TWO DIFFERENT COMPLEMENT RECEPTORS ON HUMAN LYMPHOCYTES*

One Specific for C3b and One Specific for C3b Inactivator-Cleaved C3b

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In the study of the role of lymphocytes in the immune response it is becoming increasingly apparent that lymphocyte membrane-bound receptors represent the recognition link between an antigen on the outside of the lymphocyte and the genetic capacity of the lymphocyte to respond to stimulation. The first described antigen receptor was surface-bound immunoglobulin that characterizes bone marrow-derived lymphocytes (B cells)[1] and that is absent in any appreciable quantity from thymus-derived lymphocytes (T cells) (1–4). This surface-bound immunoglobulin is similar to humoral antibody and presumably functions as an antigen receptor with a high degree of specificity for individual antigens. B cells also have receptors for both the antibody (5, 6) and complement, C3, (7, 8) contained in immune complexes. The probable function of these latter two receptors is less clear and it was thought that since they require preformed antibody in order to react with antigen, they might function only in detection of antigens in the secondary immune response. Recently, however, it was shown that a complement receptor could bind fluid phase C3 (9) and other workers demonstrated that fluid phase C3 was apparently required for B cell activation in the primary immune response (10). The complement receptor may therefore play a key role in the primary immune response.

Previously we had studied complement receptors on human lymphocytes and had noted that there was a difference in the complement receptors detected on normal as compared with those on leukemic lymphocytes (11). With normal lymphocytes, EAC1-

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[1] *Abbreviations used in this paper:* B cells, lymphocytes derived from bone marrow; CRL, complement receptor lymphocytes; EA, sheep erythrocytes sensitized with γM rabbit antibody to sheep cells; EAC, sheep erythrocyte-antibody-complement complexes; GVB, gelatin Veronal buffer with 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (12); HBSS, Hanks' balanced salt solution; hu, human; mo, mouse; T cells, lymphocytes either originating in the thymus or in other lymphoid tissues under the control of the thymus; TRFC, human thymus-derived rosette-forming cells (T cells).

3hu (EAChu) detected just slightly more lymphocytes possessing the complement receptor than did EACmo, but with most chronic lymphatic leukemia lymphocytes, EACmo detected 2–20-fold more complement receptor lymphocytes than did EAC1-3hu. It was also noted in that study that EAC1-3hu reacted 20-fold better in immune adherence[2] with human erythrocytes than did EACmo. The human erythrocyte C3 receptor (immune adherence receptor) thus appeared to possess activity that was opposite to the complement receptor activity observed with leukemic lymphocytes.

In the present study complement receptors on normal lymphocytes, chronic lymphatic leukemia lymphocytes, and erythrocytes were compared. It was shown that normal peripheral lymphocytes have two different complement receptors that are antigenically distinct: one is a receptor for cell-bound C3 (C3b) and the other, a receptor for C3b that has been cleaved by the C3b inactivator. The first of these two receptors appears to be identical with the immune adherence receptor found on erythrocytes. Both receptors were also found in lymphoblastoid cell lines. Most chronic lymphatic leukemia lymphocytes were found to have the receptor for cleaved C3b, but only a few of these lymphocytes possessed the immune adherence receptor. Granulocytes, on the other hand, were found to have the C3b receptor but not the receptor for cleaved C3b.

Materials and Methods

Lymphoid Cells and Granulocytes.—Blood from normal adults and patients with a confirmed diagnosis of chronic lymphatic leukemia was collected in heparin. Cultured lymphoblastoid cells, initiated from normal buffy coat cells, were a generous gift from Dr. Noburu Kashiwagi and Dr. Thomas Hütteroth.

