing cells only in the first of three serial observations, and one showed IgM dominance in one single determination. These patients included one with acute cholecystitis (no organisms isolated), one with *Klebsiella* pneumonia, one with *Klebsiella* urinary tract infection, and one individual with culture-negative empyema.

One case with exudative tuberculous pleuritis was studied in addition to the patients with acute infections. This case is not included in the figures. The cells of the pleural effusion were studied about 6 weeks after onset of symptoms. Close to 100% were small lymphocytes, 87% of which were T-cells. No Ig-bearing cells were detected, and only 4% were EAC3 binding. Simultaneous determinations of peripheral blood lymphocytes showed 70% T-, 21% EAC3-binding, and 20% Ig-bearing cells.

In most instances, proportions of B-cells as determined by the C3 receptor technique produced values relatively close to those obtained by using sums of cells staining for IgG, IgA, and IgM. However, four exceptions to this were noted in the individuals showing the highest values for B-cells by surface immunoglobulin

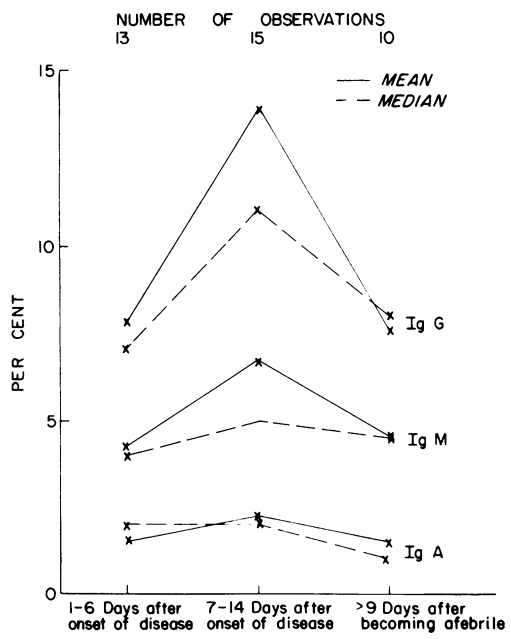

FIG. 3. *Mean and median percentages of B-lymphocytes carrying IgG, IgA, or IgM in 18 patients with acute bacterial infections.*

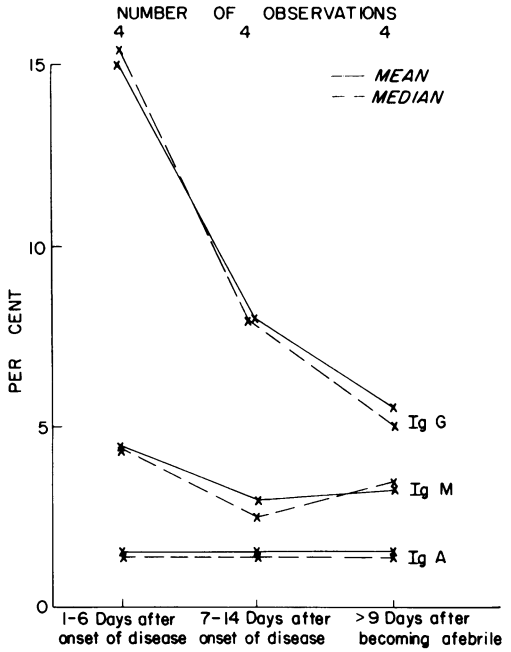

FIG. 4. *Mean and median percentages of B-lymphocytes carrying IgG, IgA, or IgM in four patients with acute viral or mycoplasmal infections.*

TABLE 3. *Percentage of Ig-bearing and E-binding lymphocytes in four patients with viral or mycoplasmal infections*

Patient	Ig-bearing lymphocytes (%)[a]												E-binding lymphocytes (%)[a]		
	IgG			IgA			IgM			Σ-Ig					
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
J.B.	11	10	7	2	1	2	5	2	3	18	13	12	53	66	64
M.C.	21	8	9	1	2	2	4	3	4	26	13	15	62	13	83
T.F.	20	8	3	2	1	1	5	1	2	27	10	6	24	35	60
J.O.	8	6	4	1	2	1	4	6	4	13	14	9	60	55	78
Mean	15	8	5.5	1.5	1.5	1.5	4.5	3	3.2	21	12.2	10.2	49.7	42.2	71.2

[a] Groups I, II, and III were as in Table 2.

summation, whereas at the same time C3 determination showed much lower percentages (Table 4).

Correlation between T- and B-lymphocytes. Although there was a tendency to inverse relationship between the percent of T- and B- cells, the increase in T-cells could usually not be accounted for by a corresponding decrease in B-cells, and vice versa. This was particularly striking in the five patients with unusually low T-lymphocyte proportions. Four of them also had among the highest B-cell recordings, but the lowest T-cell count coincided with the highest B-cell count in only one patient. In other words, the sum of the percentage of T and B lymphocytes was not constant. In three patients, the sum exceeded 100%. All of these individuals had high B-cell percentages and are included in Table 4.

Null lymphocytes. Cells which could not be classified as either T- or B-cells were called "null cells" (19). The mean percentage of null cells in the patients with various infections was 31.6% in the first determination, 27% in the second, and 22.1% in convalescence. In normal controls, the average percentage of null cells was 24.5%.

Differential white blood counts were performed in most acutely ill patients. No correlation existed between the percentage of T- and B-cells and relative or absolute numbers of polymorphonuclear leukocytes, monocytes, or lymphocytes, nor was there any apparent correlation between T- and B-cell levels and severity of disease, drug therapy, age, or concomitant other diseases.

DISCUSSION

Our studies indicate interesting serial changes in relative proportions of T- and B-lymphocytes during the course of acute infections in man. These changes must in some way be correlated with important events in the host immune response.

