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R D Lueker, … , Z H Abdin, R C Williams Jr

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Research Article

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Peripheral Blood T and B Lymphocytes during Acute Rheumatic Fever

RICHARD D. LUEKER, Z. H. ABDIN, and RALPH C. WILLIAMS, JR.
with the technical assistance of KATHLEEN A. KILPATRICK

From the Department of Medicine, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131 and Department of Pediatrics, Free Rheumatic and Heart Center, Cairo, Egypt

ABSTRACT Proportions and total numbers of thymus-derived (T) and bone marrow-derived (B) peripheral blood lymphocytes were studied in 53 patients with acute rheumatic fever, diagnosed on the basis of modified Jones criteria. An elevation in both proportions and absolute numbers of cells bearing surface Ig was found in most patients, particularly during the first 7 days after onset. Conversely, T-cell proportions and numbers were often found to be depressed early in the acute phases of rheumatic fever. Proportions of cells bearing surface Ig did not correlate with another B-cell marker, the aggregated gamma globulin receptor, suggesting that such cells bearing surface Ig were not all B lymphocytes. Incubation for 20 h at 37°C of cells showing high proportions of surface Ig-bearing cells resulted in diminutions of proportions of cells bearing surface Ig in both normal and rheumatic fever subjects, although there was no appreciable increment in proportions of lymphocytes expressing T-cell markers. Patients with initial attacks showed higher percentages and total numbers of Ig-bearing lymphocytes ($P < 0.01$) than did those with rheumatic fever recurrences. Elevations in numbers and proportions of peripheral blood lymphocytes bearing Ig appeared to correlate with the relative acute nature of the rheumatic fever attack.

INTRODUCTION

Acute rheumatic fever is a disease characterized by polyarthritis, serositis, and carditis, in which many cells in the inflammatory lesions appear to be lymphocytes. Indeed, the basic cellular constituents of rheumatic subcutaneous nodules, cardiac Aschoff bodies, or acute valvular lesions are often characterized by a predominance of lymphoid elements. The present study was designed to examine possible alterations in peripheral blood lymphocytes during the course of active rheumatic fever. Particular attention was directed at acute and serial studies of lymphocyte cell surface markers in this disorder. For some time it has been held as an established clinical principle that acute rheumatic fever is somehow related to the host immune response to the streptococcus (1–7). Whether this response is principally mediated by humoral immunity and thus the products of bone marrow-derived lymphocytes (B-cells)[1] (8, 9) or whether cell-mediated mechanisms operating through thymus-derived lymphocytes (T-cells) (10–12) constitute the most significant aspect of the rheumatic inflammatory response still remains to be determined. A recent report by Read, Fischetti, Utermohlen, Falk, and Zabriskie (13) emphasizes a clear relationship between cell-mediated immunity to various streptococcal cell wall antigens and acute attacks of rheumatic fever. The current study indicates that typical rheumatic fever is associated with a characteristic alteration in peripheral lymphocyte profiles that suggests intense participation of both humoral and cell-mediated immunity during the acute process. The availability of a large number of acutely ill patients at the Free Rheumatic and Heart Center in Cairo, Egypt, together with a smaller group of patients studied in Albuquerque made the current study of 53 patients possible.

METHODS

Patients

Albuquerque group. The Albuquerque group of 15 patients was derived from individuals hospitalized at Bernalillo County Medical Center, Presbyterian Hospital, or St.

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[1] *Abbreviations used in this paper:* B-cell, bone marrow-derived lymphocyte; E, erythrocyte; T-cell, thymus-derived lymphocyte.

Joseph's Hospital, Albuquerque, N. M., between September 1972 and September 1974. All of these patients were diagnosed as having acute rheumatic fever on the basis of clear evidence of migratory polyarthritis, antecedent streptococcal infection, and clinical confirmation of active carditis. All patients included in the present study satisfied the modified Jones criteria for acute rheumatic fever (14). None of the Albuquerque patients had chorea. 12 were young adults, aged 19–32; (mean age 22), 3 were teenagers, aged 13, 14, and 16. 13 gave no previous history of acute rheumatic fever; 2 had at least one antecedent rheumatic fever attack. 11 of the 12 adult Albuquerque patients were studied during the 1st wk of acute symptoms; one patient had chronic recurrent carditis and arthritis of five mo duration. One patient in the teenage Albuquerque group was studied within 1 wk of initial symptoms; two were studied between 7 and 30 days after initial symptoms.

In six Albuquerque patients serial studies over a 1–3 mo interval were possible as the initial acute rheumatic episode subsided. Five of these patients received salicylates alone. Only two of the patients in the entire Albuquerque group were treated with corticosteroids (20–60 mg of prednisone/day for fulminant carditis).

Egyptian patients. A second group of 38 patients were studied during 3 wk in May 1974 at the Free Rheumatic and Heart Center Hospital, Cairo, Egypt. Most of the Egyptian patients were individuals with acute rheumatic fever of recent onset. As in Albuquerque, the patients studied in Cairo were subdivided into three groups. 24 patients were considered very acute (group A), first being studied within 7 days after initial onset of symptoms; another group of 8 patients (subacute group B) was initially studied between 7 and 30 days after onset of first symptoms; and a third group of 6 patients was studied during what was considered chronic rheumatic activity of 1–6 mo duration (chronic group C).

8 of the 24 acute group A Cairo patients had active chorea at the time of study. 14 showed clear evidence of active carditis and arthritis at the time of study and 5 showed migratory arthritis as the major manifestation of their rheumatic activity. Three serial determinations every 5–7 days were completed on most patients in the acute group. Among the 24 patients included as acute, 11 were experiencing what appeared to be an initial attack of rheumatic fever, whereas 13 gave a history of previous attacks. The patients studied in Cairo constituted a unique group, since because of the referral pattern to the Free Rheumatic Heart Center, patients with prior attacks had often been previously hospitalized at the same facility. Age range in the acute Egyptian patients was from 5 to 17, with a mean age of 10 yr. This group included eight boys and 16 girls.