Separation of Lymphocytes.—With normal peripheral blood, dextran sedimentation of erythrocytes was followed by centrifugation on Hypaque-Ficoll in which most of the phagocytes were pelleted, due to having been previously weighted with carbonyl iron. Poly-L-lysine-sensitized carbonyl iron particles were purchased from Technicon Instruments Corp., Tarrytown, N. Y. The carbonyl iron from one bottle of Technicon Lymphocyte Separation Reagent was resuspended in 15 ml of 1.6% dextran T-250 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in Hanks' balanced salt solution (HBSS) containing twice the normal amounts of $CaCl_2$ and $MgCl_2$, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (carbonyl iron reagent). Heparinized blood was mixed in a ratio of 2:3 with the carbonyl iron reagent for 1 h at 37°C on an inversion rotator at 15 rpm. After allowing the erythrocytes to sediment at room temperature for 20 min, the leukocyte-rich supernate was removed and diluted with an equal volume of ice-cold normal saline and 20 ml was layered onto 5 ml of ice-cold Hypaque-Ficoll (12% Hypaque in 8% Ficoll, d = 1.095 g/ml) in siliconized 23 × 105 mm glass centrifuge tubes. These were centrifuged at 4°C for 30 min at 1,000 g. A layer consisting of 91–99% lymphocytes was aspirated from the interface. After two washes in ice-cold normal saline, the cells were resuspended at 2 × 10^6/ml in RPMI-1640 (Microbiological Associates, Inc., Bethesda, Md.). No evidence of selective depletion of B or T cells was obtained when the percentages of

[2] Immune adherence, first described by Nelson in 1953 (13), is the agglutination of EAC (or any immune complex that fixes complement) with primate erythrocytes by way of the C3b (14) on the EAC and the C3b receptor found on primate (and not on nonprimate) erythrocytes.

cells with surface immunoglobulin and complement receptors and thymus-derived rosette-forming cells were checked after each purification step and also after parallel purifications by other less efficient procedures (15, 16). With leukemic peripheral blood the carbonyl iron step was unnecessary because the leukocytes were usually composed of 90–99% lymphocytes. After sedimentation of erythrocytes with 3% dextran, the lymphocytes were isolated by Hypaque-Ficoll (d = 1.08 g/ml) flotation as previously described (11).

Separation of Granulocytes.—After sedimentation of erythrocytes with 3% dextran (11), the leukocyte-rich supernate was resuspended in 30% fetal bovine serum in RPMI-1640 to a cell concentration of 2×10^6 leukocytes/ml and processed as previously described to separate adherent and nonadherent cells (11). Cells not adhering to plastic Petri dishes (lymphocytes, erythrocytes, and platelets) were discarded. The adherent cells (granulocytes and monocytes) were eluted by adding 10 ml of HBSS containing 0.04 M EDTA to each Petri dish and after a period of 1 h at 4°C, the eluted cells were recovered. After two washes with ice-cold RPMI-1640 the adherent cells were examined and typically contained 92% granulocytes, 6% monocytes, and 2% lymphocytes.

Erythrocyte-Antibody-Complement Complexes (EAC).—EA, EAC14^{oxy}23hu (EAC1-3hu), and EAC1423mo (EACmo) were prepared as previously described (11). EAC1-3huIn were prepared by treating EAC1-3hu with 8 U of purified C3b inactivator (17) for 1 h at 37°C, followed by two washes in ice-cold GVB. 1 U of C3b inactivator is that amount required for 50% inhibition of immune adherence utilizing EAC1-3hu and human erythrocyte indicator cells. When 8 U of C3b inactivator were used, inhibition of immune adherence was usually greater than 95%. However, if inhibition was less than this amount, a second treatment of the complex with C3b inactivator was performed.

EA(G) were prepared by incubating EA at a concentration of 2.5×10^8/ml with 1% glutaraldehyde in saline at 37°C for 15 min. This fixation procedure was previously shown to permit complement activation on the surface of the cell but to prevent hemolysis even when a large excess of whole complement was employed.[3] EAChu(G) were prepared by incubating EA(G) at 2×10^8/ml with an equal volume of whole human serum diluted 1:2.5 for 30 min at 37°C. After this procedure the unlysed red cells were washed twice with ice-cold GVB.

Detection of Complement Receptor Lymphocytes (CRL) and Thymus-Derived Rosette-Forming Cells (TRFC).—CRL and TRFC were measured as previously described except that the medium used was RPMI-1640, instead of 0.04 M EDTA-HBSS$^=$ (11).

Immunofluorescence.—Detection of surface immunoglobulin-bearing cells was performed as previously described except that the anti-immunoglobulin treatment was carried out in the presence of 0.03 M NaN_3 (2, 11).

