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Leprosy: Altered Complement Receptors in Disseminated Disease

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We have studied the expression of the C3b receptor (CR1) on erythrocytes of 55 patients with Hansen's disease. We developed a radioimmunoassay utilizing a monoclonal antibody that recognized an epitope different from the C3b binding site, which therefore enabled us to measure total number of CR1 regardless of receptor occupancy. We observed that patients in the lepromatous pole of the disease had a mean of 310 CR1/erythrocyte, whereas the ones in the tuberculoid pole showed a mean of 577 CR1/erythrocyte; 77 normal controls had a mean of 512 CR1/erythrocyte. The number of C3b receptors on the cells of lepromatous patients was significantly decreased ($p < .001$) when compared to the normal population or tuberculoid patients. The presence of receptors for the C3b fragment of complement (CR1) on the surface of human erythrocytes enables these cells to participate in a number of immune functions including the clearance of circulating immune complexes. These findings could bear importance in the ability of the host to clear immune complexes from the circulation in patients with lepromatous leprosy.

The receptor for the C3b fragment of the third component of the human complement system (CR1) was first reported more than 30 years ago as the immune adherence receptor. This molecule was demonstrated by the binding of particles coated with complement fragments to primate erythrocytes and nonprimate platelets [1,2]. Subsequently it has been shown that CR1 is also present on the surface of a variety of human blood and tissue cells such as polymorphonuclear (PMN), monocytes, and B lymphocytes [3], subsets of T lymphocytes [4], macrophages and glomerular podocytes [5], and Langerhans cells [6].

CR1 has been isolated and characterized as a 250,000 M_r glycoprotein [7], expressing a number of regulatory properties in the function of the complement system [8]. It accelerates the decay of C3 cleaving enzymes of both the alternative [9] and classical pathways [10], and acts as a cofactor for Factor I in the degradation of the α' chain of the C4b and C3b fragments, converting them to their inactive forms, C4c–C4d and C3bi [11]. Therefore this receptor contributes to prevent the damaging effect of complement deposition on host cells.

An important biological function of CR1 is its participation in the clearance of immune complexes. It has been reported that circulating immune complexes (CIC) carrying C3b bind to the receptor on erythrocyte membranes [12,13] and are carried by these cells to the liver where they are processed by local macrophages [14]. This processing of CIC apparently can be prevented by complement depletion, under such condition they deposit in tissues liable to suffer immune complex-mediated injury [15]. Clinical relevance for these concepts has been inferred from the observation that certain autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), have a marked reduction in the expression of CR1 on their erythrocytes [16–18].

In this study we provide additional evidence for the linkage between CR1 levels and diseases which feature an altered immune response. We examined patients with various forms of leprosy, motivated by the spectrum that spans from lepromatous leprosy, known to have high levels of CIC to the tuberculoid pole, featuring low or absent levels of immune complexes [19,20]. These studies have shown that the patients with the lepromatous form of the disease show a selective reduction in erythrocyte-associated CR1.

MATERIALS AND METHODS

Patients

Fifty-five patients diagnosed as having Hansen's disease were seen and followed in the San Diego Hansen's Disease Clinic. They were classified according to clinical and histopathological features [21] as lepromatous, borderline and tuberculoid leprosy. Only 3 patients were untreated at the time of the study. The patient population groups are summarized in Table I. The control population consisted of 77 healthy race matched volunteers ranging in age from 19–71 years.

Purified CR1 and Anti-CR1 Monoclonal Antibodies

CR1 was purified to homogeneity from human erythrocytes according to published methods [9,22]. Murine monoclonal antibodies were produced by using a modification [23] of the method of Kohler and Milstein [24]. Mice were immunized by 2 i.p. injections with 10 μg of purified CR1. Spleen cells were fused with the murine myeloma line P3 × 63.AG8.653 at a ratio of 6:1. The antibodies produced by clone 543 were tested using purified CR1 in an enzyme-linked immunoabsorbent assay (ELISA). The ascites fluid was passed through a protein A-agarose column (EY, San Mateo, California) equilibrated with 0.1 M phosphate buffer pH 8.0, and the bound IgG was eluted with 0.1 M acetic acid. Fab fragments were obtained by digesting the IgG with 2% w/w pepsin (Sigma, St. Louis, Missouri) for 18 h at 37°C, pH 4, in the presence of 0.02 M Cys-HCl (Sigma, St. Louis, Missouri); 0.025 M iodoacetamide was then added and the fragments were purified by high-performance liquid chromatography (HPLC) (Waters, Milford, Massachusetts) gel filtration on a 7.5 × 600 mm TSK-250 column (BioRad, Richmond, California).