Crowther et al. (4) have studied the morphological changes in the lymphocyte population in peripheral blood in man after artificial immunization and acute infections. In nonimmunized subjects, about 80% of the lymphocytes were classified as small and 20% as medium-sized, whereas the number of large lymphoid cells was 0.5%. The cellular response to immunization with different kinds of inactivated viral and bacterial antigens involved an increase in the number of medium-sized and large lymphocytes and the appearance of about 0.5% plasma cells. The number of large cells never exceeded 4%. Similar changes were noted in patients with various viral and bacterial infections. Also, in hypersensitivity reactions (20) and in autoimmune disease (3) similar lymphoid cell responses have been reported. With the advent of methods for determination of T- and B-cells, it has become possible to study the variation in these subpopulations of lymphoid cells in response to antigenic stimulation. The large lymphocytes are not included in our study. This should not significantly influence the results, since the number of large lymphocytes was small.

Studies from this laboratory have shown a diminution of T-cells and an increase in B-cells in some patients with active systemic lupus (R. P. Messner, F. D. Lindstrom, and R. C. Williams, Jr., J. Clin. Invest. **52**:56A) and rheumatoid arthritis (19). Wybran et al. (21) recently reported low levels of rosette-forming lymphocytes in viral infections in contrast to bacterial infections. The method used, however, measured only a subpopulation of T-cells. In the current study, significant decreases have been observed in the percentage of T-lymphocytes in patients with different kinds of acute infections, whereas the proportion of B-lymphocytes was increased.

TABLE 4. *Summary of data showing unusual differences in surface Ig and C3 receptors in four patients with severe acute infections*

Patient	Diagnosis	Age	Sex	B-cells (%)[a]				T-cells (%)		Sum of Ig-bearing and E-binding cells	
				Σ-Ig		C3					
				I	II	I	II	I	II	I	II
D.M.	Tuberculous pleuritis	37	M	62	20	15	21	68	70	130	90
R.G.	Bilateral lobar pneumococcal pneumonia	30	M	56		17		54		110	
J.C.	Acute cholecystitis	79	M	35	15	23	14	73	76	108	91
C.D.	Staphylococcal abscess	24	M	32	17	15	11	39	62	71	79

[a] I, Acute stage; II, convalescence.

The cause of the T-cell depression is not known. It cannot be explained solely as a consequence of increased numbers of B-cells. The decrease in T-cells was rather concomitant with increase in the percentage of those lymphocytes which cannot be determined as either T- or B-cells, the so-called null cells. A possible explanation for the apparent disappearance of the T-cells could be a rapid dissemination of the cells to the actual tissue lesion, while the null cells could represent newly formed lymphocytes which have not yet acquired their surface immunoglobulins. We have, however, no direct evidence to support such a hypothesis. Almost all of the lymphocytes from pleural fluid from a patient with pleural tuberculosis were shown to be T-cells, but the test was done late in the disease when peripheral blood lymphocytes were normalized. Another possibility that must be considered in that some alterations in serum secondary to the infection might affect the T-cell receptors for sheep red cells. Antibody-associated lymphotoxin was recently demonstrated in acute viral infections (8). It is of interest that the lowest percentage of T-cells (9%) was recorded in a severe case of septicemia due to *Bacteroides*, which is an endotoxin producer. Endotoxin is known as a B-cell mitogen (13) and it is conceivable that the high number of null cells in this case represents young B-cells.

The increase in the proportion of B-cells occurred earlier in the small group of viral or mycoplasmal infections than in the bacterial infections. One plausible explanation for this is that the lymphocyte changes represented secondary and primary immune response, respectively. It is also possible that bacteria are less potent immunogens. Also, the morphological changes in the lymphocyte population are more pronounced in viral infections (4).

In three patients with high B-cell counts, the sum of T- and B-cells exceeded 100%, suggesting that some cells were falsely determined as B-cells. It is also suggestive that these patients showed no corresponding increase in EAC3-binding cells. Similar discrepancies have been observed in patients with active systemic lupus erythematosus (R. P. Messner, F. D. Lindstrom, and R. C. Williams, Jr., J. Clin. Invest. **52**:56A). It is possible that the EAC3 technique measures only a subpopulation of lymphocytes which is relatively unaffected during the immune response. The existence of B-lymphocytes with only one of the two surface markers has recently been documented in chronic lymphatic leukemia (18). Since the percentage of B-cells was calculated from the sum of cells bearing IgG, IgA, and IgM, incorrectly high values may be obtained if the lymphocytes contain more than one surface immunoglobulin. Double staining was not performed in this study. A recent report by Wernet et al. points to another possible source of falsely high levels of B-cells (P. Wernet, M. Fotino, R. Thoburn, A. Moore, and H. G. Kunkel, presented at the American Rheumatism Meeting, 9 December 1972. Pittsburg, Pa.). They found that cell surface Ig detected by immunofluorescence in some systemic lupus erythematosus patients may be markedly reduced by incubation at 37 C, which might be the result of elution of adsorbed immunoglobulins or endocytosis of surface Ig. The possibility of adsorbed antibodies is supported by the reports of Lee and Paraskevas (11). They found a brief but striking increase in spleen Ig-bearing cells after a single antigenic stimulation in mice. The increase was shown to be due to acquisition of cytophilic IgG by T-cells, perhaps in the form of antigen-antibody complexes.

This study is an initial attempt to describe the changes in the lymphocyte subpopulations in response to acute infections. Considerably more data are needed to evaluate the significance and reliability of current methods for determinations of T- and B-cells in disease and to confirm our findings in greater numbers of patients with different kinds of infections.

ACKNOWLEDGMENTS

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