Immune Adherence.—Rosettes of human erythrocytes and EAC were measured microscopically by a procedure similar to that used for the detection of complement receptor lymphocyte rosettes, except that human erythrocytes were substituted for lymphocytes and an incubation time of 30 min was used instead of 15 min. Rosettes with 2, 3, 4 or more attached EAC were separately enumerated and the average number of EAC bound per 100 erythrocytes was calculated.

Detection of Granulocyte Rosettes.—0.4 ml of granulocytes at 2×10^6/ml was mixed with 0.4 ml of EAC at 1×10^8/ml in a similar manner as that used for detection of CRL or erythrocyte immune adherence rosettes. Incubation on the rotator was only 5 min, since longer periods of incubation were found to produce clumps of rosettes and phagocytosis of the EAC.

Antilymphocyte and Antierythrocyte Sera.—Antilymphocyte sera were prepared in rhesus monkeys and were the generous gift of Dr. Arthur Malley. Monkeys were injected intramuscularly with whole cell suspensions containing $1–3 \times 10^8$ cells in Freund's complete adjuvant at 2-wk intervals for a period of 3–6 mo. Spleen cells were obtained at autopsy of an adult with no known gross immunological defect. The leukemic lymphocytes used for immunization were peripheral lymphocytes isolated from the whole blood of a patient with chronic lymphatic

[3] Polley, M. J. Unpublished observation.

leukemia. The brain tissue used for immunization was obtained at autopsy from the cortex region of a 2 mo old child. Thymus cells were from autopsy of a 3 mo old child. The cultured lymphoblastoid cells used for immunization were from a culture line initiated from normal buffy coat cells. Antierythrocyte serum was prepared in rabbits by immunization at 2-wk intervals with 2×10^9 human erythrocytes, first intravenously in saline, then intraperitoneally in saline, and finally intramuscularly in Freund's complete adjuvant. The rabbits were bled 4 wk after the last immunization. When tested by Ouchterlony analysis, none of the antisera reacted with normal human serum. The antierythrocyte serum was absorbed three times with 1/10 vol of human erythrocyte ghosts previously treated with papain in order to render them immune adherence negative (18).

Assay for Inhibition of Complement Receptors by Antiserum.—0.2 ml of heat-inactivated (56°C 30 min) antiserum diluted in 0.03 M NaN_3-HBSS was added to a pellet of 6×10^6 cells and allowed to stand at 0°C for 45 min, during which time the suspension was mixed with a Pasteur pipet every 15 min. The mixture was centrifuged, the supernate was discarded, and the cells, after 3 washes in ice-cold 0.03 M NaN_3-HBSS, were resuspended in 2 ml of ice-cold RPMI-1640 and tested for either CRL or immune adherence. Controls included untreated cells and cells treated with heat-inactivated normal rhesus monkey or rabbit serum.

Assay of Antilymphocyte Sera for Cytotoxicity and Immunofluorescence.—The amount of antibody directed to lymphocytes in the various sera was quantitated by ^{51}Cr release using rabbit complement and normal or leukemic lymphocyte target cells as previously described (19). The antilymphocyte sera were also studied by indirect immunofluorescence using a rabbit anti-rhesus monkey γ-globulin conjugated with fluorescein isothiocyanate (2). This antiserum was prepared in rabbits previously made tolerant to human γ-globulin (20).

Fab Fragments of Antimouse C3.—Rabbit antiserum to mouse C3 was prepared (21) and monovalent papain fragments of the γ-globulin fraction of the serum were isolated (22).

RESULTS

CRL, Surface Immunoglobulin, and TRFC in Cultured Lymphoblastoid Cells.—Lymphoblastoid cell lines were initiated from normal buffy coat cells either with no addition of virus or with the addition of a freeze-thaw lysate of a previously established culture (23). Each of the cell lines tested resembled B cells, since all had surface immunoglobulin, CRL, and totally lacked TRFC (Table I). As in tests with normal lymphocytes, EAC1-3hu usually detected

TABLE I

CRL, Surface Immunoglobulin (SIg), and Thymus-Derived Rosette-Forming Cells (TRFC) in Cultured Lymphoblastoid Cell Lines

Cell line	CRL+		SIg+	TRFC+
	EACmo	EAC1-3hu		
	%	%	%	%
Be.-C	75	82	51	0
Mo.-A	64	76	30	0
Ib.-A	88	89	31	0
Iw.-B	32	42	25	0
P.G.-L.C.	73	38	54	0
H.H.	84	97	24	0
T.M.	93	74	63	0
M.W.	93	70	46	0

slightly more CRL than did EACmo (11). However, in three out of eight cultures tested (P.G.-L.C., T.M., and M.W.), the cells resembled leukemic lymphocytes in that more cells with a complement receptor were detected with EACmo than with EAC1-3hu. In addition, the rosettes formed were clearly stronger in that more EAC were bound per rosette with EACmo than with EAC1-3hu.

