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Receptors for activated C3 on thymus-dependent (T) lymphocytes of normal guinea-pigs

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Summary. In a survey of lymphocyte subpopulations in normal guinea-pig blood, lymph node, spleen, thymus and peritoneal cavity, a considerable overlap was observed between the percentages of C3-receptor bearing lymphocytes (CRL) and of thymus-dependent (T) cells in lymph nodes. Simultaneous rosette-forming reactions with sheep erythrocytes carrying rabbit complement (EAC) and papain-treated rabbit erythrocytes (a T-cell marker) revealed that 20–50% of the lymph node CRL were T lymphocytes. These experiments and others on cell suspensions depleted of Ig-bearing (B) lymphocytes showed that between 8 and 36% of lymph node T cells have complement receptors. The frequency of T-CRL in other lymphoid tissues was lower, representing between 0 and 8% of the T-cell population. The reaction of T-CRL and EAC was not inhibited by EDTA which is known to inhibit the C3 receptor activity on macrophages.

INTRODUCTION

The characterization of C3-receptor bearing lymphocytes (CRL) has been the subject of many investigations, particularly on human and mouse lymphocytes (for reviews see Jondal, Wigzell & Aiuti, 1973; Shevach, Jaffe & Green, 1973; Nussenzweig, 1974; Ross, 1976). From studies on lymphocyte suspensions depleted of either bone-marrow-dependent (B) or thymus-dependent (T) lymphocytes, and from examination of the tissue distribution of CRL, it is generally agreed that most CRL are a subpopulation of B cells. With these techniques, however, it would be difficult to detect minor populations of CRL which were not B cells. For this, it is essential to examine simultaneous reactions with EAC and another marker for B lymphocytes or, preferably, for T lymphocytes.

Surprisingly few reports are available on either CRL or other lymphocyte subpopulations in guinea-pigs. Shevach, Ellman, Davie & Green (1972) found between 35 and 40% CRL in normal lymph nodes when rosetted with EAC coated with mouse complement. In these lymphocyte suspensions all Ig-bearing (B) cells, identified by staining with fluorescein-labelled anti-guinea-pig Fab, had C3 receptors, but it was not stated whether any CRL were Ig-negative. A T-cell marker for guinea-pigs, employing an affinity reaction with E_{rabbit}, was first described independently by Wilson & Coombs (1973) and by Stadecker, Bishop & Wortis (1973). This marker is apparently analogous to the reaction of sheep erythrocytes with human T lymphocytes. Rosette formation is inhibited by anti-thymocyte serum, and does not occur with the L_2C B-cell leukaemia lymphocytes (Stadecker *et al.*, 1973). The reaction is not inhibited by anti-Ig serum, and, as

Abbreviations: CRL, lymphocytes with receptors for complement; E, erythrocytes; EA, erythrocytes coated with rabbit IgG antibody; EAC, erythrocytes coated with rabbit IgM antibodies and complement; FITC. flourescein isothiocyanate; iFCS, heat-inactivated foetal calf serum; Ig, immunoglobulin; PBS, phosphate buffered saline; RT, room temperature (22–26°).

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confirmed in the present report, there is little or no overlap between the E-rosetting population and Ig-bearing (B) cells (Wilson & Gurner, 1975).

When guinea-pig lymph node cells were tested by Stadecker *et al.* for simultaneous rosette formation with E_{rabbit} and EAC carrying mouse complement, no T lymphocytes with receptors for complement (T–CRL) were observed. The conditions for this mixed rosetting test were not described, however, and the results of only one experiment were reported.

A more extensive study of lymphocyte populations in guinea-pig blood, lymph nodes and thymus was made by Revell, Wilson & Coombs (1974). Unfortunately, with the techniques then available large numbers of lymphocytes did not react with any of the markers, but significant improvements have been made since that time. The sensitivity of the T-cell reaction with E_{rabbit} is greatly increased by pre-treating the erythrocytes with papain (Wilson & Gurner, 1975). Also, the direct antiglobulin rosette forming reaction (DARR) has been developed for the detection of Ig-bearing (B) lymphocytes (Coombs, Wilson, Eremin, Gurner, Haegert, Lawson, Bright & Munro, 1977), and is more sensitive than the immunofluorescent staining technique (Haegert, Hurd & Coombs, 1978). For the present investigation, EAC prepared with rabbit complement were found to give optimum results in detecting C3- receptors.

