Characterization of Patients With an Increased Susceptibility to Bacterial Infections and a Genetic Deficiency of Leukocyte Membrane Complement Receptor Type 3 and the Related Membrane Antigen LFA-1


Three children from two unrelated families had a history of recurrent bacterial infections, and their neutrophils were shown to have deficient phagocytic and respiratory responses and possible deficiencies in chemotaxis or adherence. Their neutrophils were strikingly deficient in the ability to ingest or give a respiratory burst in response to unopsonized bakers’ yeast or zymosan (Z). Tests for the ability to ingest or give a respiratory burst in response to adherence. Their neutrophils were strikingly deficient in responses and possible deficiencies in chemotaxis or shown to have deficient phagocytic and respiratory responses to Z were probably due to CR2 deficiency, because treatment of normal neutrophils with anti-CR2, but not anti-FLA-1, inhibits responses to Z by 80% to 90%. Ingestion of Staphylococcus epidermidis by normal neutrophils was shown to be partially inhibited by monoclonal antibodies to the α-chain of either CR2 or LFA-1, and monoclonal antibody to the common β-chain inhibited ingestion by 75%. Thus, both CR2 and LFA-1 may have previously unrecognized functions as phagocyte receptors for bacteria. The absence of this type of nonimmune recognition of bacteria by these children’s neutrophils may be one of the reasons for their increased susceptibility to bacterial infections.

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EUKOCYTE CR1 (C3b/C3b receptor) and CR2 (iC3b/C3d/C3d receptor) have been isolated and characterized structurally, and tests with antibodies raised to the purified receptors have given important clues to the functions of CR1 and CR2. CR3 (iC3b receptor) has a 165,000 M, α-chain that is linked noncovalently to a β-chain of 95,000 M, and is a member of a family of three membrane antigens that have an identical β-chain structure linked to one of three distinct α-chains. The other two family members are LFA-1 (175,000 M, α-chain) and p150,95 (150,000 M, α-chain). Several monoclonal antibodies are specific for the α-chain of CR3; anti-Mac-1, anti-Mo, OKM1, and MN-41. Quantitation of C receptors with 125I mononclonal anti-CR3, and anti-CR3, indicated that neutrophils from each patient expressed normal amounts of CR1 per cell but less than 10% of the normal amount of CR3. Examination of neutrophils by sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that a normal glycoprotein of ~165,000 daltons was missing. Immunoblotting of these gels indicated that the missing band was CR3.

MATERIALS AND METHODS

Patients. Patients No. 1 and 2 were an 11-year-old Caucasian girl and her 6-year-old brother. Detailed clinical histories of these two patients have been reported separately. A third male sibling of this family was healthy, and there was no other family history of disease. The two affected children suffered from recurrent bacterial infections of Staphylococcus aureus. Blood cultures from patients 1 and 2 were positive for Staphylococcus aureus. In addition, the patients had skin infections with Staphylococcus aureus and Staphylococcus epidermidis. The patients were admitted to the hospital for treatment of recurrent skin infections and for evaluation of their susceptibility to infection.

Methods. Peripheral blood was collected from patients No. 1 and 2 and a healthy family member. Blood was drawn after informed consent was obtained, and the study was approved by the Health Sciences Ethical Committee of the University of North Carolina. Peripheral blood samples were drawn into vacutainer tubes containing EDTA. Blood samples were used for studies of phagocytosis, respiratory burst, and adherence.

Phagocytosis. Neutrophils were isolated by ficoll-hypaque gradient centrifugation and were used within 3 hr of isolation. Neutrophils were washed twice and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum. Neutrophils were incubated with unopsonized zymosan (Z), opsonized zymosan (OZ), or opsonized Staphylococcus aureus (OA) at 5 × 10⁶ neutrophils/ml and at a neutrophil-to-opsonin ratio of 1:20. Phagocytosis was assessed by flow cytometry using a fluorescence-activated cell sorter (FACScan) and fluoresecin-labeled goat anti-human IgG.

Respiratory Burst. Neutrophils were incubated with unopsonized zymosan (Z), opsonized zymosan (OZ), or opsonized Staphylococcus aureus (OA) at 5 × 10⁶ neutrophils/ml and at a neutrophil-to-opsonin ratio of 1:20. Respiratory burst was assessed by measuring the production of superoxide anion (O²⁻) using nitroblue tetrazolium as the indicator dye.