Group B or the subacute Egyptian patients ranged in age from 6 to 15 (mean 11 yr) with three girls and five boys. Two patients in this group had active chorea, six had clear evidence for carditis and arthritis, and one patient showed migratory polyarthritis alone. Two patients in group B were experiencing what appeared to be their first attack of rheumatic fever and six gave a history of previous rheumatic episodes.

The six patients in group C (three boys and three girls, mean age 11) all showed clear evidence of chronic carditis. One patient showed persisting active chorea and two patients continued to experience arthritis. Three of these six chronic patients were in their first attack; the other three had previous history of rheumatic activity.

Among the Cairo patients, prolonged serial studies beyond 3 wk were not possible; however, on the whole the degree of pancarditis and intensity of clinical involvement were judged to be somewhat more severe than most of the patients studied in Albuquerque. Because of this and because a majority of the Albuquerque patients were young adults, somewhat older than the Egyptian patients, the two groups of patients were considered separately in most instances.

35 normal adult controls were drawn from students, staff, and laboratory personnel in both Albuquerque and Cairo. In addition, a group of 28 normal children, matched insofar as possible for age and sex primarily with the patients studied in Egypt, was included. No differences were noted in normal control values recorded among adult or child controls studied in Albuquerque and Cairo. Normal control children were either hospitalized for elective orthopedic surgery or drawn from normal healthy subjects, since no published data were available for B- and T-cell values in this age group.

Lymphocyte determinations

All lymphocyte determinations in both Albuquerque and Egypt were made by the same individual (Ms. Kilpatrick). Peripheral blood samples were drawn in heparinized syringes and lymphocytes separated at room temperature (22°C) with Hypaque-Ficoll gradient centrifugation (15, 16). No attempt was made to remove monocytes; however, total white blood cell counts and differentials were performed on all samples tested. No patient with acute rheumatic fever had more than 5% monocytes on peripheral smear. Moreover, direct estimation of phagocytic cells present in Ficoll-Hypaque lymphocyte preparations was performed with small polyacrylamide beads added directly to such preparations, as previously described (16), and monocytes were thus identified and excluded directly under phase microscopy or during immunofluorescence microscopy.

Cells bearing surface immunoglobulin and initially presumed to be B cells were determined with direct immunofluorescence and staining for surface IgG, IgA, and IgM, with fluorescein-conjugated anti-Ig antibodies isolated from specific immunoabsorbent columns (9, 16). Initially, the sum of cells staining for IgG plus IgA plus IgM was taken as an estimate of the percent B-cells, on the basis of previous work demonstrating that most B-cells bear one major class of surface immunoglobulin (16–18). In some instances, when values for B-cells were adjudged to be high, total numbers of cells staining for kappa plus lambda light chain determinants were also recorded.

In addition, in many patients studied in Cairo, concurrent B-cell determinations were performed by the method of Dickler and Kunkel (19), with fluoresceinated IgG aggregates. After initial studies had indicated what appeared to be uniformly high values for cells showing surface Ig, some patients' cells were studied before and after overnight incubation in balanced Hanks' solution at 37°C in a 5% CO_2 incubator, and surface Ig characteristics were reassessed after 20 h to determine if cell-associated Ig remained associated with the membrane or was eluted off and not regenerated during such short-term culture. Careful evaluation of percent recovery and viability of such cultured lymphocytes was performed in all instances by direct counting and supravital dye staining before and after culture.

T-cells were enumerated by two independent methods. Indirect immunofluorescence with rabbit anti-human fetal thymocyte antiserum, exhaustively absorbed with human B-cells as previously described (20–23), represented one method of T-cell identification. Anti-human thymocyte antiserum used at 1:2–1:5 dilution was followed by fluorescein-con-

TABLE I

Comparative Normal Values for Percentages and Total Numbers of T- and B-Cells in 29 Child and 35 Adult Controls

	Percentages	Total numbers
	%	mm^{-3}
Children		
B-cells	26±7.9	956±474
T-cells		
Anti-T cell	72±7.5	2,552±751
E-binding	67±6.0	2,362±740
Adults		
B-cells	22±5.5	543±289
T-cells		
Anti-T cell	78±9.0	1,892±750
E-binding	65±10.0	1,571±607

Results are mean ±SD.

jugated goat anti-rabbit Ig and counting of cells showing distinct surface immunofluorescence under incident ultraviolet light.

In addition, the technique of erythrocyte (E)-binding or sheep cell rosette formation, adopted from methods previously described (24–26), was used as a second independent method for enumeration of T-cells. Sheep cell rosette formations were always performed in phosphate-buffered saline, pH 7.4, without the addition of exogenous human or fetal calf serum. Lymphocytes were incubated with sheep erythrocytes for 12–14 h at 4°C and rosettes subsequently scored as positive in any cell showing three or more adherent erythrocytes. Sheep cells from the same sheep were obtained fresh each week and were never used when more than 7 days old. In general, as previously noted (21, 23, 27), T-cell values from the indirect immunofluorescence technique were slightly higher than those obtained with the sheep cell rosette method. Total absolute numbers of T and B lymphocytes were calculated from absolute lymphocyte counts done concurrently with each sample.

To assess the reliability of the methods used, sums of T- plus B-cells were performed. In all normal adult and children's controls such summation gave values ranging between 95 and 102% (mean 98.9%). However, in a considerable number of the patients with acute rheumatic fever, sums of T- plus B-cells were somewhat greater than 100%, by either T-cell values by indirect immunofluorescence or sheep cell rosette binding. Because it appeared that extremely high values for lymphocyte surface immunoglobulin (presumably B-cells) were present, an attempt was made to see if the sera from such patients contained cytophilic Ig that might produce artificial or apparent increases in cell surface Ig on lymphocytes. Direct immunofluorescence with polyvalent fluorescein-conjugated anti-Ig with reactivity for IgG, IgA, and IgM, as well as for kappa and lambda determinants, was performed before and after incubation of serum or plasma samples for 4 h at 37°C with a panel of six normal subjects' lymphocytes.