C3b Dimers

C3 was purified according to Tack and Prahl [25]. C3b dimers were generated by incubating C3 with 0.6% w/w trypsin for 6 min at 37°C, and subsequent addition of an excess of soybean trypsin inhibitor (Sigma) as described [26]. Separation of C3b monomers and dimers was achieved by gel filtration on Sephadex G-150 equilibrated with 1 M NaCl 50 mM Na acetate. C3b eluted from the column in two peaks; one consisted of a high molecular weight protein of 360,000 daltons

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Abbreviations:
BB: Borderline leprosy
BL: borderline lepromatous
BT: borderline tuberculoid
CIC: circulating immune complex(es)
DGVB: veronal-buffered saline (0.075 M NaCl) containing 2.5% dextrose and 0.5% gelatin
EDTA: 0.02 M ethylenediamine tetraacetate
LL: lepromatous leprosy
RA: rheumatoid arthritis
SLE: systemic lupus erythematosus
TT: tuberculoid leprosy

representing C3b dimers, while the second peak contained C3b monomers of 180,000 daltons, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Radiolabeling of Anti-CR1 Fab and C3b Dimers with ^{125}I

The Fab fragments of anti-CR1 and C3b dimers were labeled with ^{125}I (New England Nuclear, Boston, Massachusetts) using iodogen-coated tubes (Pierce, Rockford, Illinois), according to the manufacturer's instructions. Free and bound ^{125}I were separated by centrifugation through tubes of Bio-gel P6 (BioRad) [27].

Quantitation of CR1

A radiobinding assay was developed to measure CR1 on erythrocyte surfaces using ^{125}I-Fab fragments to the monoclonal anti-CR1 IgGl (MoAB 543). Ten μl of venous blood were drawn in 0.02 M ethylenediamine tetraacetate (EDTA, Fisher Scientific, Fairlawn, New Jersey), filtered over glass wool and washed 3× in veronal-buffered saline (VBS), removing any remaining buffy coat after each wash. The erythrocytes were then resuspended at a concentration of 2.5×10^9/ml in VBS (0.075 M NaCl) containing 2.5% dextrose (Mallinkrodt, Paris, Kentucky), and 0.5% gelatin (J. T. Baker, Phillipsburg, New Jersey) (DGVB). Fifty μl of erythrocytes were incubated on ice with 50 μl of increasing dilutions of ^{125}I-Fab anti-CR1 in DGVB ranging from 6.5 to 0.4 μg/ml. After 60 min, the mixtures were layered onto 200 μl of dibutylphthalate (Eastman Kodak, Rochester, New York) in 400 μl polypropylene microfuge tubes (BioRad). Following centrifugation for 1 min at 8000 *g* in a microfuge B (Beckman, Palo Alto, California), the tubes were sectioned and the sections containing pellets and supernatants were analyzed for bound and free ^{125}I-Fab. Nonspecific binding was determined by incubating erythrocyte samples with a 200-fold excess of unlabeled anti-CR1 IgG for 60 min before adding the ^{125}I-Fab. The number of CR1 molecules per cell was calculated by binding isotherms and Scatchard analysis of the binding data corrected for nonspecific binding.

TABLE I. *Clinical distribution according to patient's origin*

Mexico	Philippines	Vietnam	Other
LL: 18	LL: 3	LL: 1	LL: 3
BL: 1	BL: 3	BL: 1	BL: 0
BB: 1	BB: 4	BB: 2	BB: 0
BT: 1	BT: 2	BT: 1	BT: 2
TT: 0	TT: 2	TT: 8	TT: 2

Abbreviations: LL = lepromatous, BL = borderline lepromatous, BB = borderline, BT = borderline tuberculoid, TT = tuberculoid.

RESULTS

The mean number of CR1 molecules per erythrocyte for each group of patients is shown in Fig 1. Patients with lepromatous leprosy had a mean number of CR1 per erythrocyte of 310 ± 33 SEM, significantly different ($p = < 0.001$), from the number of CR1 measured in an age- and sex-matched group of healthy individuals (512 ± 36 SEM). The values obtained in the patients with tuberculoid leprosy (577 ± 76 SEM) did not differ significantly from the control population ($p = > 0.3$). Patients with borderline leprosy had a lower number CR1 (443 ± 41 SEM); however this difference was not statistically significant ($p = 0.1$). CR1 levels on erythrocyte surface of normal individuals as well as patients tested at various times throughout 6 months showed no significant variation.