Adherence. Neutrophils were incubated with unopsonized zymosan (Z), opsonized zymosan (OZ), or opsonized Staphylococcus aureus (OA) at 5 × 10⁶ neutrophils/ml and at a neutrophil-to-opsonin ratio of 1:20. Adherence was assessed by flow cytometry using a fluorescence-activated cell sorter (FACScan) and fluoresecin-labeled goat anti-human IgG.

 Studies with monoclonal antibodies have suggested a role for LFA-1 in the cytotoxic function of T cells, but the function of LFA-1 on phagocytic cells is unknown.

Described in this report are three patients who have an apparent genetic deficiency of CR2, LFA-1, and the common β-chain shared also with p1 50,95. These patients are probably similar or identical to other patients described recently whose neutrophils have defects in either phagocytosis or adherence and chemotaxis. The present study of the leukocytes from three of these patients suggests that CR2 might be a receptor for yeast in addition to being an iC3b receptor. However, because the patients had skin infections with bacteria and not with yeast, it is perhaps more significant that studies of anti-CR2- and anti-LFA-1-treated normal neutrophils suggest that both CR2 and LFA-1 might have a role in the phagocytosis of unopsonized Staphylococcus epidermidis.
infections, occasionally of life-threatening nature, but there were also periods of one to three years when both were healthy except for minor skin infections and persistent gingivitis. Not mentioned in the original report was the observation that resected tissue from the borders of infections occasionally showed scanty polymorph infiltration on histologic examination.

Patient No. 3 was a Caucasian man aged 22, who developed a septic purulent rash shortly after birth. He then developed recurrent skin infections with ulceration and scarring, which were mainly confined to his legs and arms and from which Staphylococcus aureus was grown. He did not suffer from systemic bacterial infections and had uneventful attacks of varicella and measles in childhood. From age 9 he was treated with prednisolone and then levamisole with little clinical evidence of a response to these agents. Growth and development were normal until the institution of prednisolone, after which there was retardation of skeletal growth. He was one of five children of nonconsanguineous parents. One sibling died of septicemia at 6 months of age. Two siblings and the patient suffered from a hair condition with pilonidal, but his siblings were otherwise well. Three months after the last series of investigations on this patient, he developed a further skin ulcer on his thigh, which spread and resisted all attempts at treatment. He eventually developed septicemia and died.

Isolation of neutrophils, monocytes, and lymphocytes. Blood from normal volunteers or patients was drawn into either preservative-free heparin or acid-citrate-dextrose anticoagulant. After sedimentation of erythrocytes with dextran, mononuclear cells and neutrophils were separated by centrifugation on a two-step Ficoll-Hypaque gradient. For natural killer (NK) cell assays, mononuclear cells, monocytes, and B lymphocytes were removed by adherence to nylon wool and glass. Before C receptor assay, portions of each cell type were washed and resuspended at 4 x 10⁶/mL in either HBSS/1% bovine serum albumin (BSA) or BDVA (3.5 mmol/mL veronal buffer with 10 mmol/L sodium chloride, 1% BSA, 3.5% dextrose, 0.2% sodium azide, and 2 mmol/L each of calcium chloride and magnesium chloride, pH 7.2, and 4 mS at 22 °C).

Preparation of sheep erythrocyte–C3 complexes (EC3). EC3b were prepared with purified C3, and two steps of C3 fixation with trypsin and a nickel-stabilized C3-converase formed with purified factors B and D. EC3bi and EC3dg were prepared by treatment of EC3b with purified factor H and factor I in either isotonic or low-ionic strength buffer, respectively, as previously described. The amount and type of fixed C3 fragments on the EC3 was determined by assays for uptake of ¹²⁵I-labeled monoclonal anti-C3c, C3g, and C3d. EC3 were prepared to contain 2.0 to 2.5 x 10⁹ molecules of C3 per Et. EC3b contained <500 molecules of iC3b/C3dg contamination per cell. EC3bi contained <3% contamination with factor I-resistant C3b, and EC3dg contained <5% contamination with resistant C3b and iC3b.

Antibodies to C receptors, LFA-1, and NK cells. Rabbits were immunized with purified C3r and IgG F(ab')₂ fragments were prepared. E11 monoclonal anti-CR3, CR2, and NK cells were assayed by EC3 rosette formation in either HBSS/1% BSA or BDVA. CR3 and CR4 on neutrophils were quantitated with ¹²⁵I E11 and ¹²⁵I MN-41, respectively. To prevent Fc receptor binding of ¹¹¹I IgG antibodies, neutrophils were incubated with 100 to 300 μg/mL of heat-aggregated IgG for 15 minutes before the assay for uptake of the ¹¹¹I antibodies. Heat-aggregated IgG was prepared from DEAE-isolated human IgG (10 mg/mL in phosphate-buffered saline [PBS]) that was heated at 63 °C for 30 minutes.

