COMPLEMENT RECEPTOR (CR_1) DEFICIENCY IN ERYTHROCYTES FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS*

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Recently, it was shown that a human red cell (RBC)[1] membrane glycoprotein of 200,000 mol wt is a powerful inhibitor of one of the central enzymes of the complement system, the C3-convertase of the alternative pathway (1). This protein, identified as the immune-adherence receptor for the complement fragments C3b and C4b (CR_1), (2) was later shown to inhibit the classical pathway C3- and C5-convertases (3).

In human peripheral blood, CR_1 is found on erythrocytes, lymphocytes, neutrophils, and monocytes (4, 5). Although normal human RBC bear $<2 \times 10^3$ molecules of CR_1 (2, 6), their presence can be detected by the immune-adherence assay based on the agglutination of erythrocytes by C3b (7, 8) or C4b-bearing (9) immune complexes. Miyakawa et al. found that RBC from most patients with systemic lupus erythematosus (SLE) failed to exhibit immune-adherence and suggested that the erythrocyte defect, presumably involving CR_1, might be inherited rather than acquired (10).

To study this phenomenon further, we developed an immunoradiometric assay for human CR_1, using monoclonal antibodies, and measured its concentration in RBC from normal individuals and from patients with SLE and other diseases.

Materials and Methods

Monoclonal Antibodies. Monoclonal antibodies against human CR_1 were produced as described by Kohler and Millstein (11). CR_1 was purified to homogeneity from human erythrocytes (1), and 6 μg was incorporated into Freund's complete adjuvant and injected into mice. 3 wk later, the mice were boosted with 6 μg of CR_1 intraperitoneally, and the spleens were removed 3 d later and used for fusion with the mouse plasmacytoma cell line SP2 (12).

The assay for the detection of antibody to CR_1 secreted by the hybrids into the culture medium was performed as follows. The wells of plastic plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with C3b by incubation with 50 μl of purified C3b (13), 50 μg/ml in 0.02 M phosphate buffer, pH 7.6, at room temperature for 2 h. The wells were washed three times with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.4, containing 0.02% sodium azide (BSA-PBS). Then 25 μl of partially purified CR_1 was added to the wells at a concentration that saturated the CR_1 binding sites of the solid-phase C3b. Partially purified CR_1 was obtained as described by Fearon (1), except that the steps involving affinity chromatography on Sepharose C3b and Sepharose-lentil lectin were omitted. After incubation for 1 h at room temperature, the wells were washed three times with 0.05% Tween

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; C, complement; CR_1, complement receptor for C3b and C4b (immune-adherence receptor); NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PMN, polymorphonuclear cells; RA, rheumatoid arthritis; RBC, erythrocytes; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; Staph A, *Staphylococcus aureus.*

20 (Fisher Scientific Co., Pittsburgh, PA) in BSA-PBS. Then 25 μl of the culture supernatants was added to the individual wells. After incubation for 2 h at 4°C, followed by three washes with Tween-BSA-PBS, 25 μl (1 ng) of ^{125}I-radiolabeled affinity-purified goat anti-mouse immunoglobulin (Ig) (5×10^7 cpm/μg) was added to each well. The antibodies were radiolabeled using Enzymobeads (Bio-Rad Laboratories, Richmond, CA), as described by the manufacturer. After an additional incubation of the plates for 1 h at room temperature, the wells were washed four times with Tween-BSA-PBS, cut individually, and counted in a gamma counter. The cells yielding positive supernatants were cloned by limiting dilution in the presence of mouse thymocytes; the clones were expanded and then injected intraperitoneally into Pristane (Aldrich Chemical Co., Milwaukee, WI)-treated mice to obtain ascites fluid.

Ig were purified to homogeneity by ammonium sulfate precipitation, followed by column chromatography on DEAE Sephacel and Sephadex G-200 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ). Both monoclonal antibodies used in this study, 57F and 44D, were typed as IgGl,K. A monoclonal antibody (3D11) against a malaria antigen was also typed as IgG1,K and was used in control experiments (14).

Preparation of Cells. Lymphocytes were obtained by centrifugation of suspensions of tonsil cells on Ficoll-Paque (Pharmacia Fine Chemicals) cushions (15). Cells were obtained from fresh, citrated peripheral blood of normal individuals and patients. Mononuclear cells were first separated by Ficoll-Paque. A population of lymphoid cells enriched for T cells was then obtained by passage of the mononuclear cells through a nylon wool column (16), followed by repeated panning on petri dishes coated with affinity-purified $F(ab')_2$ fragments of rabbit anti-human IgG (17). The final preparation contained 2.7% lymphocytes that formed rosettes with $EAC14^{oxy}23b$. Polymorphonuclear cells (PMN) were separated by centrifugation on cushions of Percoll (Pharmacia Fine Chemicals) (18). Erythrocytes were washed four times with 0.15 M NaCl, carefully removing the buffy coat after each centrifugation.

