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Research Article

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Autoantibody to the C3b/C4b Receptor and Absence of this Receptor from Erythrocytes of a Patient with Systemic Lupus Erythematosus

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Abstract

A 29-yr-old woman with systemic lupus erythematosus (SLE) was found to have no detectable C3b/C4b receptors (CR1) on her erythrocytes (E) when they were assayed by the binding of rabbit polyclonal and murine monoclonal (Yz-1) anti-CR1. Analysis by two-color fluorescent flow cytometry of CR1 expression on the patient's B lymphocytes that had been stained indirectly with monoclonal anti-B1 and rabbit F(ab')$_2$ anti-CR1 also revealed a marked deficiency of CR1. Total cellular CR1 of neutrophils, assessed by a sandwich radioimmunoassay, was about half that of neutrophils from normal individuals. Because her E had expressed 173 sites/cell 2 yr before, the CR1 deficiency was considered to be acquired and a possible mechanism was sought.

Autoantibody to CR1 was measured by a radioimmunoassay in which serum or its fractions were incubated in microtiter wells that had been coated with purified CR1, and binding of immunoglobulin to the wells was quantitated with ^{125}I-labeled goat IgG antihuman F(ab')$_2$. The CR1-specific binding of immunoglobulin from the patient's serum was 19.1 ng/well of the detecting antibody when her E had eight CR1 sites per cell; that of 28 healthy donors was 1.3±0.5 ng/well (mean±SEM), and that of 34 additional patients with SLE was 0.5±0.3 ng/well. The activity was present also in purified IgG and its F(ab')$_2$ fragment, indicating that the binding of serum immunoglobulin to CR1 was not mediated by C3 fragments. The specificity of the patient's IgG for CR1 was confirmed when pretreatment of the CR1-coated wells with affinity-purified rabbit F(ab')$_2$ anti-CR1 was shown to inhibit by 68% the binding of the IgG. The autoantibody also interacted with CR1 in cell membranes, as assessed by its capacity to inhibit the binding of indirectly fluoresceinated Yz-1 to neutrophils, and, when combined with goat IgG antihuman F(ab')$_2$, to diminish the binding of dimeric C3b to normal E.

During the period of the marked deficiency of CR1 the patient experienced an exacerbation of disease activity which was treated with prednisone. Clinical improvement was accompanied by a decrease in the serum concentration of anti-CR1 to levels present 2 yr earlier, and an increase of CR1 to 170 sites/E. The temporal association between high titers of an autoantibody to CR1, absence of CR1 from E, and heightened activity of SLE suggest that the former may have had a role in the other manifestations of the patient's disease.

Introduction

Systemic lupus erythematosus (SLE)[1] is an autoimmune disease characterized by the presence of autoantibodies to multiple antigens. Among these are antibodies to the nuclear antigens, single- and double-stranded DNA (dsDNA), ribonucleoproteins and histones (1), cytoskeletal proteins (2, 3), cell surface constituents such as Ia (4), β-2 microglobulin (5), and asialo-GM$_1$ (6), and plasma proteins such as immunoglobulin and the classical pathway C3 convertase, C4b,2a (7–9). The biologic effects of some autoantibodies are direct, such as the prolongation of the half-life of C4b,2a by its autoantibody, whereas the pathophysiologic effects of other antibodies, such as those directed to DNA, may be mediated indirectly by the formation of immune complexes (10).

Patients with SLE have recently been recognized as having a relative deficiency of the C3b/C4b receptor (CR1) on their erythrocytes (E) (11–16) that impairs the capacity of these cells to bind DNA–anti-dsDNA complexes in vitro (17). In addition, patients with proliferative glomerulonephritis of SLE have no detectable CR1 on podocytes in affected glomeruli, although individuals with other types of endocapillary proliferative glomerulonephritis have apparently normal expression of CR1 on their podocytes (18, 19). Two general mechanisms have been shown to account for the abnormality on E: a primary deficiency caused by inheritance of an allele(s) associated with low CR1 number (13, 16), and a secondary deficiency caused by accelerated catabolism of CR1 in association with complement activation at the cell surface (15). The deficiency of CR1 on E may contribute to the elevated plasma levels of immune complexes that occur in SLE because the capacity of E to clear complexes from the circulation depends on their expression of this receptor (20).

In the present study another possible mechanism for an acquired deficiency of CR1 is described in which a patient with SLE was found to have autoantibodies directed to this protein. A temporal association in this patient between heightened activity of disease, absence of CR1 from E, and high titers of auto–anti-CR1 suggests that the latter may have had a role in the clinical manifestations of her disease.

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1. *Abbreviations used in this paper:* ANA, antinuclear antibody; B1-biotin, murine monoclonal IgG, directed to the B1 antigen of B lymphocytes, that has been conjugated with biotin; CR1, the human C3b/C4b receptor; dsDNA, double-stranded DNA; E, erythrocyte(s); FITC, fluorescein isothiocyanate; FITC-GaM, FITC-conjugated goat IgG anti-mouse IgG; FITC-GaR, FITC-conjugated goat F(ab')$_2$ anti-rabbit F(ab')$_2$; HBSS-BSA, HBSS containing 0.1% BSA; HBSS-1% BSA, HBSS containing 1% BSA; NHS, normal human serum; RaCR1, affinity-purified rabbit F(ab')$_2$ directed to CR1; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; XRITC, rhodamine 600.