The improved markers, together with EA (carrying rabbit IgG) for measuring IgG–Fc receptors, formed the basis of the present investigation, in the course of which an overlap was found in the percentages of T cells and C3-receptor bearing cells in guinea-pig lymph nodes. By mixed rosette forming reactions and by depletion studies, the presence of complement receptor-bearing T cells was confirmed, sometimes in high proportions, in these organs.

MATERIALS AND METHODS

Animals

Guinea-pigs were males and females of the Hartley strain, and ranged in size from 200 to 1100 g (3 weeks to 2 years old).

Lymphoid cell suspensions

Except where indicated to the contrary, the medium used was RPMI 1640 (Flow), buffered to pH 7·2 with 20 mM HEPES (Sigma Co.) and containing 5% iFCS. All lymphoid cells were washed three times and adjusted to 2×10^6 per ml before testing.

Blood. Lymphocytes were isolated from defibrinated blood by treating with methyl cellulose and carbonyl iron, according to the method of Wilson & Coombs (1971). Erythrocytes were lysed by brief exposure (20 s) to distilled water, and the final suspension usually contained at least 90% lymphocytes plus basophils and a variable number of Kurloff cells.

Lymph nodes and thymus. Lymph nodes and thymus were teased into RPMI 1640/10% iFCS, filtered through wire gauze to remove clumps and treated with carbonyl iron (37°, 30 min) to separate macrophages.

Spleen. Spleen cells were teased out and treated with carbonyl iron. The erythrocytes were then removed by distilled water lysis (20 s). The lymphocyte suspensions usually contained large numbers (up to 30%) of Kurloff cells and some basophils.

Peritoneal. The peritoneal cavity was washed out as described by Butterworth, Coombs & Wilson (1976) to give a suspension containing macrophages, eosinophils and lymphocytes. A high proportion of macropages and some eosinophils were removed by incubating the cells in 4 inch plastic Petri dishes (Falcon) for 1 h at 37°. Non-adherent cells, including lymphocytes, were gently swirled off. In some tests, the remaining macrophages were removed by feeding carbonyl iron and these suspensions contained approximately 70% lymphocytes and 30% eosinophils.

Rosette-forming reactions

Rosettes were prepared by mixing equal volumes (usually 25 μl) of lymphocytes (2×10^6 per ml) and 1% (v/v) erythrocytes in 5×50 mm plastic tubes, and centrifuging at 1700 r.p.m. (350 ***g***) for 2 min at 4°. The percentages of reacting lymphocytes (with three or more erythrocytes adhering) were counted in toluidine blue stained suspensions mounted on siliconed microscope slides. Variable numbers of the Kurloff cells found in blood and spleen-cell suspensions react with the T-cell marker (Wilson & Gurner, 1975) and have receptors for IgG–Fc. These cells were excluded from the counts of reacting lymphocytes.

Papain-treated rabbit erythrocytes for T-cell marker. Rabbit erythrocytes were treated with papain (Wilson, Gurner & Coombs, 1975), washed three times and resuspended in RPMI 1640/5% iFCS. For rosetting, equal volumes of lymphocytes and E_{rabbit} were mixed and left for 15 min at RT. They were centrifuged (as above) and left for 1 h at 4° before resuspending.

Direct antiglobulin rosette forming reaction (DARR) for Ig-bearing lymphocytes. E_{ox} were treated with trypsin and coupled by chromic chloride ($CrCl_3$) to the IgG fraction of a rabbit antiserum (R191) to guinea-pig γ-globulin. The method was modified from that described by Coombs *et al.* (1977). Washed E_{ox} were treated with 0·25% trypsin (30 min, 37°) and, after washing, were resuspended in an equal volume of 0·025% soya bean trypsin inhibitor (10 min, RT). They were washed three times in 0·9 NaCl and the packed erythrocytes were then mixed with an equal volume of rabbit IgG anti-guinea-pig γ-globulin (2 mg protein per ml in 0·9% NaCl). Two volumes of 0·02% $CrCl_3$ were added while mixing on a Vortex stirrer, and the mixture was rotated for 1 h at RT. The cells were washed three times with PBS and stored at 4° for up to 2 weeks. The coupled E_{ox} were tested by agglutination with titrations of (a) goat antiserum to rabbit IgG, and (b) guinea-pig γ-globulin.

Lymphocytes were washed twice with PBS containing 1% bovine serum albumin (PBS/BSA) at 4° immediately before rosetting with the antiglobulin-coupled E_{ox}. As a control E_{ox} coupled to normal rabbit IgG by the same method was included in every test.