RESULTS

The values for T- and B-cells obtained with the normal children's control group were slightly different from those recorded for adult controls. Of some importance were the higher values for cells bearing surface immunoglobulin as well as total T-cell numbers in children. Our comparative normal values for adults and children are shown in Table I.

Albuquerque group. Studies among the 12 young adults and 3 teenage patients seen in Albuquerque indicated a distinct elevation among over half the patients in proportions of peripheral blood lymphocytes bearing surface Ig during the initial phases of acute rheumatic fever. In addition, total numbers of peripheral blood lymphocytes bearing surface Ig were increased in most patients. These data are shown in Table II. Whether or not all of these cells represented true B-cells appeared problematical, since summation of cells bearing surface IgA, IgG, and IgM in two patients gave values of 68% and 97%, respectively. Summation of cells staining for kappa and lambda determinants in these instances gave values of 35 and 46% respectively.

Studies of initial proportions of peripheral blood T-cells by both immunofluorescence and E-binding showed a tendency to lowered values among a considerable fraction of patients ($P < 0.01$–0.05). These data, along with values for total numbers of peripheral blood T-cells, are also shown in Table II.

Serial studies of proportions and total numbers of peripheral blood lymphocytes present during the evolution of acute rheumatic episodes were performed in six

TABLE II

Percentages and Absolute Numbers of T and B Cells in 12 Adult Albuquerque Patients with Acute Rheumatic Fever and 35 Controls

			T cells			
	B cells		Anti-T		E-binding	
	%	mm^{-3}	%	mm^{-3}	%	mm^{-3}
Rheumatic fever patients	30±14	842±530	62±13	1,732±694	57±12	1,578±543
Controls	22±5	543±289	78±9	1,892±750	65±10	1,571
P	NS	<0.02	<0.01	NS	=0.05	NS

Results are means ±SD.

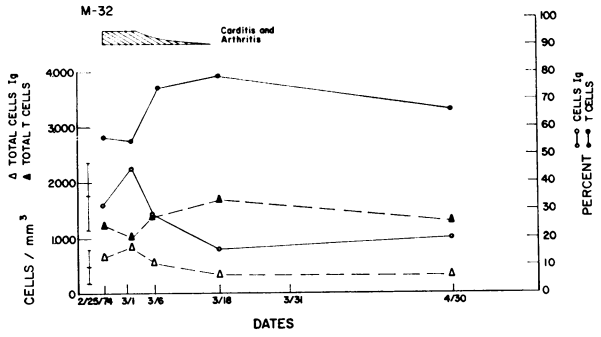

FIGURE 1 Serial studies in a 32-yr-old man with acute rheumatic fever of proportions and total numbers of peripheral blood lymphocytes bearing surface Ig, as well as T-cells as measured by indirect surface immunofluorescence.

patients. In all patients studied, initial total numbers of T-cells were either slightly depressed or at the lower range of normal, increasing slightly or steeply over the 3 wk–2 mo observation periods after the acute attack. In all six of the Albuquerque patients followed with serial studies, initial proportions or percentages of T-cells were moderately decreased and gradually increased towards normal as the rheumatic attack subsided.

All six patients serially studied showed an initial high value or peak for total numbers of lymphocytes bearing surface Ig during the first 14 days of study, with gradual decline in total numbers of cells bearing surface Ig as the attack subsided. Similarly percentages of cells with surface Ig showed highest values during the initial 2 wk of rheumatic fever, again gradually subsiding during the acute attack. Representative examples of such serial studies are shown in Fig. 1 and in Table III. Only one patient, M. C. (Table III), received corticosteroids; the remainder were treated with rest and salicylates.

Egyptian group. The principal finding among the patients studied in Cairo was a marked elevation in proportions of lymphocytes bearing surface Ig. It can be seen from Fig. 2 that the highest values for cells showing surface Ig were encountered among the acute group A patients, with apparent progressive diminution of proportions of lymphocytes showing surface Ig in the subacute and chronic patients (groups B and C, respectively). In addition, values for total absolute

TABLE III

Serial Studies in Six Patients of T-Cells and Cells Bearing Surface Ig during Acute Rheumatic Fever

Patient	Age	Sex	Date	Sum of percentages of cells with Ig	T-cells		T & B	Total B cells	Total T-cells	
					AT‡	E-binding‡			AT	E-binding
	yr				%		%			
F. Y.	29	M	1/05	35	51	63	86/98	476	693	856
			1/09	42	61	43	103/85	1,685	2,447	1,725
			1/16	18	82	—	100/—	629	2,866	—
J. W.	23	F	10/18	42	48	61	90/103	1,517	1,733	2,203
			10/24	21	40	47	60/67	518	984	1,156
			11/29	21	68	72	89/93	—	—	—
L. C.	18	F	12/07	39	60	31	99/70	1,135	1,746	902
			12/12	46	51	58	97/104	1,288	1,428	2,216
			1/28	31	70	62	101/93	1,249	2,821	2,498
			3/11	25	71	61	96/86	720	2,044	1,756
M. C.	24	F	12/11	68*	46	53	114/121*	2,176	1,472	1,696
			12/17	72*	49	73	121/145*	1,578	1,487	—
			1/11	46	56	48	102/94	1,288	1,568	1,344
B. G.	32	M	2/25	31	56	65	87/96	653	1,223	1,370
			3/01	45	55	36	100/81	826	1,010	660
			3/06	29	74	71	103/100	546	1,394	1,338
			3/18	16	78	72	94/88	337	1,647	1,520
			4/30	20	63	62	83/82	328	1,312	1,016
M. C.	14	M	1/17	97*	37	53	134/150*	1,407	1,037	1,491
			1/23	52	52	55	104/107	1,914	1,766	2,024
			1/30	45	60	57	105/97	2,635	3,514	3,338
			3/20	51	52	48	103/99	2,040	2,080	1,920

* In instances where patients showed very high proportions of cells bearing surface Ig, total numbers of B-cells were estimated with 100 minus percent T-cells as relative B-cell value.