Assay for Fc receptors and Fc receptor-mediated phagocytosis. EA₉₀ were prepared with sheep E and subagglutinating dilutions of rabbit IgG anti-sheep E antibody, were suspended at 2 x 10⁹/mL in BDVA and tested for rosette formation with neutrophils suspended at 4 x 10⁹/mL in BDVA. One hundred micromolar of EA₉₀ was mixed with 100 μL of neutrophils in a 10 x 65-mm plastic tube and incubated on a rotator for 15 minutes at 37 °C. The EA₉₀ preparation giving the highest proportion of rosettes was used for studies of phagocytosis. After formation of EA₉₀ rosettes with neutrophils or monocytes, the tubes were mixed on a vortex mixer (Scientific Industries, Bohemia, NY) to disrupt rosettes, and the cells were examined by phase contrast microscopy for ingested EA₉₀. Two hundred cells were scored as having either 0, 1, 2, 3, or 4+ ingested EA₉₀, and the number of EA₉₀ ingested per 100 phagocytes was calculated.

Immunofluorescence staining. IgG antibodies were centrifuged at 8,000 g for ten minutes, and, in some experiments, treated with 2:1 wt ratio of protein A-fluorescein isothiocyanate (SPA-FITC, Pharmacia Fine Chemicals, Piscataway, NJ) to minimize binding to Fc receptors. Pellets of 1 x 10⁶ cells were mixed with 1 μg of monoclonal antibody in 1% BSA/PBS/0.2% sodium azide or 50 μL of hybridoma tissue culture supernatant and incubated on ice for 30 minutes. Unbound antibody was removed by layering the suspension onto 3 mL of 6% BSA/PBS/0.2% azide in 10 x 65-mm and centrifuging at 400 g for five minutes at 4 °C. After aspiration of the supernatant, 50 μL of a 1:10 dilution of F(ab')₂-anti-mouse (or rat) IgG (or IgM) coupled to FITC (Cappel Laboratories, West Chester, Pa) was added, and the cells were incubated for 30 minutes on ice. Unbound fluorescent antibody was removed in the same way as monoclonal antibody, and the cells were examined for membrane fluorescence.

Evaluation of immunofluorescence staining by cytofluorographic techniques. Immunofluorescence staining was analyzed with the Cambridge MRC custom-built dual laser flow cytometer for which descriptions have been published. The instrument was set up to excite fluorescence with the Spectrophysics 16405 argon laser (Spectrophysics, Mountain View, Calif) tuned to 488 nm and to analyze forward light scatter together with 90-degree scatter and fluorescence simultaneously. Three measurements from each photodetector—pulse height, width, and area—were collected from each cell sample. The data were recalled from disk and gated on the widths of the forward and 90-degree scatter pulses to exclude debris and clumps. Pulse width is the most reliable size measurement in this system. Fluorescence was then gated on total 90-degree light scatter and monodimensional histograms were generated. The 90-degree light scatter detector response is used as an internal instrument control to check that there has been no increase in light scatter that could contribute to the fluorescence signal.

Assay for neutrophil phagocytosis of yeast or staphylococcus. Neutrophils were tested for phagocytosis of unopsonized heat-killed bakers’ yeast (Saccharomyces cerevisiae), or unopsonized heat-killed and FITC-labeled S epidermidis or S aureus. With unopsonized yeast, the neutrophils or monocytes were pelleted together with the yeast at 200 g for two minutes, incubated as a pellet for two minutes at 37 °C, vortexed vigorously, and examined for ingested yeast particles. Bacteria (3 x 10⁹) and neutrophils (5 x 10³) were added to each tube and incubated in the presence of 200 ng of ¹₂⁵I-125I or ¹₁¹I labeled yeast. The presence of ingested yeast was determined by autoradiography.
10^3 in 30 μL HBSS/1% BSA were incubated together in suspension for 30 minutes at 37 °C and then examined for ingestion after mixing on a vortex mixer to disrupt bacteria rosettes and removal of uningested bacteria by centrifugation of neutrophils through a single-step gradient of 6% BSA/PBS. For experiments that examined the effect of antibodies on ingestion, 5 x 10^6 neutrophils were treated with 5 μg of monoclonal IgG, 96 μg of F(ab')2 anti-CR3, or 100 μg of heat-aggregated human myeloma IgG, for 20 minutes at room temperature before addition of bacteria.