EAC for C3-receptor bearing lymphocytes. The EAC used in most experiments were E_{sheep} coated with a sub-agglutinating dose of IgM rabbit antibodies and incubated in undiluted C6-deficient rabbit serum (pre-absorbed at 4° with sheep erythrocytes) as a source of complement (EAC_R). The EA_{IgM} were incubated with complement so as to prepare EAC carrying either C3b, C3d, or C3b + C3d (Wilson, Kanski & Coombs, 1978). Control indicator cells were EA_{IgM} incubated in heat-inactivated C6-deficient rabbit serum. The presence or absence of C3b on EAC was confirmed by the immune adherence of human erythrocytes.

In a few experiments, the reaction of lymphocytes with EAC_R was compared with that of EAC prepared with either mouse complement on E_{ox} (Eremin, Plumb & Coombs, 1976) or guinea-pig complement on E_{sheep} (Revell *et al.*, 1974).

Lymphocyte rosettes with EAC were incubated either at 37° or at 4° (see text) for 30 min before resuspending and counting.

EA_{IgG} for Fc-receptor bearing lymphocytes. The indicator cells were E_{ox} treated for 45 min at RT with rabbit antiserum (pool 13/10/65) containing a high titre of IgG anti-E_{ox} antibodies. The cells were washed three times with PBS and stored at 4° for up to 1 week in RPMI 1640/0·2% iFCS.

Rosettes with lymphocytes were left at 4° for 30 min before resuspending.

Mixed rosette-forming reactions

For these experiments, lymphocytes were centrifuged with equal volumes (usually 25 μl) of a fluorescein-stained and a non-stained indicator erythrocyte. The percentages of lymphocytes reacting with either type of indicator cell alone or with both cells ('mixed' rosettes) were counted by examining on a Leitz Orthoplan microscope fitted with a Ploem illuminator. Erythrocytes were stained with fluorescein-isothiocyanate (FITC) as described by Wilson *et al.* (1975), and were thoroughly washed to remove unconjugated FITC.

The conditions for mixed rosetting were sometimes modified, of necessity, from those described above for rosetting with only one erythrocyte marker. To test for C3 receptors on T cells, the lymphocytes were mixed with E_{rabbit} and EAC, left for 15 min at RT, centrifuged, and kept for 1 h at 4° before resuspending. Where necessary to facilitate blood lymphocyte reactivity with EAC (see text), the centrifuged cell pellets (lymphocytes, E_{rabbit} and EAC) were incubated at 37° for 30 min before leaving 1 h at 4°.

Careful checks were made to ensure that neither the FITC-staining nor the altered conditions for rosette preparation had any effect on the percentages of lymphocytes reacting with a particular indicator cell.

Depletion of Ig-bearing lymphocytes from lymph node cell suspensions

Two aliquots (1 ml) of lymph node lymphocytes were rosetted with E_{ox} coupled to IgG anti-guinea-pig γ-globulin. The pelleted cells were resuspended and the two aliquots pooled. They were layered over 2 ml Ficoll–Hypaque (rel. density 1·09) and centrifuged (400 *g*) at RT for 10 min. The interface layer of non-rosetted lymphocytes was harvested, washed twice and finally adjusted to 2×10^6 cells per ml in either RPMI 1640/5% iFCS or PBS/1% BSA (for DARR test).

RESULTS

Preliminary experiments on guinea-pig lymphocyte receptors for C3

Comparison of reactions with EAC_M, EAC_{GP} and EAC_R. A comparison was made between EAC

prepared with either mouse, guinea-pig or rabbit complement. The relative numbers of CRL detected by these three indicator erythrocytes varied according to the source of the lymphocytes (Table 1). Splenic lymphocytes reacted equally well with all three types of EAC. Lymphocytes from lymph node and blood, however, reacted optimally only with EAC_R. The EAC_{GP} formed almost as many rosettes as EAC_R with lymph node lymphocytes, but their reaction with blood lymphocytes was relatively much weaker. The EAC_R was therefore used for the remainder of the investigation.

Table 1. Comparison of the reactions given by blood, lymph node and spleen lymphocytes with EAC_M, EAC_{GP} and EAC_R

Origin of lymphocytes	Percentage of lymphocytes rosetted with: EAC_M carrying C3b+C3d	EAC_{GP} carrying C3b+C3d	EAC_R carrying C3b	EAC_R carrying C3b+C3d	EAC_R carrying C3d
Blood*	4		14		14
		0·5	18		20
		3	13		10
Lymph node	11		49	47	55
	12			52	
	16			35	
	21			40	
		32	39		45
Spleen	24		29		39
	32			30	
	29			27	
	24			23	
		40	39		45

* EAC rosettes on blood lymphocytes were incubated at 37° for 30 min before resuspending. All other rosettes were kept at 4°.