Methods

Antibodies. Affinity-purified rabbit IgG and F(ab')$_2$ antihuman CR1 (RaCR1) were prepared as described (13, 21). The F(ab')$_2$ fragment was radiolabeled to a specific activity of 1.2 × 10^6 cpm/μg (22). Murine monoclonal Yz-1 IgG anti-CR1 (23) was purified from ascites by precipitation with 50% saturated ammonium sulfate and chromatography on DEAE Sephacel (Pharmacia Fine Chemicals, Piscataway, NJ). Fab fragments were produced by papain digestion of purified Yz-1 (24). Yz-1 IgG and Fab were radiolabeled with ^{125}I (Amersham Corp., Arlington Heights, IL) to specific activities of 1.8–3.1 × 10^6 cpm/μg (22).

Biotin-conjugated monoclonal anti-B1 (B1-biotin) (Coulter Immunology, Hialeah, FL), which recognizes a 35,000 M_r antigen present on the surface of B lymphocytes (25), human isotype- and light chain-specific affinity-purified goat IgG (Southern Biotechnology Associates, Birmingham, AL), fluorescein isothiocyanate (FITC)–conjugated goat F(ab')$_2$ antirabbit F(ab')$_2$ (FITC-GaR; Cappel Laboratories, West Chester, PA), and FITC-conjugated goat IgG antimouse IgG (FITC-GaM) (Southern Biotechnology Associates) were obtained. The isotype- and light chain-specific goat antihuman immunoglobulins were radiolabeled with ^{125}I to specific activities of 0.9–1.4 × 10^6 cpm/μg (22). The FITC-GaR was preabsorbed with human IgG that had been coupled to CNBr-activated Sepharose (Sigma Chemical Co., St. Louis, MO).

Purification of CR1. CR1 was purified as described (26, 27). Purified CR1 was radiolabeled using the Bolton-Hunter reagent (New England Nuclear, Boston, MA) and analyzed by electrophoresis on a gradient gel of 3 to 10% acrylamide in the presence of sodium dodecyl sulfate (SDS-PAGE) (28) followed by autoradiography. Some studies used CR1 of similar purity that had been prepared by a modified procedure using affinity chromatography on Yz-1-Sepharose.

Quantitation of CR1. The number of CR1 on E was determined by incubation of washed E for 1 h at 22°C with incremental concentrations of either ^{125}I-Yz-1 (from 0.03 to 1.0 μg/ml) or ^{125}I-RaCR1 (from 0.16 to 5.13 μg/ml) followed by centrifugation of E through cushions of dibutylphthalate (Eastman Kodak Co., Rochester, NY) (13). The preparation and binding of ^{125}I-dimeric C3b to E was performed as described (13). Erythrocyte CR1 was also analyzed by immunoblotting as described (29, 30). Total cellular CR1 in detergent lysates of purified neutrophils (31) was measured by a modification of the sandwich protocol of Unkeless and Healy (29, 32).

For quantitation of CR1 on B lymphocytes by cytofluorography, 4 × 10^6 mononuclear leukocytes were incubated for 30 min at 0°C with 15 μg/ml of either RaCR1 or nonimmune rabbit F(ab')$_2$ in 0.065 ml Hanks' balanced salt solution (M. A. Bioproducts, Walkersville, MD) containing 0.1% bovine serum albumin (Miles Scientific, Naperville, IL) (HBSS-BSA) and 40% heat-inactivated pooled normal human serum (NHS). The cells were washed in 2.5 ml HBSS-BSA and incubated at 4°C for 30 min with 125 μg/ml FITC-GaR in HBSS-BSA containing NHS. The cells were washed, resuspended in HBSS-BSA containing B1-biotin, and incubated for 30 min at 0°C. After the cells were washed they were incubated for 20 min at 0°C with 60 μg/ml XRITC (rhodamine 600)-conjugated avidin (Vector Laboratories, Burlingame, CA). After a final wash, the cells were fixed in 0.1 ml 2% paraformaldehyde and held at 4°C in the dark until they were evaluated by flow cytometry.

Neutrophils were stained with Yz-1 by incubation of 4 × 10^6 cells in 0.05 ml HBSS-BSA with 5 μg/ml Yz-1 or UPC-10, an irrelevant, anti-levan monoclonal antibody (Litton Bionetics, Charleston, SC), for 30 min at 0°C. The cells were washed and incubated for 30 min with 15 μg/ml FITC-GaM in the presence of 33% NHS, washed again, and fixed in paraformaldehyde and held at 4°C until analysis by flow cytometry.

Immunofluorescent staining was analyzed with a Cytofluorograf system 50-H (Ortho Diagnostic Systems, Westwood, MA) equipped with an argon laser operated at 488 nm and 250 mW and a krypton laser operated at 568.2 nm and 200 mW, and interfaced with a model 2150 Data Handler (Ortho Diagnostic Systems). The relative fluorescence intensity was standardized from day to day by adjustment of the gains to yield a constant mean channel of fluorescence for stock suspensions of calf thymocyte nuclei that had been conjugated with FITC or XRITC, respectively (Ortho Diagnostic Systems).