Blood samples were obtained from 34 SLE patients from Bellevue Hospital-New York University Medical Center, and The Rockefeller University, New York. All patients fulfilled at least four of the preliminary criteria for the diagnosis of SLE (19). There were 5 males and 29 females, ranging in age from 19–55 yr old (mean, 34 yr). The duration of the illness was from 4 mo to 15 yr (mean, 8 yr). At the time of the study, 20 patients were being treated with prednisone, with daily doses ranging from 5–100 mg. 13 of these patients had active disease. Blood samples were also obtained from 7 asthmatics, males and females, who were receiving daily doses of >40 mg of prednisone or equivalent, and from 10 patients with sero-positive rheumatoid arthritis. The controls consisted of 52 healthy male and female blood donors and laboratory personnel, ranging in age from 20 to 55 yr old.

Immunoradiometric Assay for CR_1 in Cell Extracts. The cell extracts were prepared as follows: 4×10^9 packed, washed erythrocytes were lysed with 200 μl of 1% Nonidet P-40 (NP-40, Particle Data Inc., Elmhurst, IL) in PBS containing 50 μg/ml of the synthetic elastase inhibitor Suc (OMe)-Ala-Ala-Pro-Val-MCA (Peninsula Laboratories, Inc., San Carlos, CA) and 1 mM phenylmethylsulfonyl fluoride (PMSF). After 1 h of incubation with occasional agitation by vortexing, the volume was brought to 2 ml with BSA-PBS containing the same protease inhibitors and centrifuged at 50,000 *g* for 30 min.

In the case of other cell populations, an identical procedure was used, except that the lysing buffer also contained 200 U/ml of deoxyribonuclease I (Worthington Biochemical Corp., Freehold, NJ). The final concentration of neutrophils or mononuclear cells in the extracts was 2×10^7/ml, and the concentration of T cells in the extracts was 10^8/ml.

The immunoassay was performed as follows: 25 μl of two dilutions of the cell extracts in BSA-PBS containing 0.1% NP-40 were added to C3b-coated plastic wells that were prepared as described in the assay for the detection of monoclonal antibodies. After 2 h of incubation at 37°C, the wells were washed three times with Tween-BSA-PBS. Then 25 μl of a 2 μg/ml solution of ^{125}I-labeled monoclonal antibody to CR_1 (57F) was added to the wells. The monoclonal antibody was also radiolabeled using Enzymobeads. After an additional incubation for 1 h at room temperature, the wells were washed four times with Tween-BSA-PBS, cut individually, and counted in a gamma counter. The amounts of CR_1 in the extracts were calculated from a standard curve obtained with purified CR_1, which was included in each series of determinations (Fig. 1).

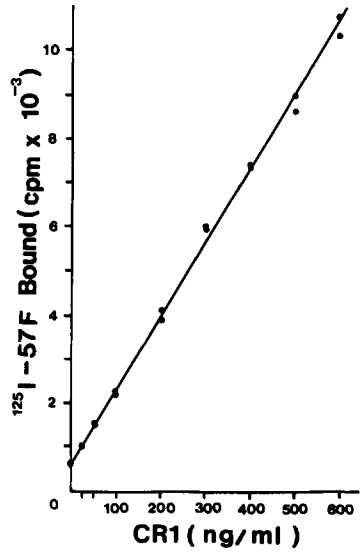

FIG. 1. Standard curve obtained by titrating purified CR_1 by means of the immunoradiometric assay.

Immunoassay for Measuring CR_1 on Cell Surfaces. 10^7 erythrocytes were incubated with 100 ng of ^{125}I-labeled monoclonal (57F) in 200 μl of BSA-PBS at 37°C. After incubation for 30 min, triplicate samples of 50 μl of the reaction mixtures was layered onto 300 μl of a mixture of 8 vol of dibutyl phthalate (Fisher) and 2 vol of dinonylphthalate (Eastman Kodak Co., Rochester, NY) in Beckman microtest tubes (Beckman Instruments Inc., Fullerton, CA). The tubes were centrifuged at 8,000 *g* for 90 s in a Beckman B microcentrifuge (Beckman Instruments, Inc.). The tips of the tubes were cut and counted in a gamma counter.