It is possible that the variations in rosette forming ability of the EAC was not only associated with the species of origin of the complement, but also with the relative amounts of activated C3 on their surfaces. Michlmayer & Huber (1970) showed that the numbers of CRL detected in human blood was dependent on the concentration of complement used to prepare the EAC. In preparing EAC for the present work, the guinea-pig complement had to be used at a much higher dilution (1/250) than either the mouse (1/10) or the rabbit (undiluted) complement because of its high lytic titre.

Effect of temperature on rosette formation with EAC_R. Lymphocytes from guinea-pig blood, lymph nodes and spleen were mixed with equal volumes of 1% EAC_R and centrifuged. The pellets containing rosettes were then incubated for 30 min at either 4°, 22° (RT) or 37° before resuspending and counting.

Again, differences were found in C3-receptors on lymphocytes from the various tissues. Blood lymphocytes showed a marked temperature dependence and reacted maximally with EAC_R only at 37°, while lymphocytes from lymph node and spleen gave equally strong reactions at all temperatures. For example, in one test on blood lymphocytes the percentage of EAC rosettes increased from 6% at 4° to 12% at 22° and 19% at 37°. In all further experiments on blood CRL, therefore, the rosettes were incubated at 37° prior to resuspending.

In similar tests with EA_{IgG}, variations in temperature had no effect on the numbers of rosettes formed by blood, lymph node or spleen lymphocytes.

The percentages of lymphocytes with receptors for C3b were usually similar to those reacting with C3d, and simultaneous rosette formation with the two EAC_R indicator erythrocytes showed that usually between 90 and 100% of C3-receptor bearing lymphocytes reacted with both C3b and C3d. Control experiments using EA_{IgM} treated with heated complement were always negative.

Lymphocyte sub-populations in blood, lymph node, spleen, peritoneum and thymus, with special reference to CRL

Counts were made of lymphocytes belonging to various subpopulations (Ig-positive, C3 or Fc-receptor bearing, or T cells) in suspensions of cells isolated from different compartment of the lymphoid system. The results, shown in Table 2, were obtained in tests on lymphocytes from ten guinea-pigs.

Few CRL were found in either the peritoneal cavity or the thymus, both of which have very high percentages (usually 90% or more) of T lymphocytes. In blood lymph nodes and spleen, however, where a greater proportion of lymphocytes are Ig-bearing (B) cells, the numbers of CRL were much increased. The percentages of blood and spleen CRL were usually similar to those of lymphocytes with receptors for IgG-Fc, and when compared to Ig-bearing lymphocytes the CRL showed a slightly lower frequency in blood but were equally numerous in spleen.

A very different pattern of lymphocyte subpopula-

Table 2. Lymphocyte subpopulations in blood, lymph node, spleen, peritoneum and thymus of normal guinea-pigs

Origin of lymphocytes	Percentage of total lymphocytes*				
			Carrying receptors for		
	T cells	Ig-bearing	C3b	C3d	IgG–Fc
Blood	78	22	19	18	13
	65	25	15	12	11
	53	43	41	41	36
	80	16	12	14	8
	56	44	29	31	40
	37	53	41	37	44
	(61·5 ± 16·3)	(33·8 ± 14·8)	(26·2 ± 12·8)	(25·5 ± 12·4)	(25·3 ± 16·3)
Lymph nodes†	64	31	48	45	23
	78	21	36	36	12
	81	14	28	24	7
(animals aged	70	27	48	42	15
1–3 months)	85	12	19	15	6
	90	7	14	16	9
	87	9	24	28	4
	89	10	17	16	10
	51	46	44	57	36
	(77·2 ± 13·2)	(19·7 ± 12·9)	(30·9 ± 13·5)	(31·0 ± 14·9)	(13·6 ± 10·1)
Lymph nodes†	62	39	63	60	45
	60	38	62	67	17
(animals aged	69	26	51	55	13
1–2 years)	70	32	51	52	33
	80	19	33	41	26
	(68·2 ± 7·9)	(30·8 ± 8·4)	(52·0 ± 12·1)	(55·0 ± 9·7)	(26·0 ± 13·0)
Spleen	43	54	56	58	58
	40	50	50	38	34
	61	40	46	44	44
Peritoneum	88	4	4	4	19
	90	3	0	1	NT
	93	6	6	1	3
Thymus	92	1	3	3	1
	98	2	1	1	2