‡ AT refers to T-cells determined by indirect immunofluorescence and E-binding to T-cells determined by sheep cell rosettes.

numbers of lymphocytes with surface Ig were significantly elevated ($P < 0.001$) above control values in acute group A as well as in group B patients ($P < 0.05$). These data are shown in Fig. 3A. Confirmation of the specificity of surface Ig staining was achieved by blocking experiments with unlabeled specific anti-IgG, -IgA, or -IgM. Preincubation of test cells from patients with acute rheumatic fever with unconjugated specific antisera blocked subsequent surface Ig staining. In addition, absorption of specific anti-Ig conjugates with their respective insolubilized antigens eliminated surface Ig staining on test lymphocytes.

Assay for proportions of B-cells in the entire Cairo group with human aggregated γ-globulin and the aggregate receptor gave values considerably lower than those obtained with summation of cells staining for IgG, IgA, and IgM. Examples of such comparative determinations are shown by the open circles in Fig. 2, as well as in Fig. 3B and in Table IV. Our values for normals studied concurrently, however, were considerably lower than those originally reported by Dickler and Kunkel (19) and generally ran one-half to one-third the values for surface Ig fluorescence (7–12%, as opposed to 22% of cells by surface immunofluorescence).

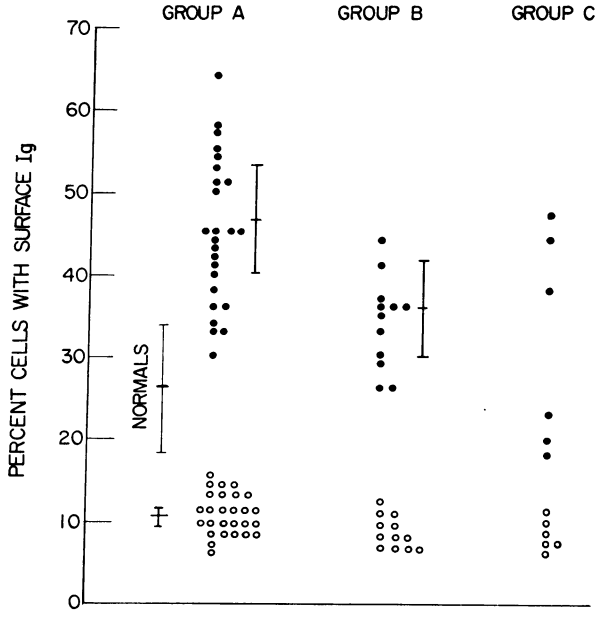

FIGURE 2 Scattergram showing percentages of lymphocytes showing surface Ig (●) in acute patients, group A, subacute group B, and chronic Cairo patients, group C. Mean and one SD for normal children's controls are shown to left. Differences between normals and group A, $P < 0.001$, and for group B, $P < 0.02$. Shown below in open circles are the proportions of cells with aggregate or Fc receptors. No significant differences were noted between normals 10.5±1 and rheumatic fever subjects with this concomitant B-cell marker.

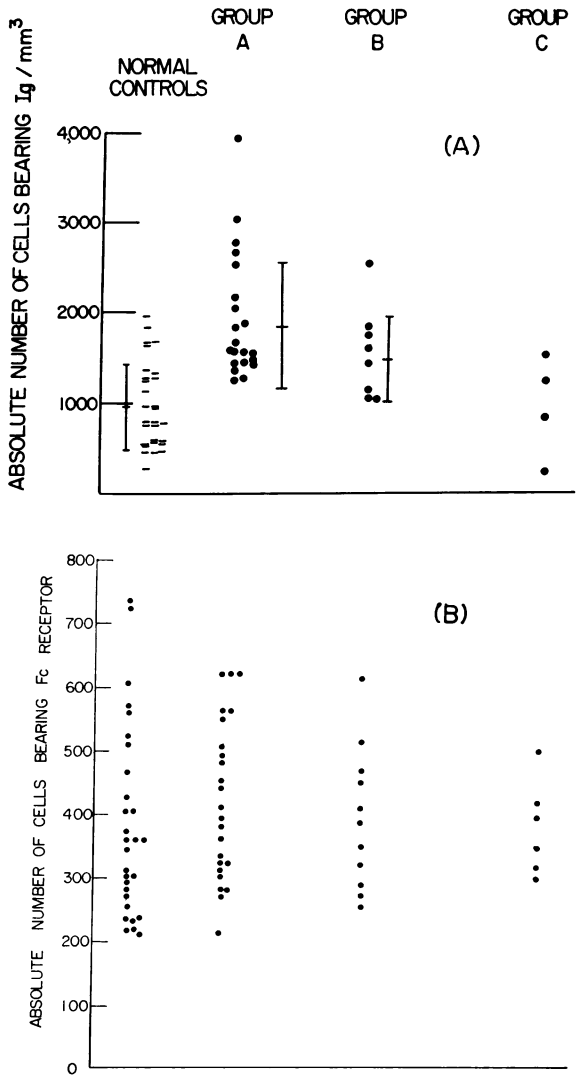

FIGURE 3 (A) Scattergram showing total numbers of cells bearing surface Ig in Cairo Groups A, B, and C as compared to normal controls. (Group A, $P < 0.001$ and group B, $P < 0.05$). (B) Similar comparison is shown for numbers of peripheral blood lymphocytes reacting with the aggregate or Fc receptor. No difference was noted between normals shown on left and patient groups A, B, and C studied.

These data appeared to indicate that cells bearing surface Ig were not all B-cells; therefore, whenever sums of T- and B-cells ran above 105%, the values for T-cells were subtracted from 100 to obtain estimates used for calculating total B-cells or cells with Ig, since we felt from results obtained with the aggregate-binding method that some cells showing surface Ig might be T-cells bearing adsorbed immunoglobulins.