Purification of human IgG and preparation of F(ab')$_2$. IgG was purified from human serum by precipitation in 50% saturated ammonium sulfate and subsequent dialysis against 0.0175 mM sodium phosphate, pH 6.3, and application to a column of DE-52 cellulose (Whatman Chemical Separation Inc., Clifton, NJ) equilibrated in this buffer (24). Proteins that did not bind to the column were subjected to gel filtration high-performance liquid chromatography using a TSK 250 column (Bio-Rad Laboratories Inc., Richmond, CA). Purified IgG was digested with pepsin (Worthington Biochemical Corp., Freehold, NJ). Analysis of the IgG and F(ab')$_2$ in the reduced state by SDS-PAGE and staining of the gels with Coomassie Blue revealed only polypeptides of apparent molecular weights of 50,000 and 25,000–30,000 for IgG and 25,000 for F(ab')$_2$.

Radioimmunoassay for antibodies specific for CR1. Replicate wells of flexible polyvinyl chloride U-bottom microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were incubated overnight at 4°C with 250 ng of purified CR1 in 0.025 ml HBSS containing 1 mM magnesium chloride, or with this buffer alone (33). The supernatants were removed and the wells were washed three times and incubated for 1 h at room temperature with HBSS containing 10 mg/ml BSA (HBSS-1% BSA) to block nonspecific protein binding sites. Wells prepared in this manner were shown to be able to bind specifically ^{125}I-Yz-1 monoclonal anti-CR1. Dilutions of human serum, purified IgG, or its F(ab')$_2$ fragment in 0.025 ml HBSS-1% BSA containing 10 mM EDTA were added to CR1-coated wells and incubated for 1 h at room temperature. The supernatants were removed, the wells were washed three times with HBSS-1% BSA, and the amount of immunoglobulin that had bound was determined by incubation of the wells for 1 h at room temperature with a saturating concentration of ^{125}I-goat IgG antihuman F(ab')$_2$. The developing antibody was removed, and the wells were washed, cut out, and assessed for radioactivity. CR1-specific binding of human immunoglobulin was calculated by subtraction of the amount of ^{125}I-goat IgG antihuman F(ab')$_2$ bound to wells that had not been coated with CR1 from the amount of developing antibody that was bound to CR1-coated wells. Retrospective analyses of serum concentrations of autoantibody to CR1 used serum that had been maintained at −70°C.

Clinical assays. Total hemolytic complement (34), C3 (35), C4 (35), immune complexes (36), and antinuclear antibodies (ANA) were assayed as described (37). The presence of precipitating antibodies to dsDNA, single-stranded DNA, NP, CTN, Sm, RNP, Ro, and La was assessed by counterimmunoelectrophoresis (37). HLA (38) and C4 (39) haplotypes were determined as described.

Case report. Patient R is a 29-yr-old caucasian woman with SLE. At age 24 she was noted to have a positive ANA during hospitalization after a spontaneous abortion. She subsequently developed butterfly rash, macular rash over her chest, patchy alopecia, wrist synovitis, lymph node enlargement, and splenomegaly. In 1980 the ANA titer was 1:640, total hemolytic complement was 108 U (normal range, 150–250 U), and the serum immune complex concentration was 2,500 μg/ml (normal, <23 μg/ml). Subsequent analyses revealed the presence of antibodies to CTN, Sm, and RNP, but not to double- or single-stranded DNA, Ro, or La. The patient's HLA and C4 haplotypes were determined to be A1,B7,DR2,C4A3,C4B1/A11,Bx,Cw3,DR4, C4A4,C4B2. In October 1981, the binding of ^{125}I-dimeric C3b to her E showed 173 sites/cell. She improved clinically in response to hydroxychloroquine and remained relatively well until May 1983, when she developed pleurisy and proteinuria. She improved in response to prednisone which was tapered during 7 mo and discontinued in January 1984. On 13 February 1984, she had increased severity of rash and anemia, and recurrence of proteinuria. On this date, she was found to have no detectable CR1 on her E as assayed by the binding of ^{125}I-Yz-1.

Results

Analysis of the CR1 deficiency on cells of patient R. The number of antigenic sites on E taken from patient R on 29 February 1984 was determined by assay of the binding to these cells of ^{125}I-Yz-1, a monoclonal antibody directed to CR1, and of polyclonal ^{125}I-RaCR1. There was no specific binding of either antibody to E from patient R (Fig. 1). Scatchard analysis of the equilibrium binding studies performed in parallel with cells from a healthy donor revealed 533 sites/E and 2,775 sites/E for the monoclonal and polyclonal anti-CR1 antibodies, respectively. When these antibodies had been used in previous analyses of E from 164 patients with SLE and 213 healthy donors in the United States and France (13, 40, 41), the analyses had failed to identify another individual whose E were wholly lacking CR1.

When the number of CR1 on E of patient R's first-degree relatives was determined, the mother, father, brother, and sister were found to have 694, 738, 902, and 670 sites/E, respectively, for binding of Yz-1. For comparison, 50 unrelated healthy donors were shown to have an average of 550±243 (mean±SD) Yz-1 binding sites/E.

The inability to detect CR1 on the patient's E could have been caused by masking or internalization of the receptors. Therefore, membrane proteins of 1×10^{10} E from patient R and from a healthy donor, whose intact E expressed 900 ^{125}I-Yz-1 binding sites/cell, were subjected to SDS-PAGE, transfer to nitrocellulose paper and immunoblotting with ^{125}I-Yz-1, and then autoradiography. CR1 in the E lysate from the healthy donor was clearly demonstrated, but the receptor was not detectable in the lysate of E from patient R (Fig. 2). Additional low molecular weight bands that were present in the E lysates of both donors were also observed when ^{125}I-3G8, a monoclonal anti-Fc receptor antibody, was used to develop immunoblots of E, and these bands probably represent nonspecific binding of the developing antibody to bands 4.2, 5, and 6, as described by others (42).