Rosette Formation between CR_1-bearing Cells and Sheep Erythrocytes Sensitized with Antibody and Complement. To prepare EAC14^{oxy}23b, sheep erythrocytes (E) were sensitized with rabbit IgM antibodies (A) to sheep E (Cordis Laboratories Inc., Miami, FL) and reacted sequentially with complement (C) components, guinea pig C1 (20), human C4 (21), and ^{oxy}C2 (22) at concentrations calculated to form 300 hemolytic sites per cell. To this cellular intermediate, limiting amounts of human C3 (23) were added to generate about two hemolytic C3 sites per cell. These cells (EAC14^{oxy}23b) were suspended at 1×10^8 cells/ml in RPMI 1640 (Gibco Laboratories, Grand Island Biologic Co., Grand Island, NY) with 1% Hepes buffer and 0.1% sodium azide, pH 7.4. To form rosettes, 300 μl of this suspension was mixed with 150 μl of the same buffer and 150 μl of lymphocytes (4×10^6) and was incubated, rotating continuously, for 30 min at 37°C. 300 lymphocytes were counted under the microscope and scored for rosette formation. Controls incubated with EAC14^{oxy}2 did not form rosettes. To determine the effect of the monoclonals 57F and 44D on rosette formation, the lymphocytes were preincubated at 37°C for 30 min with 150 μl of various antibody dilutions and then mixed with 300 μl of EAC14^{oxy}23b for rosette formation.

Surface Labeling of Lymphocytes and Immunoprecipitation of Membrane Proteins. 3×10^7 tonsil lymphocytes, purified as above and suspended in 1 ml PBS, were radiolabeled with 1 mCi of Na^{125}I in a glass tube coated with Iodogen (Pierce Chemical Co., Rockford, IL). After 10 min at room temperature, the reaction mixture was layered on 1 ml of fetal calf serum and centrifuged. The pelleted cells were further washed twice with PBS. Cells were lysed with 200 μl of 1% NP-40 in PBS containing 50 μg/ml of the elastase inhibitor Suc(OMe)-Ala-Ala-Pro-Val-MCA, 200 U/ml of DNase, and 1 mM PMSF. After 1 h at room temperature, the extracts were centrifuged at 12,000 *g* for 10 min.

20 μl of the supernatant was immunoprecipitated with 20 μl (20 μg) of various antibodies and control proteins: monoclonals 57F, 44D, polyclonal anti-CR1 rabbit IgG, and mouse

myeloma protein MOPC 21 (Meloy Laboratories, Inc., Springfield, VA). After incubation overnight at 4°C, 100 μl of a 10% suspension of *Staphylococcus aureus* (Staph A, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) was added and the mixture further incubated at 0°C for 30 min. The Staph A was collected by centrifugation and washed four times with PBS containing 0.1% NP-40 and 0.05% deoxycholate. The proteins bound to Staph A were eluted with 50 μl Tris, pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 6 M urea, and 20% 2-mercaptoethanol.

The eluted materials were subjected to SDS polyacrylamide gel electrophoresis (PAGE) according to the method of Laemmli (24). 4.5% running and 3% stacking gels were used, and radioautography was carried out by exposing the dried gel to an X-Omat R film (XR-5, Eastman Kodak Co.) at −70°C.

Titration of C4 Hemolytic Activity and Circulating Immune Complexes. C4 hemolytic activity was titrated using cellular intermediates prepared with guinea pig complement components. Clq-binding assays (25) and Raji cell assays (26) for the detection of immune complexes were kindly performed by Dr. A. Theophilopoulos (Scripps Clinic, La Jolla, CA). The above determinations were performed in plasma and sera from SLE patients with active and inactive disease.

Results

Characterization of the Monoclonal Antibodies 57F and 44D. By two criteria, the monoclonal antibodies 57F and 44D are directed against CR_1. They immunoprecipitated a single polypeptide of molecular weight identical to that of purified CR_1, from extracts of surface-labeled tonsil lymphocytes (Fig. 2), and at very low concentrations they inhibited rosette formation between RBC bearing C3b and lymphocytes (Table I).

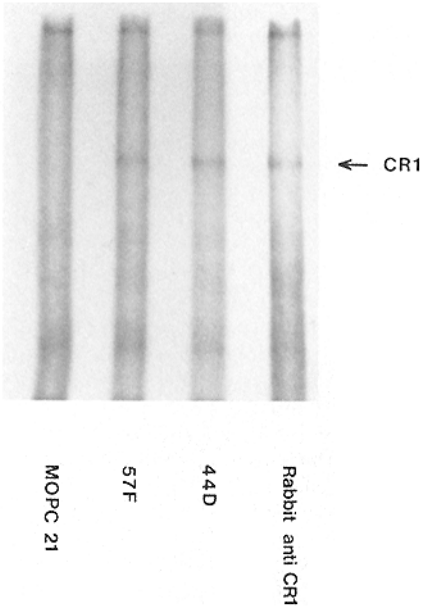

FIG. 2. Immunoprecipitation of CR_1 from the cell surface by monoclonal antibodies. The surface membranes of tonsil lymphocytes were radiolabeled, and the extracts were immunoprecipitated by various antibodies. The immunoprecipitated materials were analyzed by SDS-PAGE and radioautography. The position of purified CR_1, which was electrophoresed and stained in the same gel, is shown by the arrow. As seen, both the monoclonal antibodies 57F and 44D as well as a polyclonal rabbit anti-CR_1 (1) immunoprecipitated CR_1.