Evidence for Two Antigenically Distinct Complement Receptors.—In order to study further the differences in the reactivity of EACmo and EAC1-3hu with different cells, antisera prepared to various whole cells were employed. Since spleen cells (11) and most cultured cell lines (in particular Ib.-A of Table I) contained large numbers of cells reacting nearly equally with EACmo and EAC1-3hu, these cells were used as immunogens in order to produce an antibody to "normal" complement receptor lymphocytes. Leukemic lymphocytes from a patient (J.M. of Table IV) whose cells were strongly reactive with EACmo (62%) but almost completely unreactive with EAC1-3hu (1%) were selected to produce an antiserum directed to the reactivity observed with EACmo. Since human erythrocytes formed strong rosettes with EAC1-3hu but were usually unreactive or poorly reactive with EACmo, rabbits were immunized with human erythrocytes to produce an antiserum with reactivity to the immune adherence receptor. As a specificity control, rhesus monkeys were also immunized with thymus and brain tissue, since T cells and brain tissue, though probably sharing many antigens with B cells, were not believed to possess complement receptors.

The rhesus monkeys were hyperimmunized over a period of 3–6 mo and all of the antisera produced were shown by indirect immunofluorescence and cytotoxicity to contain antibodies of high titer directed to antigens shared by both B and T cells. When the antisera were tested for their ability to inhibit complement receptors, only the antisera produced with immunogens containing B cells had such activity. No inhibition was obtained with the antithymus, antibrain, or normal (rhesus monkey or rabbit) sera. Complement receptor inhibition activity was found only in late bleedings from animals that at this time had also developed a high cytotoxic titer. With the rabbit antierythrocyte sera, no complement receptor inhibition activity was detectable when the sera were used in subagglutinating doses. However, when 90% of the agglutinating antibody in these sera was absorbed with papain-treated, immune adherence negative erythrocytes, one out of three antierythrocyte sera inhibited immune adherence with untreated erythrocytes.

The results of the antisera inhibition assays (Table II) indicated that the complement receptors for EACmo and EAC1-3hu were antigenically distinct. Whereas the antibodies to spleen and lymphoblastoid cells inhibited rosettes with both EACmo and EAC-3hu, antibody to leukemic lymphocytes only inhibited rosettes formed with EACmo and no inhibition was detected with EAC1-3hu. With cultured cells and leukemic target cells, the antierythrocyte

serum only inhibited rosettes with EAC1-3hu. However, with normal lymphocytes and with erythrocyte target cells it inhibited rosettes with both EAC1-3hu and EACmo. This apparent dual specificity was explained later when the specificity of the two receptors was demonstrated. When the antisera to spleen and to cultured cells were absorbed with erythrocytes, the ability of the antisera to inhibit EAC1-3hu rosettes was completely removed; however, this procedure had little or no effect on the ability of these sera to inhibit EACmo rosettes with lymphoblastoid or leukemic target cells.

TABLE II

Inhibition of Complement Receptors by Antisera Directed to Various Cells

Antiserum	Target cell	Inhibition of rosette formation	
		EACmo	EAC1-3hu
		%	%
Antispleen	NPL*	87	78
	CLL	96	88
	CLB	90	87
	E	94	85
Anti-CLB	NPL	20	35
	CLL	66	57
	CLB	100	99
	E	100	94
Anti-CLL	NPL	41	0
	CLL	96	0
	CLB	52	0
Anti-E	NPL	55	88
	CLL	0	46
	CLB	0	75
	E	80	70

* NPL, normal peripheral lymphocytes; CLL, chronic lymphatic leukemia lymphocytes; CLB, cultured lymphoblastoid cells; E, human erythrocytes.