A significant number of patients in the acute Cairo group A showed what appeared to be diminished pro-

TABLE IV

Representative Individual Data on T-Cells and Lymphocytes Bearing Surface Immunoglobulin among Cairo Patients with Acute Rheumatic Fever

Date	Patient no.	Percent cells			Sum cells with Ig	Cells bearing agg.-receptor	Percent T-cells		T & Ig cells
		IgA	IgG	IgM			Immuno-fluor.	E-bind.	
		%					%		%
	4154								
5/07	(Group A)	19	23	12	54	12	65	62	119/116
5/12		14	22	20	56	15	58	32	114/88
5/16		14	24	13	51	14	61	52	112/103
	4157								
5/07	(Group A)	10	26	14	50	10	57	65	107/115
5/12		13	15	16	44	12	50	38	94/82
5/16		20	27	17	64	8	44	51	115/105
	4151								
5/08	(Group A)	19	20	12	51	14	68	69	119/120
5/13		16	33	21	60	12	50	62	110/122
5/18		15	17	20	52	10	54	51	106/103
	4162								
5/07	(Group A)	8	21	11	40	10	62	50	102/90
5/12		6	24	14	44	12	60	55	104/99
5/16		15	26	17	58	11	46	49	104/107
	4151								
5/08	(Group A)	19	20	12	51	14	54	50	105/101
5/13		16	33	21	60	12	50	54	110/114
5/18		15	17	20	52	11	52	50	104/100
	4129								
5/09	(Group B)	8	16	12	36	10	64	61	100/97
5/16		13	24	14	51	12	48	50	99/101

portions or percentages of T-cells associated with rheumatic activity ($P < 0.001$); in addition a substantial reduction in proportions of T-cells was also recorded in group B ($P < 0.05$). However, no significant reductions were recorded in absolute numbers of T-cells, as calculated either by anti-T-cell immunofluorescence or sheep cell rosette binding techniques. These data are summarized in Table V.

A particularly interesting aspect of the data obtained in Cairo dealt with an apparent difference (Fig. 4) in proportions and numbers of cells showing surface immunoglobulin between children experiencing their first attack of rheumatic fever and those with a recurrence ($P < 0.01$). There appeared to be no definite tendency for low or high cell surface Ig percentages to be associated with chorea or any other definite clinical entity. No clear difference in proportions or total numbers of T-cells between patients with initial attacks and recurrences was noted, although values for percent T-cells tended to be lower in some patients with initial attacks.

As noted above, the most prominent finding among many of the Cairo patients, as well as some studied in Albuquerque, was the relatively high values for cells bearing surface Ig. Thus, when proportions of surface Ig-bearing cells were added to T-cell values, by either the anti-T-cell or E-binding techniques, percentages falling well above 100% were obtained in as many as one-third of group A patients. Some examples of these data are shown in Table IV. The elevated proportions of cells showing surface IgA or IgM (10–20%) indicate the dramatic increase in many classes of Ig on peripheral blood lymphocytes in acute rheumatic fever.

A limited series of studies were therefore conducted to ascertain the degree of shedding of lymphocyte surface immunoglobulins that occurred after 20-h incubations of lymphocytes in Hanks' solution at 37°C in a 5% CO_2 incubator. The numbers of these studies were initially limited by technical difficulties encountered with maintenance of sterile conditions at Cairo. However, many such successful experiments were subsequently carried out in parallel with observations using

TABLE V

Relative Percentages and Absolute Numbers of T Lymphocytes among Normal Controls and Acute (Group A) and Subacute (Group B) Cairo Patients with Acute Rheumatic Fever

	By immunofluorescence			By sheep cell rosettes		
	Normal controls*	Group A acute	Group B subacute	Normal controls*	Group A acute	Group B subacute
T cells, %						
Sample size	29	23	8	28	22	8
Mean	72	62	65	67	54	63
SD	7.5	10	7	6	11.7	4.5
P-value		<0.001	<0.05		<0.001	NS
Absolute numbers of T-cells, *per mm*^{3}						
Sample size	29	20	8	28	20	7
Mean	2,552	2,471	2,584	2,362	2,121	2,324
SD	751	961	653	740	757	532
P-value		NS	NS		NS	NS

* Normal control values obtained from 28 healthy children matched for age and sex from populations studied.

normal lymphocyte donors. Representative results of such studies (Table VI) showed a considerable drop in proportions of cells showing surface Ig among three of the five patients with rheumatic fever, while good recovery and viability of total lymphocytes incubated was maintained. However, similar declines in proportions of cells bearing surface Ig were also observed after parallel incubations of normal donor cells. Of interest were the relative constancy of proportions of cells retaining E-binding or surface immunofluorescence characteristics of T-cells after such incubations. In no cases studied did the proportions of cells showing fluorescent staining with anti-T-cell antiserum or by E-binding increase as much as might be expected if adherent Ig were covering up important T-cell antigens. Thus it appeared that surface Ig was lost from the lymphocyte membranes of both normal subjects and those with rheumatic fever, after such short incubations. The high viability rates and good recoveries of cells appeared to be points against differing survival rates of incubated T

TABLE VI

Relative Changes in Lymphocyte Cell Surface Markers before and after Incubation at 37°C for 20 h

	Cells with surface Ig		T cells					
	Before	After	Before		After		Recovery of total lymphocytes	Viability
	Incubation	Incubation	AT	E-binding	AT	E-binding		
	%		%				%	%
Normal controls								
C. H.	21	16	76	55	58	55	90	95
P. H.	22	18	69	51	66	44	71	95
C. He.	16	8	61	66	63	65	85	85
R. S.	18	7	73	62	61	61	89	90
A. W.	15	9	80	—	62	—	90	95
K. K.	16	14	79	—	65	—	70	90
M. P.	25	24	74	61	75	51	90	95
Acute rheumatic fever								
R. T.	26	18	64	—	60	—	85	95
R. O.	30	18	63	64	65	67	65	90
S. H.	16	17	68	53	49	45	95	95
M. Mo.	23	23	50	57	55	52	80	100
W. S.	46	33	55	43	50	51	80	95

AT, anti-T cell.