TABLE I

Inhibition of Rosette Formation between EAC14^{oxy}23b and Tonsil Lymphocytes by Monoclonal Antibodies

Monoclonal antibodies	Concentration	Inhibition of rosette formation*
	ng/ml	%
44D	2,000	86
	200	89
	20	85
	2	87
	0.2	50
57F	20,000	79
	2,000	50
	200	39
	20	55
	0.2	0
3D11	20,000	0
(Control)	200	0

* 24% of cells in this preparation formed rosettes with EAC14^{oxy} 23b in the absence of antibodies; no rosette formation occurred with EAC14^{oxy} 2.

TABLE II

*Distribution of CR_1 among Cell Populations**

Cells		Treatment	CR_1 molecules/cell
Tonsil	Mononuclear	None	1.8×10^5
		Trypsin‡	0
Peripheral blood	Erythrocytes	None	1.4×10^3
		Trypsin‡	0
	Mononuclear, total	None	3.8×10^4
	PMN	None	1.4×10^5
	T lymphocytes§	None	7.0×10^2
Raji		None	0

* The concentrations of CR_1 were measured in cell extracts, as described in Materials and Methods.

‡ Cells were incubated with trypsin, 100 μg/ml for 30 min at 37°C, and the reaction was stopped by adding soybean trypsin inhibitor.

§ This population contained 2.7% of cells that formed rosettes with EAC14^{oxy}23b.

The monoclonals were also used to measure the concentration of CR_1 in cells from human peripheral blood and tonsils and in Raji cells (27). As shown in Table II, CR_1 was not detectable on Raji cells. Mononuclear cells from tonsils and peripheral blood had 1.8×10^5 and 3.8×10^4 CR_1 molecules per cell, respectively. By rosette formation with EAC14^{oxy}23b, the tonsils contained ~50% B lymphocytes. Assuming that the other cells do not contain CR_1, we calculate that the B lymphocytes have 3.6×10^5 CR_1 molecules. By the same reasoning, the CR_1-positive cells of human peripheral blood (monocytes and B lymphocytes), which constitute ~15% of the total mononuclear cells, bear an average of 2.5×10^5 CR_1 molecules. The number of CR_1 molecules in neutrophils and erythrocytes was 1.4×10^5 and 1.4×10^3, respectively. Extracts of

purified T lymphocytes contained small numbers of CR_1 molecules (7×10^2/cell), which could be accounted for entirely by the presence in this population of 2.7% of cells that formed rosettes with $EAC14^{oxy}23b$ and which could be contaminating monocytes or B lymphocytes.

An additional point of interest was that, when tonsil cells were treated with trypsin (100 μg/ml, 30 min, at 37°C), CR_1 could no longer be detected in the extracts, implying that most CR_1 molecules of B lymphocytes were surface bound.

Although both monoclonals recognized CR_1 and inhibited its function, they bound to separate epitopes of the molecule. This was determined by measuring the binding of the ^{125}I-labeled monoclonals to plastic plates coated with CR_1, as described in the legend of Fig. 3. Under saturating conditions, the specific counts bound to CR_1, using a mixture of 57F and 44D, were equal to the sum of the counts obtained with each monoclonal separately. Furthermore, the amounts of labeled 44D that bound to the

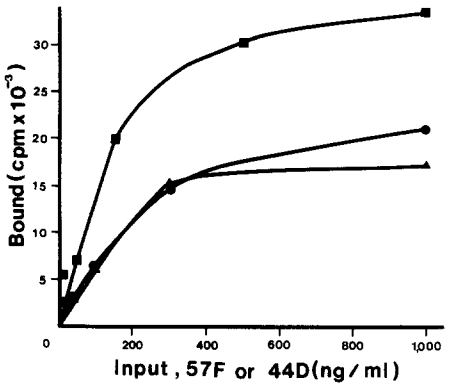

FIG. 3. The monoclonal antibodies 57F and 44D bind to separate epitopes of CR_1. CR_1 was bound to C3b-coated plates, as described in Materials and Methods. The radiolabeled monoclonal antibodies, either separately or as a mixture, were added. After 1 h of incubation, wells were washed, cut individually, and counted. The numbers in the abscissa represent the final concentrations of each monoclonal antibody in wells. The results show that under saturating conditions the number of counts bound to the wells that received a mixture of 57F plus 44D represent approximately the sum of the counts in wells that received the separate monoclonal antibody. ■, 57F + 44D; ●, 57F; ▲, 44D.