To assess whether cells other than E also were deficient in
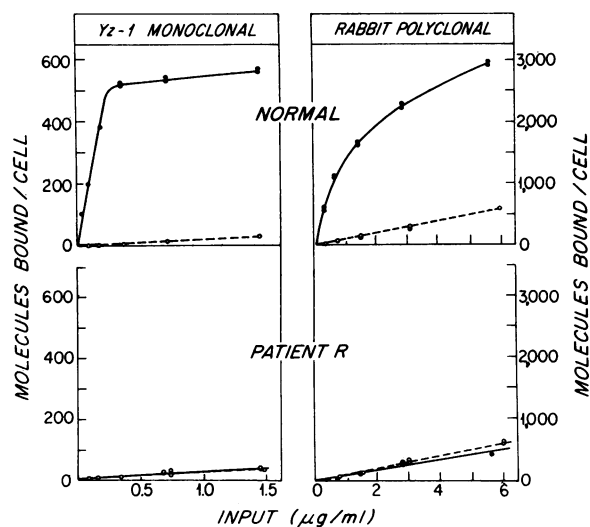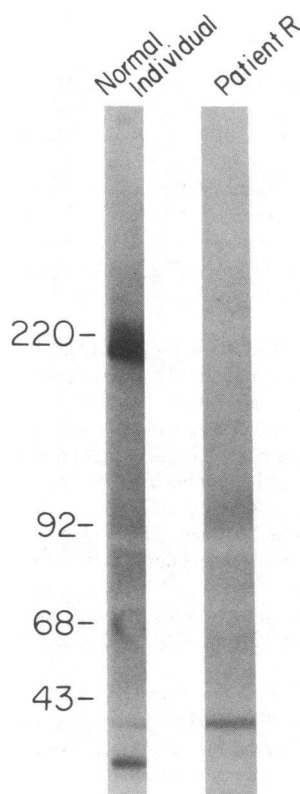
Figure 2. Analysis by immuno-blotting of E membranes for the presence of CR1 antigen. Detergent lysates of E membranes from patient R and a normal individual were subjected to SDS-PAGE under nonreducing conditions and transferred to nitrocellulose strips, which were probed with ^{125}I-Yz-1. The positions of the reduced protein standards of spectrin band 1 (220 kD), phosphorylase b (92 kD), BSA (68 kD), and ovalbumin (43 kD) are shown.

CR1, the mononuclear leukocytes from patient R on 19 March 1984 and cells from a healthy donor were stained with B1-biotin and XRITC-avidin, and with RaCR1 and FITC-GaR, and analyzed by two-color immunofluorescent flow cytometry (Fig. 3). 9% of the lymphocytes of the healthy donor and 17% of those of patient R were identified as B cells by their red fluorescence, and the respective mean channels of fluorescence were 50.1 and 53.1. The relative amount of CR1 on the B1-positive cells, expressed as the mean channel of green fluorescence, was 38.1 for the healthy donor and 5.3 for patient R. Replicate samples of lymphocytes stained nonspecifically with nonimmune rabbit F(ab')$_2$ and FITC-GaR exhibited mean channels of green fluorescence of 1.7 and 2.3, respectively. The mean fluorescent channel for CR1 staining of B1-positive lymphocytes was 35.2±7.4 (mean±SD; range, 26.6–43.9) for five other healthy donors.

CR1 of neutrophils was measured in detergent lysates of these cells by a sandwich radioimmunoassay, rather than on intact cells by flow cytometry, because most of this receptor resides in an intracellular compartment inaccessible to antibody (43, 44). On 19 March 1984, neutrophils from patient R had 18,793 receptors/cell, which was less than half of the 43,297±7,674 (mean±SD) receptors/cell measured for nine healthy donors.