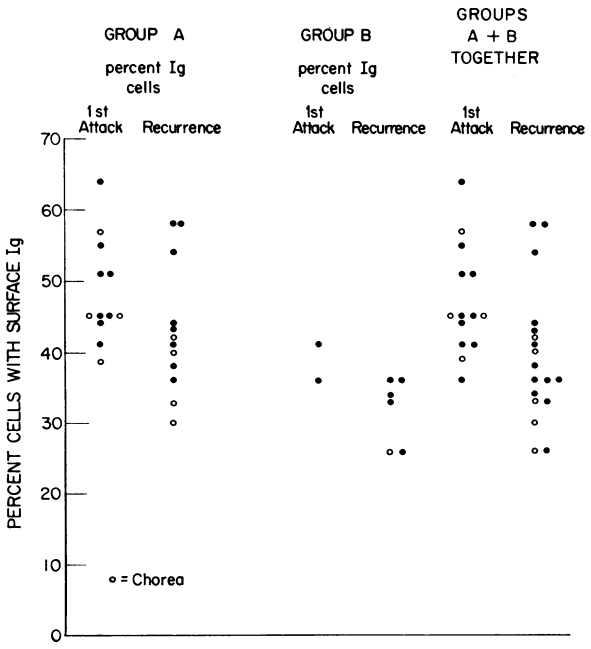

FIGURE 4 Percentages of peripheral blood lymphocytes bearing surface Ig have been plotted separately in patients undergoing an initial attack or recurrence of acute rheumatic fever. A significant elevation was recorded among subjects with initial attacks as compared to those with recurrences ($P < 0.01$). In addition (not shown) total cell numbers bearing surface Ig were significantly elevated in initial attacks ($P < 0.01$). However, no significant differences were noted when proportions or absolute numbers of cells bearing Fc or aggregate receptors were compared in these two groups.

and surface Ig cell populations. Further incubation studies with a larger number of patients with acute rheumatic fever are necessary, since we feel that adsorbed Ig is still a strong possibility to explain high levels of surface Ig in many of the rheumatic fever patients studied to date.

In addition, all sera from patients with acute rheumatic fever were studied for the presence of cytophilic Ig or immunoglobulins that might be capable of binding to normal lymphocyte cell surfaces. Test sera were incubated at 37°C, as described above, with a panel of different normal lymphocytes and cells examined before and after such serum incubations for relative changes in percentages of cells showing surface immunoglobulin. Only 2 of the 53 sera examined showed significant cytophilic antibody, increasing proportions of test lymphocytes showing surface immunoglobulin by 30–50%. In one of these patients, no relative increase in lymphocytes bearing surface immunoglobulin had been recorded on initial study, whereas the other showed 40% lymphocytes with surface Ig. It was clear that the presence of demonstrable cytophilic Ig or Ig-bearing complexes could not explain the substantial elevations of lymphocytes showing surface Ig in many of the acute rheumatic fever patients studied by this particular technique.

DISCUSSION

The present studies indicate that one of the major changes during the early phase of acute rheumatic fever is a striking elevation in both proportions and absolute numbers of peripheral blood lymphocytes bearing surface immunoglobulins. A substantial number of the patients originally studied in Albuquerque showed this change, and it was confirmed in a much larger group of individuals studied later at the Free Rheumatic and Heart Center in Cairo. Of considerable interest was the apparent correlation between relative proportions and total numbers of lymphocytes bearing surface immunoglobulins and the relatively acute nature of the individual rheumatic attack, as shown in Fig. 2 and 3. Lymphocyte proportions of cell surface immunoglobulins tended to be higher during first attacks than in recurrences. These findings appear to link increased proportions and total numbers of peripheral lymphocytes bearing surface Ig to acute attacks of the disease. It is still not clear, however, whether the increased proportions of such Ig-bearing lymphocytes are indeed all B-cells. That this is unlikely is indicated by the concurrent data obtained by the aggregated IgG binding receptor method, whereby much lower values for B-cells were obtained than by summation of cell surface IgG, IgA, and IgM (Fig. 2). Moreover, the prolonged cell incubation studies appeared to indicate some degree of active shedding of cell-adsorbed surface Ig in several rheumatic fever patients successfully studied. In addition, summation of cells stained for kappa and lambda immunoglobulin determinants in some instances gave values lower by at least one-half than those determined by summation of IgG + IgA + IgM. These latter data appear to indicate that more than one class of immunoglobulin is present on single cells. The phenomenon of active shedding of adsorbed cell surface Ig has been recently emphasized by Winchester et al. (28) in the study of patients with active systemic lupus erythematosus. In this latter study the effect of cytophilic cold-reactive IgM antilymphocyte antibodies was clearly defined. We could detect no prominent cold-reactive cytophilic antibodies in our rheumatic fever patients studied to date, although these were searched for with incubation of cells with test sera at low temperature. Despite the failure to demonstrate substantial proportions of adsorbed Ig on cells during the incubation studies described above, we consider it highly likely that cytophilic anti-lymphocyte antibody may account for some of the data presented here. A large number of additional patients must be similarly studied with long-term incubations and possibly cell-surface enzymatic strip-

ping before such a phenomenon can be completely ruled out.

The use of aggregated IgG to detect the Fc receptors of B-cells, originally introduced by Dickler, recorded values of 22% in normal human peripheral blood determinations (29, 30). However, recently this value has been modified downward approximating 10% after simultaneous studies of several B-cell markers, including surface Ig and the Fc or aggregate receptor, by Winchester and coworkers.[2] In these latter studies it was found that the use of pepsin-digested F(ab)$_2$ antibodies to surface Ig produced considerably lower values for total percent cells with surface Ig (10%) than had been previously reported by many other groups of workers. This was felt to represent binding of fluoresceinated whole rabbit IgG antibody both by B-cell Fc receptors and by specific anti-Ig reactions. Pepsin-digested anti-Ig reagents were not utilized in our study and thus our normal values ranged from 22 to 26%. Of interest, however, were our values of 7–12% for binding of aggregates, which may be much closer to a true estimate of true B-cells than the figures we obtained with surface immunofluorescence of Ig. It seems likely that the use of pepsin-digested anti-immunoglobulin reagents may produce lower values in subsequent studies; however, the *relative* elevation in proportions and total numbers of lymphocytes bearing surface Ig in the patients already studied with acute rheumatic fever still represents a finding of considerable interest.