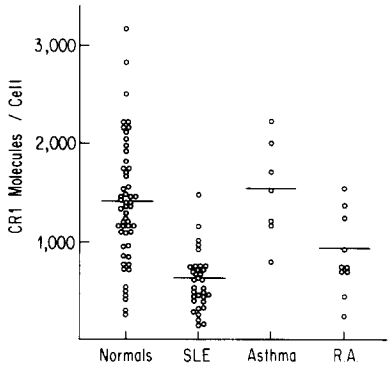

FIG. 4. Number of CR_1 molecules on erythrocytes from normal individuals and patients. CR_1 were measured in extracts of RBC from 52 normal individuals, 34 SLE, 10 RA, and 7 asthmatic patients, using an immunoradiometric assay. The CR_1 levels of the SLE patients are significantly diminished.

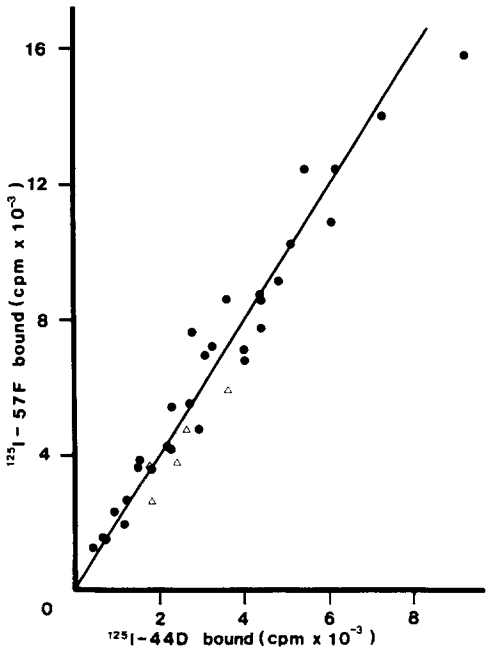

FIG. 5. Correlation between CR_1 measurements in RBC extracts using two monoclonal antibodies. CR_1 titrations in extracts of RBC from normal individuals (●) and SLE patients (△) were performed by immunoradiometric assay. Two assays were performed in each sample, using either antibody 57F or 44D. The correlation coefficient between the two measurements was 0.96 ($P < 0.001$).

CR_1-coated plates were not influenced by the presence of high concentrations of cold 57F and vice versa (data not shown).

CR_1 Levels in Normal Individuals and Patients. CR_1 was measured in extracts of RBC of 52 healthy volunteers using monoclonal 57F (Fig. 4), and the number of CR_1 molecules per cell was estimated as 1,410 ± 620. No significant differences in the receptor levels were observed when the individuals were grouped by sex, blood group, (A, B, O), or age (20–29, 30–39, and 40–55 yr of age).

As shown in Fig. 5, we also measured CR_1 in the extracts, using monoclonal 44D instead of 57F. The correlation coefficient between the results of the two measurements was 0.96 ($P < 0.001$). A separate immunoassay was used to estimate the amounts of CR_1 on cell surfaces (see Materials and Methods). When the results were compared with the CR_1 concentrations in the corresponding extracts, the correlation coefficient was 0.98 ($P < 0.001$).

In 34 SLE patients, the CR_1 levels in RBC extracts (600 ± 307 molecules/RBC) were significantly lower than those of normals ($P < 0.001$). To determine whether CR_1 varies in the course of disease, we performed sequential determinations in some patients. Four individuals were tested during disease activity and in remission. Two patients showed large increases (80% and 176%) of CR_1 levels when in remission, and in the others, the low CR_1 value did not change.

In 10 patients with rheumatoid arthritis, the CR_1 levels were 903 ± 417 molecules/RBC, a value that is also significantly below normal ($P < 0.02$). To verify whether the administration of steroids lowers the levels of CR_1 on erythrocytes, we studied seven asthmatics undergoing long-term treatment with prednisone or an equivalent drug.

The CR_1 values in this group (1,560 ± 500 molecules/RBC) did not differ significantly from those of normal individuals.

Correlation between C4 Levels, Circulating Immune Complexes, and CR_1 Values in Patients with SLE. C4 hemolytic titrations were performed in plasmas from 24 patients with SLE and in 18 age- and sex-matched normal volunteers. The C4 levels in the patients and controls were 29,500 units ± 20,400 and 39,000 ± 15,400, respectively. The correlation coefficient between CR_1 and C4 levels was 0.56 ($P < 0.01$) in patients and 0.038 ($P \geq 1$) in normal individuals.