A table of our patient population shows a distinct pattern of clinical expression associated with the ethnic background of the patient. Of our 55 patients, 18 were of Mexican origin and had lepromatous leprosy. Nine of 14 Filipinos had borderline leprosy (3 BL, 4 BB, 2 BT) and 8 of 13 Vietnamese had tuberculoid leprosy. The remaining 7 patients studied were of other origins.

To determine if the monoclonal antibody was bound to an epitope of the CR1 different from the C3b binding site, fresh erythrocytes were incubated with a 10-fold saturating dose of unlabeled C3b dimers, and the CR1 expression was measured with anti-CR1 Fab as detailed in *Materials and Methods*. As shown in Fig 2, the saturation of erythrocytes with C3b dimers did not interfere with the measurement of CR1 molecules utilizing ^{125}I-Fab. The inverse, that is, the saturation of the cells with the antireceptor antibody did not affect the saturable binding of ^{125}I-C3b dimers (not shown). These experiments showed that the monoclonal anti-CR1 used measures total number of CR1, regardless of receptor occupancy.

At the time of the study only 3 patients were without treatment; when assayed after treatment the number of CR1 per erythrocyte did not change, although, due to the small size of this population, no conclusions can be drawn. However, since both the lepromatous and the tuberculoid patients received similar antibacterial treatment (dapsone, rifampin), and patients receiving thalidomide did not differ in CR1 expression from other lepromatous patients, it is unlikely that therapy had

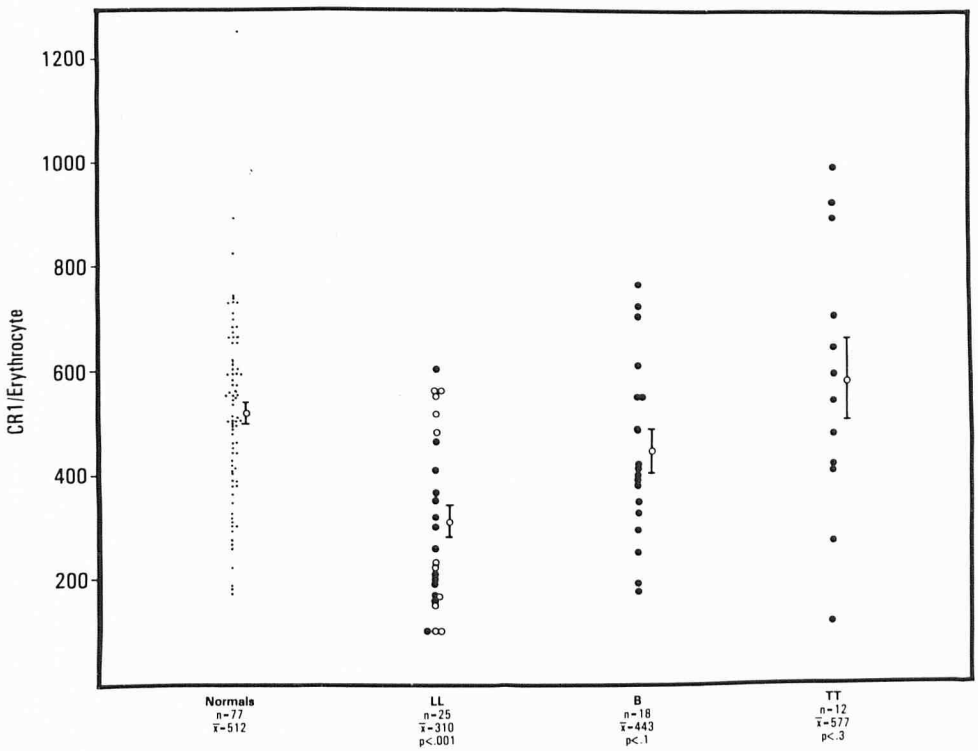

FIG 1. Number of CR1 (C3b receptors) on erythrocytes of *normal* volunteers, lepromatous (*LL*), tuberculoid (*TT*), and borderline (*B*) patients. *Open circles* represent patients with lepromatous leprosy that had erythema nodosum leprosum at the time of the study. Analysis of the data by *t*-test and nonparametric tests showed a significant difference ($p < .001$) between LL and TT patients as well as between LL patients and normals. *Bars* represent means ± SEM for each group.