The clinical importance of the rise in cells bearing surface Ig was further borne out by the serial studies recorded in patients followed over a 30–90-day period. That other extraneous influences, such as salicylates, corticosteroids, or concurrent medications, might be partially involved in the results recorded cannot be entirely eliminated. Several recent reports (31, 32) have stressed the substantial effects of prednisone and other immunosuppressive drugs on peripheral blood T and B lymphocyte profiles. However, none of the patients studied in Egypt were receiving corticosteroids, and elevation of numbers and proportions of peripheral blood lymphocytes bearing surface Ig was observed in at least 10 rheumatic fever patients before therapy of any kind was instituted.

It would seem that the exact character or precise profile of cells bearing surface Ig in acute rheumatic fever remains to be completely elucidated. If a sizable proportion of such cells are indeed B-cells, it would suggest that an intense humoral immune response has been initiated. It will now be of interest to apply methods designed to test for specific anti-streptococcal or anti-cardiac antibody activity directly on such cell surfaces, possibly by radioautographic or direct labeling methods recently described (33). The observations of Shulman and Ayoub (34) concerning differences in avidity of antibody to streptococcal group A carbohydrates between patients with rheumatic fever and glomerulonephritis or streptococcal infections are of considerable interest in this regard.

If on the other hand, a substantial proportion of peripheral blood lymphocytes bearing cell-surface immunoglobulin in rheumatic fever are actually activated T-cells (35, 36) instead of B-cells, then their presence would be compatible with a predominant cell-mediated mechanism in the disease itself. The real questions of the current studies are how best to define precursors of humoral immunity or B-cells, and how to quantitate activated T-cells. This problem was previously encountered during studies related to systemic lupus erythematosis, where large percentages of cells from some patients showed surface Ig (21). Other workers have emphasized this problem (28) and precise methods for B-cell quantitation continue to evolve. A multiplicity of B-cell markers as well as T-cell markers is now available to enumerate peripheral blood lymphocytes (37). Since the identity of all of the cells in acute rheumatic fever bearing surface Ig is not yet clear, additional B-cell markers, possibly with such techniques as C3 rosettes (21), may be of help. Perhaps application of the use of pepsin-digested anti-Ig reagents, as introduced by Winchester,[2] will help to clarify the problem of absolute B-cell identification.

The importance of cell-mediated immunity in rheumatic fever has recently been emphasized by the studies of Read and coworkers (13), showing a clear temporal relationship between migration inhibition to streptococcal cell wall products and acute rheumatic fever attacks. Other studies reported by McLaughlin, Patterson, Hartz, and Emburg (38) have failed to demonstrate cellular hyperactivity to heart homogenates or purified myocardial fractions, as measured by lymphocyte transformation in vitro. Further studies are necessary to define the interactions of cellular and humoral immunity in this disorder.

The demonstration of cell-mediated immunity to streptococcal antigens (13) may be related in some way to our finding of increments in peripheral blood lymphocytes bearing surface immunoglobulins during the acute rheumatic fever attack. The profile of elevated cells bearing surface immunoglobulins, either as B-cells or activated T-cells, could be compatible with a loss of suppressor T-cell function during the acute rheumatic episode. Until recently, loss of suppressor cell function has been mentioned only as an hypothesis to explain or encompass various autoimmune reactions (39, 40).

[2] Winchester, R. J., and H. G. Kunkel. 1974. Report presented at conference on "The Immunological Bases of Connective Tissue Disorders". V Lepetit Colloquium, 11–13 November 1974. Madrid, Spain.

However, recently developed methods may allow some quantitative estimation of human suppressor T-cell function (41). It will now be of considerable interest to apply such methods to study such diseases as acute rheumatic fever. If the presently observed elevations of lymphocytes bearing surface Ig represent an unbridled response of activated autoaggressive B- and/or T-cells ungoverned by suppressor mechanisms, then quantitation of suppressor activity might be predicted to show a marked deficit.