Raji cells and solid-phase Clq-binding assays for circulating immune complexes were performed in 20 serum samples from SLE patients. The mean values were 955 ± 756 (normal values, <17) for the Raji cell assay and 181 ± 122 (normal values, <13) for the Clq-binding assay expressed in μg of aggregated human gamma globulin equivalents. When these values were compared to CR_1 levels in the same patient, a negative correlation was found with the results of the Clq-binding assays ($r = -0.49$, $P < 0.05$) but not with the Raji assay ($r = 0.32$, $P > 0.2$).

Discussion

The present study reports quantitative information on the distribution of complement receptor (CR_1) molecules in normal individuals and in patients. The CR_1 measurements were made using monoclonal antibodies. The antibody specificity was confirmed by immunoprecipitation of CR_1 from extracts of surface-labeled cells, by inhibition of rosette formation between B lymphocytes and the RBC intermediate $EAC14^{oxy}23b$, and by the characteristic distribution of the antigen among cells of human peripheral blood.

According to these measurements, we estimate that B lymphocytes bear the highest number of CR_1 molecules (3.6×10^5) on their membranes, followed by neutrophils (1.4×10^5) and RBC (1.4×10^3), whereas T lymphocytes are negative. However, as pointed out by Siegel et al. (28) and Medof and Oger (29), if the ratio of RBC to leukocytes is taken into consideration, >90% of CR_1 in human peripheral blood is erythrocyte associated. Therefore, in theory, complement-fixing immune complexes formed in circulation might first encounter CR_1 of RBC. Indeed, Medof and Oger (29) have added immune complexes prepared with labeled antigen to whole human blood and found that in <5 min, 80–85% of the counts became RBC associated. These experimental findings and the observations of Nelson (30), that RBC seem to be involved in the clearance of complexes from circulation, might provide an explanation for the decrease in the number of CR_1 observed in SLE patients (see below).

The number of CR_1 in extracts of RBC from 52 normal individuals 20–55 yr of age varied from 250–3,000 molecules per erythrocyte. Because of the wide range of values obtained, we performed several experiments to exclude some possible sources of systematic error. For example, the assay for CR_1 in RBC extracts involved two successive specific steps, i.e., the binding of the receptor to solid-phase C3b, followed by the titration of the bound receptor using a radiolabeled monoclonal antibody (57F). It could be argued that the wide differences in CR_1 levels in RBC from normals is artefactual and a reflection of the presence in the population tested of structural variants (allotypes) of CR_1 with different affinities for C3b and/or for the monoclonal 57F.

To study the influence of the monoclonals, we repeated the titrations in RBC extracts using monoclonal 44D instead of 57F. In other experiments, we measured CR_1 on the membrane of intact cells, using an assay that is independent of the affinity of the receptor for C3b. The correlation between the results of these measurements performed simultaneously in samples of RBC from the same individuals was highly significant. Because the two monoclonals are directed against separate epitopes of CR_1, it is unlikely that the large variation in CR_1 values is caused by the presence of structural variants of CR_1 in the population studied.

We also found that the CR_1 levels of RBC in several normal volunteers were remarkably stable. In four individuals, two of whom had low CR_1 levels (<300 molecules per cell), we performed a series of determinations during a period of 6 mo. In every case, the values of CR_1 varied <10% around the mean (results not shown).

Of some interest is the finding that the Raji cell line does not have detectable CR_1. Raji cells have been used to measure circulating immune complexes (26) in humans, and the nature of their membrane-associated complement receptor has been the subject of some controversy (31, 32). In light of the present observations, Raji cells either have a membrane receptor for C3b different from CR_1 or, more likely, the receptor recognizes a modified form of C3b, perhaps resulting from its interaction with control proteins (32).

We also measured CR_1 levels on RBC from patients with autoimmune diseases. A low number of CR_1 molecules was found on erythrocytes of individuals with SLE. This finding explains previously reported observation (10, 33) of defective immune-adherence properties of the patients' erythrocytes. The cause of the diminution in the expression of CR_1 is unknown. The defect is not uniquely characteristic of SLE patients because low CR_1 levels were also observed in patients with rheumatoid arthritis. It is unlikely that corticosteroids are responsible because CR_1 levels were normal in extracts of RBC from asthmatic individuals receiving high doses of prednisone.

The defect could represent a secondary manifestation of the presence in circulation of immune complexes and/or autoantibodies to CR_1. The ligands could block the receptors, mediate the removal of some of the modified RBC from circulation, or promote the internalization of the receptors from the membrane of RBC precursors (34). Some of our findings are compatible with this hypothesis. For example, significant correlations were found between CR_1 and the levels of circulating immune complexes as well as between CR_1 and hemolytic titers of C4 in serum. Also, in two out of four patients with SLE, the levels of CR_1 increased substantially after remission, showing that the deficiency is at least in part reversible.