**Assays for NK cell cytotoxicity.** Adherent cell-depleted mononuclear cells from patients and normal individuals were tested for killing of 3^1Cr-labeled K562 target cells in a standard four-hour assay.39

**Analysis of neutrophil glycoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** Neutrophils (1 x 10^6) in 1 mL PBS were treated with 50 μL of 0.1 mol/L diisopropylfluorophosphate in ethylene glycol for ten minutes on ice, pelleted, and solubilized with 1 mL PBS containing 0.5% Nonidet P40 and 2 mmol/L phenylmethylsulphonyl fluoride. After boiling in SDS and 2-mercaptoethanol, the entire sample was electrophoresed on 7.5% polyacrylamide gel slabs.24 Proteins were detected with Coomassie blue. For immunoblotting, proteins were transferred to nitrocellulose, incubated with 125I-MN-41 (0.35 μCi/μg) overnight at 4 °C, and placed on x-ray film for eight days at –70 °C for identification of the gel band representing the α-chain of CR3.

**RESULTS**

**Review of early clinical laboratory tests.** All three patients had been examined since infancy for possible immune deficiency.24 The most striking abnormality had been the neutrophil respiratory burst response to unopsonized zymosan, that was shown to be <10% of normal in patients No. 1 and 224 and subsequently also in patient No. 3.24 Although the motility of the patient’s neutrophils measured in vitro appeared to be normal,24 the absence of polymorph infiltrates in wound margins suggested the possibility of some type of in vivo defect in either chemotaxis or adherence.

**Immunofluorescence assays.** The finding of absent surface staining for the OKM1 epitope of CR3,24 suggested that the patients’ neutrophils might be deficient in the entire CR3 antigen. Neutrophils, monocytes, and lymphocytes were examined for surface staining with OKM1 and other antibodies known to be specific for the α-chain of CR3 (anti-Mol, anti-Mac-1, and MN-41) (Table 1). The majority of normal neutrophils and monocytes and an average of 10% of lymphocytes were stained with anti-Mac-1, OKM1, anti-Mol, and MN-41. The lymphocytes stained with anti-Mac-1, OKM1, and anti-Mol are known to include the cells that express NK activity,30,31 and accordingly, a similar 10% of normal lymphocytes were also shown to stain with anti-Leu-7 that identifies an NK-associated antigen (Table 1). By contrast, none of the monocytes, neutrophils, or lymphocytes from patients No. 1 and 2 stained with anti-Mac-1, anti-Mol, or OKM1. Negative results were also obtained when cells from patient No. 3 were tested with MN-41, OKM1, and anti-Mol. Among lymphocytes, 3% to 20% of anti-Leu-7-staining cells were observed, but no cells staining with anti-Mac-1, anti-Mol, MN-41, or OKM1 were detected (Table 1).

**Immunoblotting assay of neutrophil SDS-PAGE gels for CR3 α-chain.** To determine whether the glycoprotein missing from gels of the patients’ neutrophils24 was the α-chain of CR3, SDS-PAGE gels of neutrophils were blotted onto nitrocellulose and tested for reaction with 125I-MN-41 (Fig 1). A radioactive band with 150,000 estimated M, developed only in the normal neutrophil control tracks and not in the blotted gel tracks from either of the patients. The position of the radioactive band correlated exactly with the position of the missing band in the patients’ neutrophils, indicating that the missing glycoprotein was the α-chain of CR3.

**Assay of C receptors by EC3 rosette formation.** It had been postulated that the Mac-1/OKM1/Mol/MN-41 antigen was CR3 because anti-Mac-1,5 anti-Mol,10 and MN-4112 each inhibited CR3 activity and the isolated OKM1 antigen bound to EC3bi but not to EC3b.11 Thus, the patients’ neutrophils should have been deficient in CR3 activity, and demonstration of this would provide additional confirmation of the identity of CR3 with the Mac-1/OKM1/Mol/MN-41 antigen.