Measurement of autoantibody to CR1 in serum of patient R. The unprecedented, complete absence of CR1 from E of this patient, the previous finding of 173 CR1/cell on her E, and the relatively high numbers of receptors on E of her first-degree relatives suggested that a unique mechanism may have caused the acquired deficiency of CR1 on her peripheral blood cells. Because patients with SLE may produce autoantibodies that are reactive with cell surface constituents, an assay was
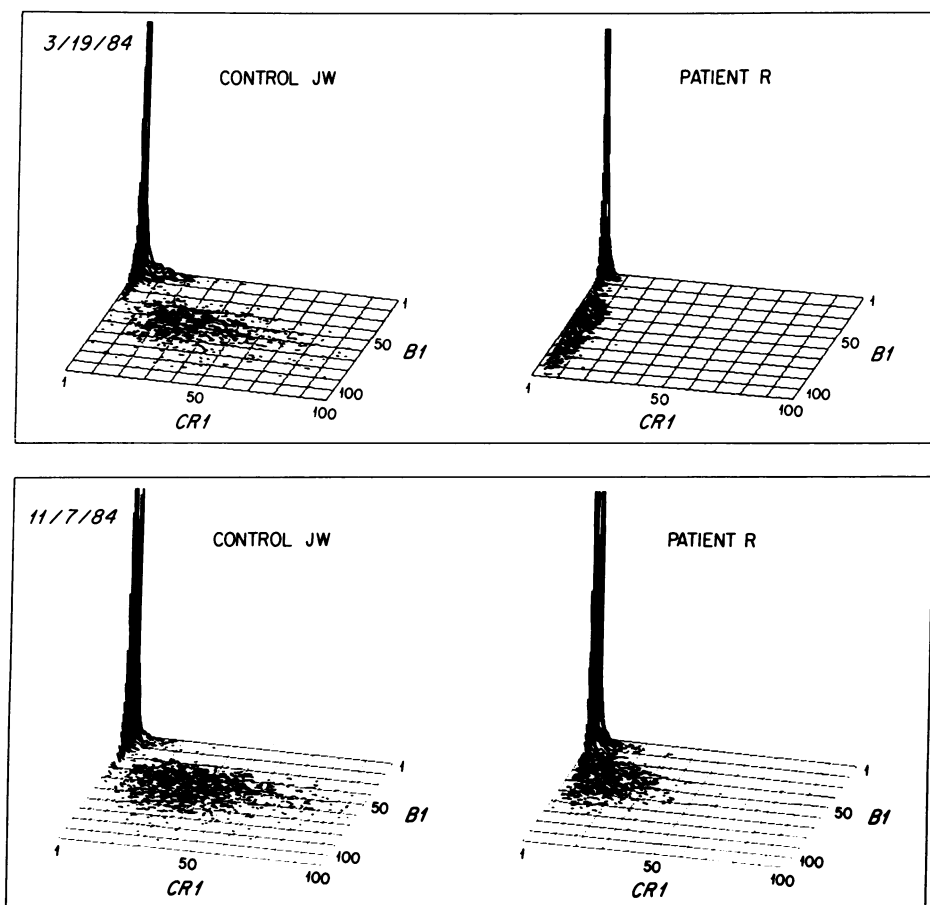

Figure 1. The binding of ^{125}I-Yz-1 murine monoclonal anti-CR1 and rabbit ^{125}I-F(ab')$_2$ anti-CR1 in the absence (●) and presence (○) of excess unlabeled antibodies to E from a normal individual and from patient R.

Figure 3. Isometric display of the distribution of peripheral blood lymphocytes according to their fluorescence intensity, as assessed by two-color cytofluorography, after indirect staining with XRITC-B1 and FITC-anti-CR1. The analyses of lymphocytes taken from patient R and the normal control, JW, on 19 March 1984 and 7 November 1984 are shown.

developed to detect autoantibodies to CR1 in the serum of patient R. CR1 was purified and analysis by SDS-PAGE showed the preparation to contain only the two major allotypic forms of the receptor (Fig. 4) (45, 46). Replicate samples of IgG purified from the serum obtained from patient R on 1 May 1984 were incubated in microtiter wells that had been prepared with the purified CR1 or buffer alone. The wells were washed, incubated with ^{125}I-goat IgG antihuman F(ab')$_2$, and assessed for the amount of detecting antibody that was bound. At each concentration of the patient's IgG there was greater binding of immunoglobulin to the CR1-coated wells than to the buffer-treated wells, with the highest concentration of IgG resulting in the specific binding of ~8 ng of the detecting antibody (Fig. 5).

A portion of the IgG and of purified C3 were subjected to digestion with pepsin. The C3 was degraded to peptides that migrated with the buffer front on 3 to 10% SDS-PAGE. The F(ab')$_2$ fragment was shown to bind specifically to CR1-coated microtiter wells (Fig. 5), indicating that the binding of the patient's immunoglobulin to CR1 was mediated by the F(ab')$_2$ region of the molecule and not by C3 fragments that may have been bound to the immunoglobulin.

To obtain additional evidence that the binding of the patient's immunoglobulin to CR1-coated wells was due to specific interaction with the antigen, the capacity of RaCR1 to inhibit this interaction was assessed. Wells that had been prepared with CR1 or buffer alone were preincubated with either nonimmune rabbit F(ab')$_2$ or RaCR1 and washed, and

the CR1-specific binding of the patient's purified IgG that had been preabsorbed against rabbit F(ab')$_2$-Sepharose, and of ^{125}I-Yz-1, was measured. In four separate experiments, the specific binding of the patient's IgG to microtiter wells pretreated with RaCR1 was 32±9.6% of the specific binding to wells pretreated with nonimmune F(ab')$_2$. Inhibition of the CR1-specific binding of ^{125}I-Yz-1 by RaCR1 was at least 95% in each experiment.

The anti-CR1 present in serum obtained from patient R on 29 May 1984 was characterized using ^{125}I-labeled, isotype-specific goat antihuman immunoglobulin preparations as the developing antibodies in the microtiter assay. Immunoglobulin in the patient's serum that bound specifically to CR1-coated plates contained IgG, IgA, and IgM, but not IgD, and both κ- and λ-light chains.