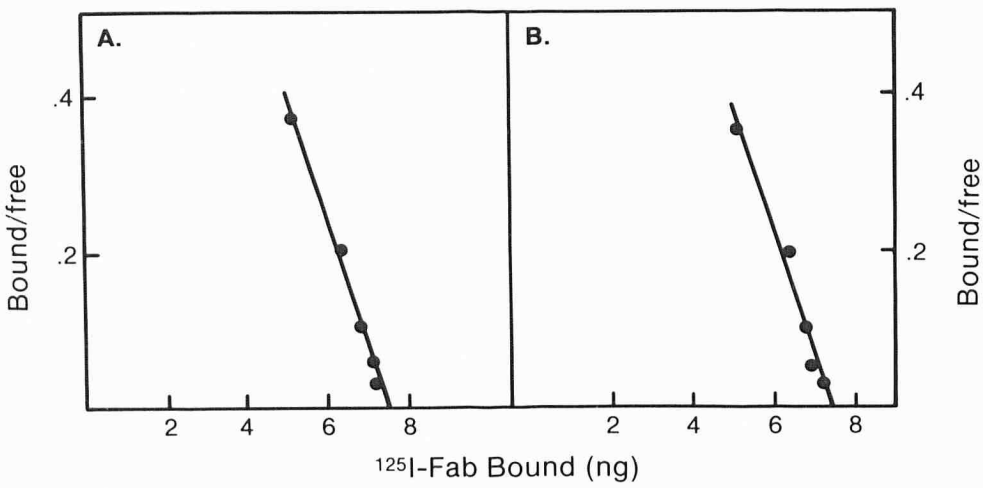

FIG 2. Effect of cell-bound C3b dimers on erythrocyte CR1 quantitation. Scatchard analysis of binding of anti-CR1 ^{125}I-labeled Fab to cells previously incubated with buffer (*A*) or C3b dimers (*B*).

any effect in the diminished expression of CR1 in lepromatous individuals.

DISCUSSION

The receptor on human erythrocytes for C3b, the activation product of the third component of complement, has been shown to play an important role in the clearance of CIC. The relative high concentration of erythrocytes in the blood stream assigns them the major role in the binding of circulating C3b bearing particles [28], in spite of having less CR1 on their surface than leukocytes. Indeed, it has recently been shown that white cells do not contribute significantly to the binding of CIC [15]. A number of reports show a reduction of CR1 molecules on the surface of erythrocytes of patients with systemic lupus as well as RA, suggesting that this alteration could result in an impairment in the clearance of CIC. Genetic studies performed in normal subjects and SLE patients have shown that the CR1 levels are inherited in a codominant fashion [18]. These results raise the possibility that the decrease in levels of CR1 on erythrocyte surfaces serve as a marker that may predispose individuals to certain diseases. Contrary to this notion, Iida, Mornaghi and Nussenzweig [17] reported in SLE patients an increase in CR1 expression on erythrocytes when their disease evolved favorably, implying the acquired nature of the changes in receptor density.

In the present study we investigated the levels of erythrocyte CR1 in a group of normal individuals and in patients diagnosed as having Hansen's disease. We found that the average number of CR1 (512 ± 25 SEM), as well as the wide scatter of values, in our normal population is in agreement with that reported by Wilson et al [18]. Although the over-all distribution of the number of receptors according to high, intermediate, and low, is also in agreement with published data [18], our results failed to show clear evidence of clustering of these groups. We observed that patients with lepromatous leprosy have a decreased number of CR1 molecules on their erythrocyte surface (310 ± 33 SEM), whereas tuberculoid patients do not differ from the normal population (577 ± 76 SEM). The levels of CR1 in patients with borderline leprosy were 443 ± 41 SEM, not significantly different from the healthy age- and sex-matched control group ($p = < 0.1$). Patients assayed 2 or more times during 6 months did not show substantial variation in the expression of erythrocyte-associated CR1. This period is probably insufficient considering the extended course of this particular disease. Long-term studies will provide evidence as to whether the levels of CR1 in borderline patients will correlate with the possible shift in clinical and histopathological features towards one of the poles in the disease process.

Studies to measure the presence of CIC in the blood of the same group of patients showed that those individuals diagnosed as having the lepromatous form of Hansen's disease have high levels of immune complexes which contain IgG and IgM immunoglobulins. In contrast, patients with tuberculoid leprosy did not (unpublished observation). These findings may suggest that the reduction in the number of CR1 per erythrocyte observed in patients with lepromatous leprosy is due to receptor occupancy. The observation that the monoclonal used to measure CR1 recognizes an epitope in the receptor distinct from the binding site (Fig 2) excludes this possibility.

These studies could support the notion that the number of CR1 on erythrocytes is a predictive marker for susceptibility to acquire a given clinical expression of a disease, or in the case of Hansen's disease determine the clinical form of the disease.

The decreased levels of CR1 on erythrocytes of patients with lepromatous leprosy could result in an impairment to correctly process CIC.

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