An alternative explanation, however, is that the defect is genetically controlled and that individuals with low CR_1 levels are more prone to develop certain immune complex diseases. In other words, the CR_1 defect might precede disease and contribute to the accumulation of immune complexes in circulation.

Whatever the mechanisms involved, it seems likely that they will also affect CR_1 of other cells. Indeed, Kazatchkine et al. (35) and Emancipator et al.[2] have recently shown that CR_1 antigen is diminished or absent in glomeruli of SLE patients with proliferative glomerulonephritis.

[2] Emancipator, S. N., K. Iida, V. Nussenzweig, and G. Gallo. Monoclonal antibodies to human complement receptor detect defects in glomerular diseases. Manuscript submitted for publication.

CR_1 plays an important role in phagocytosis (4) and probably participates in events leading to the clearance of certain types of immune complexes from circulation (36, 37). A defect in CR_1 might lead to alterations in the fate of immune complexes and of associated antigens. These abnormalities may be particularly severe in SLE patients whose Fc receptors presumably are functionally altered (38), and may contribute to an enhanced deposition of immune complexes in tissues.

Our findings imply that SLE should be added to the growing list of disorders whose pathophysiology involve defects in membrane receptors. Additional studies, however, are necessary to clarify the nature of the CR_1 defect and its role in disease. It would also be of interest to determine whether CR_1 levels are a good indicator of disease activity. The availability of sensitive and quantitative assays to measure the CR_1 antigen in extracts of cells and on cell surfaces should facilitate the experimental approach to these problems.

Summary

This study reports quantitative information on the concentration of complement receptor for C3b and C4b (CR_1) on erythrocytes from normal individuals and patients with immune complex disease. The measurements were performed by an immunoradiometric assay using monoclonal antibodies against CR_1. The antibody specificity was confirmed by immunoprecipitation of CR_1 from extracts of surface-labeled cells, by inhibition of rosette formation between B lymphocytes and the erythrocytes intermediate $EAC14^{oxy}23b$, and by the characteristic distribution of the antigen among cells of human peripheral blood.

The number of CR_1 molecules in erythrocytes from 52 normal individuals was estimated as 1,410 ± 620. No significant differences in CR_1 levels were observed when individuals were grouped by sex, age, or blood groups. In patients with SLE and rheumatoid arthritis, the number of CR_1 molecules per RBC was significantly lower, i.e., 600 ± 307 and 903 ± 417, respectively. CR_1 levels were normal in asthmatics undergoing long-term treatment with prednisone. In SLE patients, significant correlations were found between CR_1 levels, C4 hemolytic titers, and levels of circulating immune complexes. In two out of four patients with SLE, CR_1 levels increased significantly during remission, showing that the deficiency is, at least in part, reversible. The deficiency in CR_1 could be genetically controlled or could represent an epiphenomenon caused by the interaction of the receptor with a ligand present in the circulation of patients.

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References

1. Fearon, D. T. 1979. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *Proc. Natl. Acad. Sci. U. S. A.* **76**:5867.
2. Fearon, D. T. 1980. Identification of the membrane glycoprotein that is the C3b receptor

of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. *J. Exp. Med.* **152:**20.