The capacity of purified IgG from patient R to bind to CR1 in a plasma membrane was assessed indirectly by examining the inhibition of the binding of Yz-1. Replicate samples of 4 × 10^6 neutrophils in 0.1 ml HBSS-BSA were preincubated for 60 min at 0°C with 1.2 mg IgG purified from serum obtained from patient R on 1 May 1984, and from each of three healthy individuals. After being washed with ice-cold buffer, the cells were incubated with 5 μg/ml Yz-1 in HBSS-BSA for 30 min at 0°C, washed, stained by incubation with FITC-GaM for 30 min at 0°C, and analyzed by cytofluorography. In two separate experiments, IgG from patient R inhibited the binding of Yz-1 to neutrophils by 46 and 35%, whereas the IgG purified from the serum of the healthy donors reduced Yz-1 binding by only 0 to 11% (Table I).
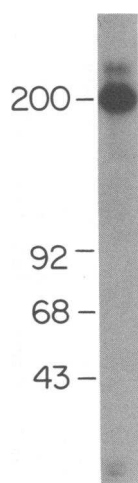
Figure 4. Analysis by SDS-PAGE and autoradiography of purified CR1 that had been labeled with ^{125}I. Numbers to left of gel are molecular weights in thousands.

Figure 5. The binding of purified IgG from patient R (*A*) and its F(ab')$_2$ fragment (*B*) to CR1-coated microtiter wells. IgG or F(ab')$_2$ was incubated in microtiter wells that had been pretreated with purified CR1 (●) or with buffer (○), the wells were washed, and bound Ig was detected by subsequent incubation of the well with ^{125}I-labeled goat IgG antihuman F(ab')$_2$. CR1-specific binding of the human IgG and F(ab')$_2$ (△) was calculated by subtracting the binding to wells that had been pretreated with buffer from that to wells coated with CR1.

The ability of serum from patient R to inhibit CR1 function was examined by incubating replicate samples of 2.5×10^8 normal E in 0.05 ml of either HBSS-BSA, serum from a healthy donor, or serum obtained from the patient on 29 February or 1 May 1984. After 60 min at 0°C, the cells were washed with ice-cold HBSS-BSA, resuspended in this buffer containing 20 μg/ml goat IgG anti–human F(ab')$_2$, and incubation was continued for 60 min more at 0°C. After they were washed the cells were assessed for their capacity to bind ^{125}I-dimeric C3b. E that had been pretreated with buffer or with normal serum bound 143 and 130 C3b molecules/cell, respectively. In contrast, pretreatment of the cells with the two serum samples taken from patient R decreased the binding to 69 and 54 C3b molecules/cell, representing 45 and 52% inhibition, respectively. In a separate experiment, sequential pretreatment of normal E with plasma taken from patient R on 29 February 1984 and with goat IgG antihuman F(ab')$_2$ caused 37% inhibition of subsequent ^{125}I-dimeric C3b binding relative to the uptake observed with buffer-treated cells. No inhibition occurred with E pretreated only with patient plasma but not with antihuman F(ab')$_2$.

28 healthy donors, 34 additional patients who fulfilled the revised criteria for the diagnosis of SLE (47), and the four first-degree relatives of patient R were compared with patient R for the presence of anti-CR1 in their undiluted sera containing 10 mM EDTA. The CR1-specific binding of serum immunoglobulin was 1.3±0.5 ng/well (mean±SEM) of ^{125}I-goat IgG antihuman F(ab')$_2$ for the healthy donors, 2.8±1.3 ng/well for the family members of patient R, and 0.5±0.3 ng/well for individuals with SLE, none of whose sera exhibited anti-CR1 activity that approached the amount, 19.1 ng/well, observed with serum taken from patient R on 29 May 1984 (Fig. 6). The four normal individuals with the most immunoglobulin binding to CR1-coated plates had CR1/E ranging from 475 to 688. Their antibodies to CR1 may have been directed to cryptic regions of the receptor that are not expressed in its native form, as is the case with autoantibodies in normal individuals to band 3 (42).

Temporal relationship between cellular CR1, serum anti-CR1, and activity of disease. On 13 February 1984, when the patient was first noted to lack CR1 on E, prednisone was reinstituted at a dose of 20 mg/d because of rash, anemia, and
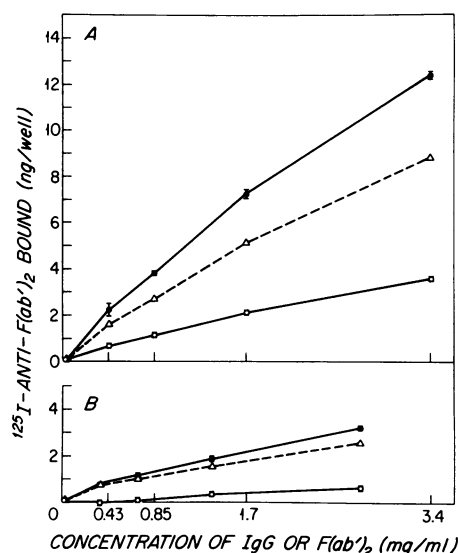
recurrence of proteinuria (Fig. 7). The dose of prednisone was eventually increased to 90 mg/d in June because of persistent disease activity with increased anemia and positive Coombs' test. The patient then improved and prednisone was decreased.