* Figures in parentheses = mean values ± SD.
† Cervical and mesenteric lymph nodes tested.
NT = not tested.

tions was found in lymph nodes. In every suspension tested, the percentage of CRL exceeded that of both the Ig-positive and the Fc-receptor bearing lymphocytes, and overlapped to a varying extent with the T-cell population. This indicated that a proportion of guinea-pig lymph node T cells have surface receptors for C3.

The specificity of the T-cell marker was confirmed by testing lymphocytes from blood, lymph nodes and spleens in simultaneous rosette forming reactions with antiglobulin-coupled E_{ox} and FITC-stained E_{rabbit}. The percentages of lymphocytes reacting with both markers was usually less than 1% and never exceeded 2%, showing that they were distinct populations. Also, the papain-treated E_{rabbit} do not form rosettes with macrophages, which, in any case, were rarely seen as they had been removed from all lymphocyte suspensions by carbonyl iron treatment.

Receptors for C3 on T lymphocytes

Mixed rosette forming reactions. Lymphocytes were rosetted simultaneously with the T-cell marker, E_{rabbit}, and EAC_R. In each test, one of the indicator cells was stained with fluorescein, and similar results were obtained whether the stain was used for the E_{rabbit} or for the EAC. The percentages of T lymphocytes having receptors for C3 (T-CRL) were calculated from counts of lymphocytes forming rosettes with E_{rabbit} only or with both indicator cells (Table 3).

The percentages of T-CRL in blood and peritoneal cell suspensions were low, representing less than 3% of the total T lymphocytes, and spleen cells showed an average of 4–6% T cells with C3 receptors. Although the lymph nodes of some guinea-pigs contained less than 10% of T cells capable of reacting with C3, others had between 10 and 20%, and some had considerably more (up to 36%). No significant differences were found between the percentages of T-CRL in the cervical and mesenteric lymph nodes from any one animal. The numbers of T-CRL in lymph nodes apparently increased with the age of the guinea-pig, and the highest proportions, averaging between 30 and 32% of the T lymphocytes, were found in animals aged between 1 and 2 years (Table 3).

In general, the T-CRL formed 20–50% of the total C3-receptor bearing lymphocytes in lymph nodes, while in blood and spleen they formed only 0–10% of the CRL.

Simultaneous rosette-forming reactions using E_{ox} coupled to anti-guinea-pig γ-globulin (as a B-cell marker) and EAC_R supported the above findings. For example, tests with cervical and mesenteric lymph node lymphocytes from one of the older guinea-pigs showed that between 30 and 37% of the CRL were Ig-negative (presumptive T) cells. Of the Ig-bearing (B) lymphocyte populations in these suspensions, 92–96% had receptors for C3.

Receptors for C3 and IgG-Fc on lymphocytes in suspensions depleted of B cells. Further evidence that a considerable proportion of guinea-pig lymph node CRL are T cells was obtained in experiments on cell suspensions depleted of Ig-bearing (B) lymphocytes (Table 3). These suspensions contained at least 93% T lymphocytes, measured by rosette formation with E_{rabbit}, and the percentages of rosettes with EAC_R showed close correlation with the results obtained in the mixed rosetting reactions.

Manconi (1977) reported high levels of T cells with

Table 3. Mean ± SD of percentage of T lymphocytes with receptors for C3b and C3d determined (a) by mixed rosette-forming reactions with EAC and E_{rabbit}, and (b) by EAC rosette formation with lymph node lymphocytes depleted of Ig-bearing (B) cells

Origin of lymphocytes	No. of observations†	Percentage of T-CRL* shown by: Mixed EAC + E_{rabbit} reactions in whole lymphocyte suspensions C3b	Mixed EAC + E_{rabbit} reactions in whole lymphocyte suspensions C3d	EAC reactions in suspensions depleted of Ig-bearing cells‡ C3b	EAC reactions in suspensions depleted of Ig-bearing cells‡ C3d
Blood	7	2·9 ± 1·5 (2–6)	2·6 ± 1·8 (0–5)		
Lymph nodes					
(a) animals up to 3 months	11 (6)	12·4 ± 2.8 (8–16)	12·8 ± 3·8 (8–19)	12·00 ± 3·9 (7–17)	12·00 ± 4·4 (7–19)
(b) animals of 1–2 years	5 (3)	29·8 ± 3·9 (26–36)	32·0 ± 2·6 (30–36)	31·3 ± 6·1 (26–38)	29·0 ± 3·6 (26–33)
Spleen	4	4·3 ± 4·0 (0–6)	6·0 ± 4·0 (0–8)		
Peritoneum	3	2·7 ± 3·0 (0–6)	1·7 ± 2·1 (0–4)		

* Figures in parentheses = range of results.