As described previously,24 CR3 cannot be specifically assayed by EC3bi rosette formation unless steps are taken to avoid EC3bi binding to CR1 and CR2. With normal neutrophils or monocytes, EC3bi were bound simultaneously by way of both CR1 and CR3; ie, complete blockade of EC3bi

| Table 1. Indirect Immunofluorescence Staining of Isolated Neutrophils, Monocytes, and Lymphocytes With Anti-Mac-1, Anti-Mol, OKM1, MN-41, Anti-Leu-7 | Fluorescence Staining |
| --- | --- | --- | --- | --- |
| Normal controls | Anti-Mac-1 (%) | Anti-Mol (%) | OKM1 (%) | Anti-Leu-7 (%) |
| Neutrophils | 98 | 98 | 96 | 0 |
| Monocytes | 89 | 92 | 90 | 0 |
| Lymphocytes | 10 | 9 | 10 | 10 |
| Patients No. 1, 2, and 3 | | | | |
| Neutrophils | 0 | 0 | 0 | 0 |
| Monocytes | 0 | 0 | 0 | 0 |
| Lymphocytes | 0 | 0 | 0 | (5, 3, 20)* |

Cells from patient No. 3 and the normal control tested at the same time were stained with MN-41 instead of anti-Mac-1. MN-41 stained the same proportion of normal cells as did anti-Mac-1.

*Percentage of stained cells from patients No. 1, 2, and 3, respectively. This type of fluorescence analysis was done on two or more occasions with each patient.
Fig 1. Immunoblotting and autoradiography of an SDS-PAGE gel slab with \(^{125}\text{I}-\text{MN}-41\) (anti-CR\(_3\) α-chain). Panel A is a gel stained with Coomassie blue, and panel B is the autoradiogram of an immunoblot of a duplicate gel run in parallel. From left to right, the gel tracks contained normal neutrophils (N), neutrophils from patients No. 1 and 2 (Pt), the mother of these patients (Mo), and another normal neutrophil control (N). In these gels the calculated mol wt of the missing glycoprotein was 150,000 (arrow), and a radioactive band exactly corresponding to this position on the autoradiogram was seen in the normal neutrophils, and to a lesser extent in the neutrophils from the mother, but not at all in the neutrophils from the two patients. In other experiments using different SDS-PAGE gel conditions, the missing gel band in these two patients (α-chain of CR\(_3\)) has had a calculated mol wt of 175,000.

Rosettes required treatment with both anti-CR\(_1\) and anti-CR\(_3\) (Table 2). With lymphocytes, EC\(_3\)bi were bound to CR\(_1\) and CR\(_3\) on B cells and to CR\(_3\) on NK cells, so that anti-CR\(_1\) and anti-CR\(_3\) only inhibited EC\(_3\)bi rosetting partially. The 10% of lymphocyte EC\(_3\)bi rosetting in the presence of anti-CR\(_1\) and anti-CR\(_3\) was probably CR\(_3\) dependent because assay of CR\(_3\) with EC\(_3\)dg also detected an average of 10% of normal lymphocytes (Table 2). To enhance the rosetting activity, rosette assays were done in low-ionic strength buffer in addition to isotonic buffer.

With neutrophils from all three patients, the percentage of EC\(_3\)bi rosettes was in the low normal range when assayed in isotonic HBSS and nearly all cells rosetted with EC\(_3\)bi in 4 mS BDVA (Table 3). Abnormally, however, neutrophil EC\(_3\)bi rosettes were inhibited completely by anti-CR\(_1\) alone, demonstrating that EC\(_3\)bi were bound only to CR\(_1\) and that CR\(_3\) activity was absent.

Monocytes from two of the three patients rosetted poorly with EC\(_3\)bi in HBSS (Table 3) and resembled anti-CR\(_3\)-treated normal monocytes (Table 2). Monocytes from patient No. 3 did rosette normally with EC\(_3\)bi in HBSS. As with neutrophils, all of the patients' monocyte rosettes with EC\(_3\)bi were inhibited completely by anti-CR\(_1\) alone, and thus monocytes were also CR\(_3\) deficient (Table 3).

Lymphocytes from patients No. 1 and 2 formed normal percentages of rosettes with EC\(_3\)b, EC\(_3\)bi, and EC\(_3\)dg. Patient No. 3 had an elevated proportion of CR\(_1\)-bearing lymphocytes (EC\(_3\)b rosettes). Treatment of the patients' lymphocytes with anti-CR\(_1\) alone reduced the proportion of EC\(_3\)bi rosetting cells down to the same level as the EC\(_3\)dg...
rosetting cells (11%), suggesting that EC3bi were bound only to CR1 and CR2 and that CR3 was missing from the patients' lymphocytes.