Anti-CR1 activity was measured by the radioimmunoassay in serum samples that had been obtained from patient R since her initial visit to the Brigham and Women's Hospital in November 1980. The serum concentration of anti-CR1 was relatively low during the initial two and one-half years that followed the onset of SLE, even during the periods of heightened disease activity in November 1980, and May 1983. In September 1983, patient R's serum had the highest concentration of

Table I. *Inhibition of the Binding of Yz-1 to Neutrophils by Preincubation of the Neutrophils with Human IgG**

	Source of IgG	Percent inhibition	
		Experiment 1	Experiment 2
Normal donors	1	0	1
	2	3	5
	3	11	9
Patient R		46	35

* Binding of Yz-1 was assessed by subsequent staining with FITC-GaM and immunofluorescent flow cytometry. Percent inhibition = [1 − (mean fluorescent channel of neutrophils preincubated with patient or control IgG/mean fluorescent channel of neutrophils incubated in buffer alone)] × 100.

anti-CR1 observed during her clinical course. The anti-CR1 concentration had decreased four- to fivefold before the institution of high-dose prednisone therapy in June 1983, after which the antibody concentration decreased to the level observed before 1983.

After October 1981, when the number of CR1/E had been 173, as determined by the binding of ^{125}I-labeled dimeric C3b, patient R's E were not examined again until February 1984, when she was twice found to have no antigenically detectable CR1 sites. CR1 number remained at $\sim$10 sites/cell through June. By August this number had returned to 170 sites/cell, coincident with the decrease in the serum anti-CR1 concentration. Repeat measurement of neutrophil CR1 on 7 November 1984 showed 35,364 molecules/cell, twice the amount present on 19 March 1984. Reanalysis of CR1 on B lymphocytes on 7 November by fluorescent flow cytometry revealed that the mean fluorescent channel had increased to 16.8, which, however, was less than the level of 41.2 observed with stained B cells of the normal individual assayed at the same time (Fig. 3).

Serum concentration of C4 antigen (normal, 26–83 mg/dl) was 13 mg/dl when patient R presented in 1980, was in the normal range in May 1982, decreased to 21 mg/dl in 1983 and to 18 mg/dl in 1984 during the two episodes of increased clinical activity of disease, and returned to normal in June 1984. C3 concentration was normal throughout the clinical course. The serum immune complex concentration was 400–500 μg/ml throughout 1983, 820 μg/ml on 13 February 1984, fell to <20 μg/ml by 19 March 1984, and remained in this range. The hematocrit fell to 25% in May 1984 and rose to 37% by August 1984. The episode of anemia was accompanied by positive Coombs' test but not by reticulocytosis or decreased serum haptoglobin. Furthermore, E taken from patient R in February 1984, which were totally lacking CR1, did not exhibit increased lysis in Ham's test.

Discussion

A total of 300 patients with SLE and 514 normal individuals have been assessed by investigators in Japan, North America, and Europe (12, 13, 15, 16, 40, 41) for the number of antigenically detectable CR1 on their E, and before this report, none had been found to have a complete absence of this receptor. The conclusion that E from patient R in early 1984 lacked CR1 was based on there being no specific binding to her cells of either monoclonal or polyclonal anti-CR1 (Fig. 1) and on the absence of detectable CR1 antigen on transblots of
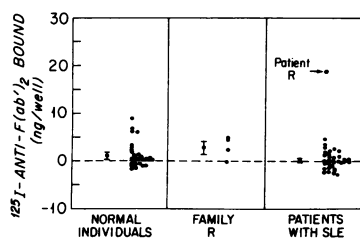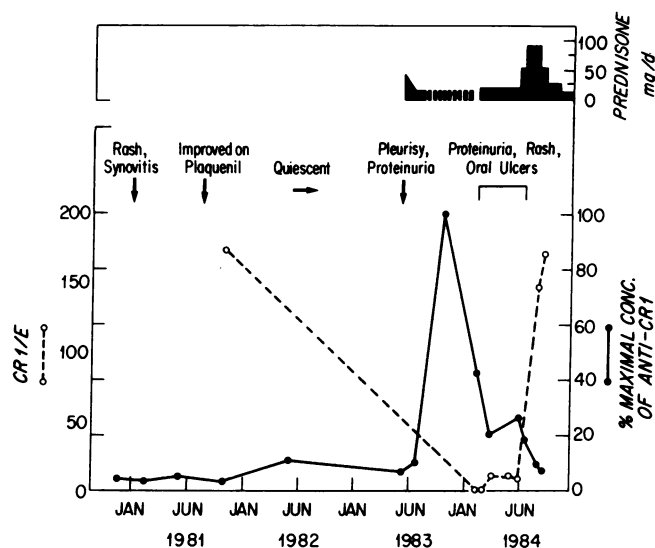
Figure 7. The number of CR1 sites/E (o) and the serum concentration (Conc.) of anti-CR1 (●) of patient R during 1981–1984.