3. Iida, K., and V. Nussenzweig. 1980. Complement receptor is an inhibitor of the complement cascade. *J. Exp. Med.* **153:**1138.
4. Bianco, C., and V. Nussenzweig. 1977. Complement receptors. *Contemp. Top. Mol. Immunol.* **6:**145.
5. Ross, G. D., and M. J. Polley. 1963. Specificity of human lymphocyte complement receptors. *J. Exp. Med.* **141:**1163.
6. Dobson, N. J., J. D. Lambris, and G. D. Ross. 1981. Characteristics of isolated erythrocyte complement receptor type one (CR1, C4b-C3b receptor) and CR1-specific antibodies. *J. Immunol.* **126:**693.
7. Nelson, R. S. 1953. The immune adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science (Wash. D. C.).* **118:**733.
8. Nelson, D. S. 1963. Immune adherence. *Adv. Immunol.* **3:**131.
9. Cooper, N. R. 1969. Immune adherence by the fourth component of complement. *Science (Wash. D. C.).* **165:**396.
10. Miyakawa, Y., A. Yamada, K. Kosaka, F. Tsuda, E. Kosugi, and M. Mayumi. 1981. Defective immune-adherence (C3b) receptor on erythrocytes from patients with systemic lupus erythematosus. *Lancet.* **II:**493.
11. Köhler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* **256:**495.
12. Shulman, M., D. Wilde, G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. *Nature (Lond.).* **276:**269.
13. Crossley, L. G., and R. R. Porter. 1980. Purification of the human complement control protein C3b inactivator. *Biochem. J.* **191:**173.
14. Potocnjak, P., N. Yoshida, R. S. Nussenzweig, and V. Nussenzweig. 1980. Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J. Exp. Med.* **151:**1504.
15. Boyüm, A. 1968. Isolation of leukocytes from human blood: further observations. *Scand. J. Clin. Lab. Invest.* (Suppl.)**21:**77.
16. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
17. Wyscocki, L., and V. L. Sato. 1978. Panning for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2844.
18. Segal, A. W., A. Fortunato, and T. Herd. 1980. A rapid single centrifugation step method for the separation of erythrocytes, granulocytes and mononuclear cells on continuous density gradients of Percoll. *J. Immunol. Methods.* **32:**209.
19. Cohen, A. S., W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Lopes, L. E. Shulman, S. L. Wallace. 1971. Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21:**643.
20. Nelson, R. A., Jr., J. Jensen, I. Gigli, and N. Tamura. 1966. Methods for the separation, purification and measurement of nine components of hemolytic complement in guinea-pig serum. *Immunochemistry.* **3:**111.
21. Bolotin, C., S. Morris, B. Tack, and J. Prahl. 1977. Purification and structural analysis of the fourth component of human complement. *Biochemistry.* **16:**2008.
22. Nagasawa, S., and R. M. Stroud. 1977. Cleavage of C2 by Cls into the antigenically distinct fragments C2a and C2b: demonstration of binding of C2b to C4b. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2998.
23. Tack, B. F., and J. W. Prahl. 1976. Third component of human complement: purification from plasma and physicochemical characterization. *Biochemistry.* **15:**4513.

24. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. **227:**680.
25. Sobel, A. T., V. A. Bokisch, and H. J. Müller-Eberhard. 1975. Clq deviation test for the detection of immune complexes, aggregates of IgG, and bacterial products in human serum. *J. Exp. Med.* **142:**139.
26. Theofilopoulos, A. N., C. B. Wilson, and F. J. Dixon. 1976. The Raji cell radioimmune assay for detecting immune complexes in human sera. *J. Clin. Invest.* **57:**169.
27. Pulvertaft, F. J. V. 1965. A study of malignant tumors in Nigeria by short-term tissue culture. *J. Clin. Path.* **18:**261.
28. Siegel, I., T. L. Liu, and N. Gleicher. 1981. The red-cell immune system. *Lancet.* **II:**556.
29. Medof, M. E., and J. J. F. Oger. 1981. Competition for immune complexes by red cells in human blood. *J. Clin. Lab. Immunol.* In press.
30. Nelson, R. A. 1956. The immune-adherence phenomenon: a hypothetical role of erythrocytes in defence against bacteria and viruses. *Proc. Roy. Soc. Med.* **49:**55.
31. Schmitt, M., H. H. Mussel, and M. P. Dierich. 1981. Qualitative and quantitative assessment of C3-receptor reactivities on lymphoid and phagocytic cells. *J. Immunol.* **126:**2042.
32. Okuda, T., and T. Tachibana. 1980. Complement receptors on Raji cells. The presence of a new type of C3 receptor. *Immunology.* **41:**159.
33. Medof, M. E., J. J. F. Oger, J. P. Antel, and G. M. Prince. 1981. Restoration of normal interactions between immune complexes and systemic lupus erythematosus blood cells by normal serum. *J. Clin. Lab. Immunol.* In press.
34. Fearon, D. T., I. Kaneko, and G. G. Thomson. 1981. Membrane distribution and adsorptive endocytosis by C3b receptors on human polymorphonuclear leukocytes. *J. Exp. Med.* **153:**1615.
35. Kazatchkine, M. D., D. T. Fearon, M. D. Appay, C. Mandet, and J. Bariety. 1982. Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in renal diseases: loss of C3b receptor antigen in proliferative nephritis of SLE. *Mol. Immunol.* (Abstr.) In press.
36. Frank, M. M., A. D. Schreiber, J. P. Atkinson. 1977. Pathophysiology of immune hemolytic anemia. *Ann. Int. Med.* **87:**210.
37. Arend, W. P., and M. Mannik. 1971. Studies on antigen-antibody complexes. II. Quantification of tissue uptake of soluble complexes in normal and complement-depleted rabbits. *J. Immunol.* **107:**63.
38. Frank, M. M., M. I. Hamburger, T. J. Lawley, R. P. Kimberly, and P. H. Plotz. 1979. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. *N. Engl. J. Med.* **300:**518.