A Receptor for C3b Inactivator-Cleaved C3b.—Since the EACmo was prepared with C5-deficient mouse serum, the complex probably contained two different forms of C3, C3b and products of C3b cleavage (C3c and C3d) produced by the C3b inactivator contained in the serum. On the other hand, since the EAC1-3hu was prepared with purified components, it contained only C3b. The observation that immune adherence with human erythrocytes and EACmo was poor compared with that with EAC1-3hu was further suggestive evidence of this postulate. Since Fab fragments of antimouse C3 completely blocked the reaction between EACmo and leukemic lymphocytes, one of the forms of C3 was clearly responsible for this activity. To determine whether the greater

activity of EACmo over EAC1-3hu with most leukemic and with some lymphoblastoid cell lines was due to a receptor for C3b inactivator-cleaved C3b, EAC1-3huIn was produced by treating EAC1-3hu with sufficient C3b inactivator to cleave more than 95% of the C3b to C3c and C3d as measured by inhibition of immune adherence. EAC was also prepared with whole human serum in an analogous manner to that used to produce EACmo, except that the EA were fixed with glutaraldehyde in order to prevent lysis subsequent to the addition of whole complement EAChu(G). The results of these experiments (Table III) indicated that the rosette-forming activity of EAC1-3hu was much increased with both leukemic lymphocytes and lymphoblastoid cells after treatment of the red cell complement complex with C3b inactivator.

TABLE III

CRL in Normal Peripheral Blood (NPB), Chronic Lymphatic Leukemia Peripheral Blood (CLL), and Cultured Lymphoblastoid Cells (CLB)

	CRL+			
	EACmo	EAC1-3hu	EAC1-3huIn	EAChu(G)
	%	%	%	%
NPB	8	10	5	7
CLL				
D.H.	79	39	77	67
F.H.	46	3	44	38
R.H.	59	6	54	59
CLB				
T.M.	98	77	95	96
M.W.	90	40	88	92

Since this activity was found to closely resemble the activity seen with EACmo, it was concluded that the receptor for EACmo demonstrated with the antiserum to leukemic cells was a receptor for C3b inactivator-cleaved C3b. Titration of purified C3 by microscopic immune adherence demonstrated that the residual 5% of immune adherence remaining subsequent to treatment of EAC1-3hu with C3b inactivator could be accounted for by as few as 100 molecules of C3b per EAC1-3huIn: an amount previously shown to be incapable of detecting complement receptor cells with normal peripheral or chronic lymphatic leukemia lymphocytes (11). On the other hand, immune adherence with EACmo and EAChu(G) was 10–70% of that obtained with EAC1-3hu, so it is probable that the EAC prepared with the whole serum either of human or mouse origin contained significant amounts of C3b as well as C3b that had been cleaved by C3b inactivator. Thus it appeared that EACmo reacted with both types of complement receptors, and when both types of receptors were present on a

single type of target cell, antisera inhibition of only one of the two types of receptors would still allow EACmo to form rosettes dependent on the other type of receptor. This was also apparently the explanation for the antierythrocyte serum causing 55% inhibition of rosettes between normal peripheral lymphocytes and EACmo (Table II). As shown in Table III, normal peripheral lymphocytes contained 10% of cells with the C3b receptor (10% CRL with EAC1-3hu) and 5% of cells with the receptor for cleaved C3b (5% CRL with EAC1-3huIn). Since 8% CRL were detected with EACmo, it was probable that this complement complex was detecting both C3 receptors and that only the C3b receptor was inhibited by the antibody to erythrocytes. Further, since human erythrocytes only contained the immune adherence receptor, EACmo reacted with erythrocytes only through the C3b contained in the complex, and therefore the antierythrocyte serum, which inhibited only the immune adherence receptor, would strongly inhibit immune adherence rosettes with EACmo when erythrocyte target cells were employed (Table II). EAC1-3hu, on the other hand, contained little if any cleaved C3b and therefore reacted only with cells having the uncleaved C3b receptor (immune adherence receptor). For this reason, the anti-chronic lymphatic leukemia lymphocyte serum, which only inhibited the receptor for cleaved C3b, did not inhibit EAC1-3hu rosettes while it completely inhibited rosettes with EAC1-3huIn (data not shown).