E membranes (Fig. 2). Therefore, it seemed likely that a process distinct from inheritance (13, 16) or complement activation (15) accounted for this unique abnormality, and an autoantibody to the receptor was sought in this patient. A radioimmunoassay was developed using CR1-coated microtiter wells that was sensitive, specific, and quantitative. With a specific radioactivity of revealing antibody of 10^6 cpm/μg, and assuming a 1:1 stoichiometry for the interaction of human Ig with the radiolabeled goat antihuman F(ab')$_2$, the specific binding of as little as 0.2 ng of IgG autoantibody in 0.025 ml of serum, or 0.0001% of the serum concentration of IgG, could be detected. The specificity of the assay, although addressed in part by the use of a CR1 antigen that had been purified to homogeneity as assessed by SDS-PAGE (Fig. 4), was further assured by the demonstration that monospecific, polyclonal RaCR1 inhibited by 68% the binding of patient R's IgG to antigen-coated wells. The noninhibitable binding of the IgG could have been to epitopes not recognized by the rabbit antibody, such as those expressed in denatured CR1 to which this antibody does not bind. The finding that both purified IgG and its F(ab')$_2$ fragment bound to the CR1-coated wells (Fig. 5) excluded the possibility that the assay was merely detecting C3-bearing immune complexes, especially when considered with the observation of the marked sensitivity of C3 to pepsin. The quantitative decrease in the binding of the F(ab')$_2$ fragment as compared with that of IgG (Fig. 5) apparently was due to partial denaturation of the autoantibody under the conditions of the pepsin digestion. IgG incubated under identical conditions but in the absence of pepsin showed a reduction in binding to CR1 of $\sim$50% as compared with that of IgG that had been held on ice. The quantitative nature of the radioimmunoassay, as demonstrated in the assay depicted in Fig. 5, was essential for the comparison of patient R's serum with that of other SLE patients and normal individuals (Fig. 6) and for the analysis of the relationship between the autoantibody and her clinical course (Fig. 7).

Antigenic analysis of the constant regions of the autoanti-

Figure 6. The amount of immunoglobulin in undiluted serum that reacted specifically with CR1 in microtiter wells, as determined by the binding of ^{125}I-goat IgG anti-F(ab')$_2$, among 28 normal individuals, 4 first-degree relatives of patient R, 34 patients with SLE, and patient R.

body demonstrated that the anti-CR1 was included among the major classes of antibody in serum and that both κ- and λ-light chains were present, indicating that it was polyclonal. The specificity of patient R's autoantibody included epitopes expressed by native CR1 present in the plasma membrane of E and neutrophils (Table I), as indirectly indicated by the capacity of serum and purified IgG to inhibit binding of dimeric C3b and Yz-1 to these cells, respectively. The requirement of a second, anti–human antibody for inhibition of dimeric C3b binding suggests that the epitopes recognized by patient R's autoantibody did not include the ligand-binding site of the receptor.

B lymphocytes resembled E in having almost no detectable CR1 (Fig. 3), whereas the CR1 content of neutrophils was ~50% of normal. Although the difference between these cells in the severity of the CR1 deficiency may have been caused by the analysis of surface CR1 of B cells and E and of total CR1 of neutrophils, it may also reflect the localization of most of CR1 in circulating, nonactivated neutrophils to compartments inaccessible to extracellular ligands (43, 44). The mechanism by which interaction of autoantibody with CR1 causes loss of the receptor has not been addressed in this study, but it is unlikely to be wholly secondary to complement activation at the cell surface because comparable deficiencies of CR1 have not been observed on E of patients with autoimmune hemolytic anemia (48).

The possibility that the receptor abnormality in patient R was a consequence of the autoantibody is supported by the inverse relationship between these two measurements before, during, and after the period of the marked deficiency on E (Fig. 7). Furthermore, analysis of 34 other patients revealed no binding of immunoglobulin to CR1-coated plates in excess of that observed in serum from normal individuals (Fig. 6), and no SLE patient has previously been found to have absent CR1 on E, which suggests a unique relationship between the presence of the autoantibody and the absence of CR1 in patient R. The autoantibody may also have been responsible for the CR1 deficiency of patient R's neutrophils and B lymphocytes, as there was improvement in these abnormalities after the decrease in serum concentration of anti-CR1 (Fig. 3). However, because there are no other quantitative data available about CR1 expression on leukocytes of patients with SLE, it is not possible to conclude that the leukocyte abnormality was unique to patient R. In addition, other pathophysiologic processes able to cause acquired CR1 deficiency may have been occurring in patient R, since the level of CR1 sites/E, 170, observed at times of low anti-CR1 concentration was lower than would have been predicted based on inheritance, as all of the patient's first-degree relatives had >600 CR1/E.

The possible biologic consequences of the autoantibody include the inability of the CR1-deficient E to clear immune complexes, and perhaps some impairment in the capacity of granulocytes to ingest C3b-bearing particles, such as complement-opsonized bacteria. The effects on B lymphocytes of the autoantibody may have been either stimulatory or inhibitory, depending on whether cross-linking of receptors by the autoantibody directly elicited a biologic response, as has been demonstrated for the interaction of human B cells with rabbit anti-CR1 (49), or blocked the ability of the cell to respond to natural ligand by creating a CR1 deficiency.

Among diseases thought to be caused by the production of autoantibodies, SLE has been considered unusual in that the disease process depends more on formation of immune complexes that cause inflammatory tissue damage than on the particular biologic functions or tissue site of the antigen recognized by the autoantibody. In contrast, antigenic specificity is critical to the effects of the anti-receptor antibodies that are responsible for myasthenia gravis (50), Graves' disease (51), and diabetes mellitus associated with anti-insulin receptor antibodies (52). Therefore, the present finding in a patient with SLE of an autoantibody to CR1 may provide an exception to this generalization because the biologic effects of this autoantibody could include impaired clearance of immune complexes and altered function of B lymphocytes. The inability to detect antibody of similar specificity in other patients with SLE may indicate either that patient R is indeed unique qualitatively or that other patients are in antigen excess, making difficult the detection of free antibody.

Acknowledgments

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