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REFERENCES

1. Green, C. A. 1942. Haemolytic streptococcal infections and acute rheumatism. *Ann. Rheum. Dis.* **3**: 4–41.
2. Taran, L. M., J. M. Jablon, and H. N. Weyr. 1945. Immunologic studies in rheumatic fever. I. Cutaneous response to type-specific proteins of the hemolytic streptococcus. B. Response to "purified M" proteins from forty known types of the hemolytic streptococcus-group A. *J. Immunol.* **51**: 53–64.
3. Murphy, G. E., and H. F. Swift. 1949. Induction of cardiac lesions, closely resembling those of rheumatic fever, in rabbits following repeated skin infections with group A streptococci. *J. Exp. Med.* **89**: 687–698 and plates 37–42.
4. Wannamaker, L. W., C. H. RammelKamp, Jr., F. W. Denny, W. R. Brink, H. B. Houser, E. O. Hahn, and J. H. Dingle. 1951. Prophylaxis of acute rheumatic fever by treatment of the preceding streptococcal infection with various amounts of depot penicillin. *Am. J. Med.* **10**: 673–695.
5. Kaplan, M. H., and K. H. Svec. 1964. Immunologic relation of streptococcal and tissue antigens. III. Presence in human sera of streptococcal antibody cross-reactive with heart tissue. Association with streptococcal infection, rheumatic fever and glomerulonephritis. *J. Exp. Med.* **119**: 651–666 and plates 70–73.
6. Zabriskie, J. B. 1967. Mimetic relationships between group A streptococci and mammalian tissues. *Adv. Immunol.* **7**: 147–188.
7. Zabriskie, J. B. 1969. The relationship of streptococcal cross-reactive antigens to rheumatic fever. *Transplant. Proc.* **1**: 968–975.
8. Unanue, E. R., H. M. Grey, E. Rabellino, P. Campbell, and J. Schmidtke. 1971. Immunoglobulins on the surface of lymphocytes. II. The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulins. *J. Exp. Med.* **133**: 1188–1198.
9. Pernis, B., L. Forni, and L. Amante. 1970. Immunoglobulin spots on the surface of rabbit lymphocytes. *J. Exp. Med.* **132**: 1001–1018.
10. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* **6**: 52–80.
11. Cerottini, J. C., A. A. Nordin, and K. T. Brunner. 1970. Specific *in vitro* cytotoxicity of the thymus-derived lymphocytes sensitized to alloantigens. *Nature (Lond.).* **228**: 1308–1309.
12. Crone, M., C. Koch, and M. Simonsen. 1972. The elusive T cell receptor. *Transplant. Rev.* **10**: 36–56.
13. Read, S. E., V. A. Fischetti, V. Utermohlen, R. E. Falk, and J. B. Zabriskie. 1974. Cellular reactivity studies to streptococcal antigens. Migration inhibition studies in patients with streptococcal infections and rheumatic fever. *J. Clin. Invest.* **54**: 439–450.
14. American Heart Association, Council on Rheumatic Fever and Congenital Heart Disease. 1965. Jones criteria (revised) for guidance in the diagnosis of rheumatic fever. *Circulation.* **32**: 664–668.
15. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 *g*. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97): 77–89.
16. Mellbye, O. J., R. P. Messner, J. R. DeBord, and R. C. Williams, Jr. 1972. Immunoglobulin and receptors for C3 on lymphocytes from patients with rheumatoid arthritis. *Arthritis Rheum.* **15**: 371–380.
17. Fröland, S. S., J. B. Natvig, and P. Berdal. 1971. Surface-bound immunoglobulin as a marker of B lymphocytes in man. *Nat. (New Biol.).* **234**: 251–252.
18. Fröland, S. S., and J. B. Natvig. 1972. Class, subclass, and allelic exclusion of membrane-bound Ig of human B lymphocytes. *J. Exp. Med.* **136**: 409–414.
19. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136**: 191–196.
20. Williams, R. C., Jr., J. R. DeBord, O. J. Mellbye, R. P. Messner, and F. D. Lindström. 1973. Studies of T- and B-lymphocytes in patients with connective tissue diseases. *J. Clin. Invest.* **52**: 283–295.
21. Messner, R. P., F. D. Lindström, and R. C. Williams, Jr. 1973. Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J. Clin. Invest.* **52**: 3046–3056.
22. Talal, N., R. A. Sylvester, T. E. Daniels, J. S. Greenspan, and R. C. Williams, Jr. 1974. T and B lymphocytes in peripheral blood and tissue lesions in Sjögren's syndrome. *J. Clin. Invest.* **53**: 180–189.
23. Bernstein, I. M., K. H. Webster, R. C. Williams, Jr., and R. G. Strickland. 1974. Reduction in thymus-derived lymphocytes in alcoholic liver disease. *Lancet.* **2**: 488–490.
24. Fröland, S. S. 1972. Binding of sheep erythrocytes to human lymphocytes. A probable marker of T-lymphocytes. *Scand. J. Immunol.* **1**: 269–280.
25. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136**: 207–215.
26. Wybran, J., M. C. Carr, and H. H. Fudenberg. 1972. The human rosette-forming cell as a marker of a population of thymus-derived cells. *J. Clin. Invest.* **51**: 2537–2543.
27. DeHoratius, R. J., R. G. Strickland, and R. C. Williams, Jr. 1974. T and B lymphocytes in acute and chronic hepatitis. *Clin. Immunol. Immunopathol.* **2**: 353–360.
28. Winchester, R. J., J. B. Winfield, F. Siegal, P. Wernet, Z. Bentwich, and H. G. Kunkel. 1974. Analyses of lymphocytes from patients with rheumatoid arthritis and systemic lupus erythematosus. Occurrence of interfering

cold-reactive antilymphocyte antibodies. *J. Clin. Invest.* **54**: 1082–1092.
29. Bentwich, A., and H. G. Kunkel. 1973. Specific properties of human B and T lymphocytes and alterations in disease. *Transplant. Rev.* **16**: 29–50.
30. Dickler, H. G. 1974. Studies of the human lymphocyte receptor for heat-aggregated or antigen-complexed immunoglobulin. *J. Exp. Med.* **140**: 508–522.
31. Yu, D. T. Y., P. J. Clements, H. E. Paulus, J. B. Peter, J. Levy, and E. V. Barnett. 1974. Human lymphocyte subpopulations. Effect of corticosteroids. *J. Clin. Invest.* **53**: 565–571.
32. Horwitz, D. A. 1974. Selective depletion of Ig-bearing lymphocytes by cyclophosphamide in rheumatoid arthritis and systemic lupus erythematosus: guidelines for dosage. *Arthritis Rheum.* **17**: 363–374.
33. Bankhurst, A. D., G. Torrigiani, and A. C. Allison. 1973. Lymphocytes binding human thyroglobulin in healthy people and its relevance to tolerance for autoantigens. *Lancet.* **1**: 226–230.
34. Shulman, S. T., and E. M. Ayoub. 1974. Qualitative and quantitative aspects of the human antibody response to streptococcal group A carbohydrate. *J. Clin. Invest.* **54**: 990–996.
35. Yoshida, T. O., and B. Anderson. 1972. Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. *Scand. J. Immunol.* **1**: 401–408.
36. Orr, K. B., and F. Paraskevas. 1973. Cell surface associated gamma globulin in lymphocytes. V. Detection of early cytophilic complexes reacting with T- and B-lymphocytes. *J. Immunol.* **110**: 456–464.
37. Brown, G., M. F. Greaves, T. A. Lister, N. Rapson, and M. Papamichael. 1974. Expression of human T and B lymphocyte cell-surface markers on leukemic cells. *Lancet.* **2**: 753–755.
38. McLaughlin, J. F., P. Y. Paterson, R. S. Hartz, and S. H. Embury. 1972. Rheumatic carditis: in vitro responses of peripheral blood leukocytes to heart and streptococcal antigens. *Arthritis Rheum.* **15**: 600–608.
39. Allison, A. C., A. M. Denman, and R. D. Barnes. 1971. Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet.* **2**: 135–140.
40. Gershon, R. K., and K. Kondo. 1970. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology.* **18**: 723–737.
41. Waldmann, T. A., M. Durm, S. Broder, M. Blackman, R. M. Blaese, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia. *Lancet.* **2**: 609–613.