Complement Receptors in Chronic Lymphatic Leukemia.—In most cases of chronic lymphatic leukemia tested in this study, the lymphocyte immune adherence receptor was partially or completely missing, while the receptor for cleaved C3b was retained. Table IV shows the results of testing 19 patients with chronic lymphatic leukemia. 15 of 18 patients' lymphocytes tested reacted 2–60-fold better with EACmo than with EAC1-3hu. Lymphocytes from one patient (S.V.) gave more rosettes with EAC1-3hu than with EACmo. The same lymphocytes also formed less rosettes with EAC1-3huIn (71% CRL) and EAChu(G) (72% CRL) than with EAC1-3hu, a finding that indicated the presence of a large number of cells with receptors for C3b. When tests for immune adherence receptors were performed on erythrocytes from three of the patients whose lymphocytes reacted very poorly with EAC1-3hu, it was found that their erythrocytes gave a percentage of immune adherence rosettes in the high normal range.

Complement Receptors on Granulocytes.—Since previous investigators (24, 25) have demonstrated C3 receptors on granulocytes, it was of interest to determine whether granulocytes had receptors for both C3b and C3b inactivator-cleaved C3b. In the present study, more than 90% of granulocytes formed strong rosettes with EAC1-3hu, but fewer rosettes were formed with EACmo. Rosette formation was maximum within 3–5 min after the EAC were added and during the first 5 min many of the granulocytes were observed to have

phagocytized as many as five complement-coated cells. As has been shown with lymphocytes, neither EA (prepared with γM antibody) nor the EAC14^{oxy}2 used to form the EAC1-3hu formed rosettes with granulocytes.

In contrast to the results obtained with lymphocytes, EAC1-3hu treated with C3b inactivator in an amount shown to inactivate 98% of the immune adherence activity no longer formed rosettes with granulocytes. Thus it was concluded that granulocytes have receptors for C3b but probably do not have receptors for C3b inactivator-cleaved C3b.

TABLE IV

CRL, Surface Immunoglobulin (SIg), and Thymus-Derived Rosette-Forming Cells (TRFC) in Chronic Lymphatic Leukemia

Patient	CRL$^+$		SIg$^+$	TRFC$^+$
	EACmo	EAC1-3hu		
	%	%	%	%
J.Al.	41	23	80	n.d.*
J.An.	70	18	100	n.d.
H.C.	15	n.d.	60	n.d.
A.H.	55	11	13	1
D.H.	79	39	74	18
F.H.	46	3	73	27
R.H.	59	6	47	32
T.H.	10	1	<1	3
J.M.	62	1	70	3
A.O.	84	73	26	n.d.
E.S.	61	7	5	n.d.
L.S.	10	9	68	n.d.
V.S.	13	3	4	1
W.S.	31	1	<1	<1
R.T.	62	3	12	3
W.T.	47	9	24	n.d.
R.V.	65	33	1	n.d.
S.V.	72	79	62	21
B.Z.	59	39	n.d.	7

* n.d., not done.

DISCUSSION

In previous studies (11, 26) our data suggested that human lymphocytes possessed two complement receptors, one for human C3 and one for mouse C3. Further, that in most cases, only the receptor for mouse C3 was detectable on chronic lymphatic leukemia cells. This finding concerning the receptor on leukemic cells seemed to explain the apparently contradictory findings of several previous investigators who had prepared their EAC with either whole mouse serum (27, 28) or with the purified components of complement (29). In

our own studies (11, 26) we had similarly used whole mouse serum for the detection of the mouse C3 receptor and purified components of human complement for the detection of the human C3 receptor. Thus, in addition to a species difference between the sources of complement employed, there was also a difference between the C3 fixed when complement was supplied by whole serum as compared with that fixed when purified complement components were used. The whole mouse serum contained C3b inactivator that would cleave cell-bound C3b into two fragments, C3c and C3d, in such a way that the C3c fragment was liberated from the cell while only the C3d fragment remained cell bound (30). When highly purified components of human complement were used instead of whole mouse serum, no C3b inactivator was present, and the C3 on the complex was in the form C3b. In experiments designed to distinguish between these two possibilities for the explanation of the difference in reactivity between EAC1-3mo and EAC1-3hu, it was found that the presence of the C3b inactivator during the preparation of EAC1-3mo was indeed responsible. The two complement receptors were distinguishable, not by the difference in species, mouse or human, but by the cell-bound C3 in the EAC1-3 complex being either in the form C3b or C3b inactivator cleaved C3b(C3d).