† Figures in parentheses refer to tests on Ig-depleted lymphocyte suspensions.

‡ These suspensions contained an average of 96% T cells (range 93–99%), 0·7% Ig-bearing cells (range 0–3%), and 0·6% cells with receptors for IgG–Fc (range 0–3%).

receptors for IgG-Fc in guinea-pig peritoneal cell suspensions, although they were relatively rare in blood, spleen and thymus. The purified lymph node T lymphocytes used in the present investigation were tested for rosette formation with EA, but few of these cells had detectable Fc receptors (subscript, Table 3).

Effect of EDTA on T-cell receptors for C3

EDTA will inhibit the interaction of C3 with receptors on guinea-pig macrophages, but has no effect on B lymphocyte receptor activity (Shevach *et al.*, 1972).

Purified lymph node T lymphocytes (B-cell depleted) were pre-treated with 0·01 M EDTA (37°, 30 min) and rosetted with EAC_R in the presence of EDTA. No reduction was found in the percentage of CRL, indicating that the C3 receptors on T cells resemble those found on B lymphocytes rather than those of macrophages.

DISCUSSION

Examination of the lymphocytes in various guinea-pig tissues has shown that a variable proportion of those with receptors for activated C3 are a subpopulation of T cells, as detected by rosette formation with papain-treated E_{rabbit}. In blood, spleen, thymus and peritoneum these cells are relatively rare, representing less than 10% of the total T cells, and the majority of CRL are B lymphocytes. Lymph nodes, however, often contain appreciable numbers of T-CRL which comprise up to 50% of the lymphocytes with receptors for C3. The T-CRL were found to be most numerous in lymph nodes of older guinea-pigs, in which up to 36% of the T cells formed rosettes with EAC.

As mentioned earlier, these T-CRL were not detected by Stadecker *et al.* (1973) in their experiments on guinea-pig lymph nodes. The reasons for this may be associated with differences in the indicator systems used in the present work. Stadecker *et al.* used EAC coated with mouse complement which in our hands gave fewer rosettes with both blood and lymph node lymphocytes than EAC prepared with rabbit complement. Also, papain treatment of rabbit erythrocytes has been found to improve greatly their reaction with guinea-pig T cells (Wilson & Gurner, 1975). This increased sensitivity is also reflected in the low numbers of null cells (neither Ig-bearing nor T cells) found in the present study.

High levels of T-CRL have only been described previously in mice by Arnaiz-Villena, Gyöngyössy & Playfair (1974). Using either anti-theta antigen or anti-Ig antibodies as T- and B-cell markers, respectively, and indirect immunofluorescent staining, they found that approximately 30% of mouse lymph node and spleen CRL are T cells. Ross, Rabellino, Polley & Grey (1973) reported appreciable numbers (up to 24%) of Ig-negative CRL in human spleen, but these were not tested for T-cell characteristics.

Rosette formation with EAC has been used by some workers as a marker for B lymphocytes. Although there is no doubt that C3 receptors are found on a high proportion of B cells from all compartments of the lymphoid system, the presence of these receptors can not be regarded as exclusive to this group of lymphocytes.

From the results of the present investigation, it is apparent that T lymphocytes with C3 receptors may form an important subpopulation, particularly in lymph nodes. These organs are involved in the initiation of immune responses, and C3 is apparently required for the production of antibodies to thymus-dependent antigens (Pepys, 1972; Feldmann & Pepys, 1974). This suggests that C3 may assist in the co-operation of T and B cells, either with each other or with macrophages, and that T-CRL may participate in this reaction. A further role for CRL is suggested by the work of Koopman, Sandberg, Wahl & Mergenhagen (1976) who showed that C3b causes the release of a macrophage chemotactic factor from guinea-pig spleen lymphocytes. These cells were probably B lymphocytes, but it is possible that the interaction of activated C3 with receptors on T lymphocytes may also result in lymphokine release.

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