The two receptors can only be measured individually if the EAC contain only one form of C3. In experiments in which whole serum was used as the source of complement, it was not consistently possible to prepare EACmo [or EAChu(G)] that was completely immune adherence negative, that is to say, in which all the cell-bound C3 had been cleaved by the C3b inactivator. However, utilizing purified components, the two receptors could be easily distinguished, since EAC1-3 could be prepared in which the C3 was entirely in the form C3b, and then this complex could be reacted with purified C3b inactivator to produce a second type of indicator complex, EAC1-3In, in which most of the cell-bound C3b had been cleaved into C3c and C3d. Since Ruddy and Austen (30) have demonstrated that C3b inactivator treatment of EAC1-3hu causes release into the fluid phase of most of the C3c but little of the C3d, it is most likely that the receptor for inactivator-cleaved C3b is specific for the C3d fragment of C3.

In order to further distinguish between the two receptors, experiments were performed utilizing antisera produced against various types of cells. Since these antisera were produced against whole cells, none was specific for the complement receptors. However, they were found to be capable of distinguishing between the two complement receptors. Utilizing the antiserum produced against chronic lymphatic leukemia lymphocytes, it was found that the immune adherence (C3b) receptor was antigenically missing or reduced from the lymphocytes of these individuals. However, the complement receptor on these leukemic cells was antigenically related to a receptor found on normal B cells, since it was inhibited by the antispleen serum and since the antileukemic cell serum inhibited the cleaved C3b (C3d) receptor of normal lymphocytes. Thus

it became apparent that with most chronic lymphatic leukemic lymphocytes, the only complement receptor that was readily detectable was that for cleaved C3b (C3d) and the C3b receptor was either absent or present in a much reduced amount. Both receptors were found to be present on normal peripheral lymphocytes and on cultured lymphoblastoid cells, but only the immune adherence (C3b) receptor, and not the C3d receptor, was found on erythrocytes.

In previous studies (31) a biologic activity has been attributed to the C3d fragment of C3 that is neither present in the native C3 molecule nor in C3b i.e., that it is agglutinable by conglutinin. It was found that conglutinin agglutinates immune complexes containing C3 (such as EAC1-3hu) only after treatment of the complex with conglutinogen-activating factor (KAF) (32). This factor was later shown to be identical to the C3b inactivator (33). The conglutination reaction, like the receptor for cleaved C3b on lymphocytes, is specific for C3b only subsequent to its cleavage by C3b inactivator. Studies are now under way to determine whether conglutinin is related to the lymphocyte receptor for C3d.

The finding of a receptor for C3b inactivator cleaved C3b (C3d) is of interest, since it means that it is possible for circulating immune complexes to become bound to lymphocytes despite the presence of C3b inactivator in serum. The observation that only the receptor for C3b (immune adherence receptor) is present on erythrocytes suggests that in an in vivo situation, if an immune complex became bound to an erythrocyte by the C3b receptor, it might be released by cleavage of the C3b by the C3b inactivator and then could conceivably become bound to a lymphocyte. The lack of a receptor for cleaved C3b on granulocytes is possibly balanced by their possessing a receptor for IgG (24) and one for C4[4] that is considerably more effective in binding immune complexes than that found on lymphocytes.

SUMMARY

In the present study it was shown that normal peripheral lymphocytes have two different complement receptors: one for C3b (the immune adherence receptor) and one for C3b subsequent to its cleavage by C3b inactivator. The two receptors are not cross-reactive and were shown by tests with various antisera to be antigenically distinct. Both the immune adherence receptor and the receptor for C3b inactivator-cleaved C3b were found on normal peripheral lymphocytes and on cultured lymphoblastoid cells. In 15 out of 18 chronic lymphatic leukemia patients, the immune adherence receptor was either partially or completely missing from the peripheral lymphocytes, while the lymphocyte receptor for C3b inactivator-cleaved C3b was retained. Normal erythrocytes, on the other hand, were found to have only the immune adherence

[4] Ross, G. D., and M. J. Polley, manuscript in preparation.

receptor. Granulocytes from normal peripheral blood appeared to have only a receptor for C3b and did not have a receptor for C3b inactivator-cleaved C3b.

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