**Quantitation of neutrophil CR1 and CR3.** CR3 was quantitated with 125I E11 monoclonal anti-CR3 (Table 4). Neutrophils from normal volunteers and normal family members expressed 2.5 to 4.5 × 10^4 CR3 per cell, whereas neutrophils from the three patients expressed somewhat higher amounts of CR3 per cell (Table 4). Even though IgG, rather than F(ab')2, E11 was used, binding of the 125I E11 to Fc receptors was negligible, as there was no inhibition of 125I E11 uptake by amounts of heat-aggregated IgG.

Neutrophil CR3 was quantitated with 125I-labeled MN-41 (Table 4). Cells from all three patients, their parents, four healthy siblings, and normal volunteers were examined. Normal neutrophils bound an average of 7.9 × 10^4 125I MN-41 molecules per cell, but this was reduced to 6.5 × 10^4 molecules per cell after incubation with heat-aggregated IgG, suggesting that some binding of the 125I MN-41 was Fc receptor dependent. When neutrophils from the three patients were examined in the presence of heat-aggregated IgG, only 5,500, 8,500, and 7,800 molecules of 125I MN-41 were bound per neutrophil (8% to 12% of normal). In family No. 1, the parents and normal brother did not have significantly less CR3 than did normal individuals. However, in family No. 2, normal family members had 48% to 75% (average 63%) of the normal amount of CR3 per neutrophil.

**Deficiency of LFA-1 a-chain and common D-chain.** The expression of LFA-1 a-chain and the D-chain shared by the CR3/LFA-1/p150,95 antigen family were examined by cytofluorography (Table 5). Fresh blood cells from family No. 1 were examined, whereas with family No. 2, fresh blood was unavailable and B lymphoblasts from a previously established Epstein-Barr virus-transformed line from patient No. 3 were examined instead (not shown). Little or no CR3 a-chain was detectable with OKM1 on the neutrophils or monocytes from patients No. 1 and 2. In addition, both patients had greatly reduced amounts of phagocyte LFA-1 a-chain and common D-chain. With lymphocytes from patients No. 1 and 2, <10% of the normal amounts of LFA-1 or D-chain was detectable. Likewise, the B lymphoblasts from patient No. 3 had <10% of the normal amount of LFA-1 and D-chain detectable on B cell lines derived from normal subjects.

**Selective deficiency in the ability to phagocytose yeast.** Because initial tests of patients No. 1 and 2 had suggested some kind of phagocytic defect,24 the neutrophils and monocytes from patient No. 3 were examined for ingestion of EA a, EC3b, and unopsonized yeast (Table 6). Phagocytosis of EA a by the neutrophils from patient No. 3 was equivalent to the normal control, whereas monocyte phagocytosis of EA a was 65% of normal. EC3b are not

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<tr>
<th>Table 4. Quantitation of CR1 and CR3 on Neutrophils From Patients and Their Families With 125I Monoclonal Anti-CR1 and Anti-CR3</th>
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<tr>
<td><strong>Molecules per Neutrophil of 125I</strong></td>
</tr>
<tr>
<td><strong>Anti-CR1</strong> (E11)</td>
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<tr>
<td><strong>Family No. 1</strong></td>
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<tr>
<td>Patient No. 1</td>
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<td>Patient No. 2</td>
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<tr>
<td>Normal brother</td>
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<td>Mother</td>
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<td>Normal control</td>
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<tr>
<td><strong>Family No. 2</strong></td>
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<td>Patient No. 3</td>
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<td>Father</td>
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<td>Normal control</td>
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<th>Table 5. Immunofluorescence Cytofluorographic Analysis of CR1 and LFA-1 on Neutrophils, Monocytes, and Lymphocytes From Patients No. 1 and 2</th>
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<tr>
<td><strong>Cell Type</strong></td>
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<tr>
<td>Neutrophils</td>
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<td>Patient No. 1</td>
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<td>Patient No. 2</td>
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<td>Normal brother</td>
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<td>Monocytes</td>
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<td>Normal control</td>
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<tr>
<td>Lymphocytes</td>
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<tr>
<td>Patient No. 1</td>
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<td>Patient No. 2</td>
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Net fluorescence intensity after subtraction of background nonspecific fluorescence obtained with X63 control supernatant. High-gain setting was used to evaluate lymphocyte fluorescence intensity. This assay of patients and their entire family was done only once. ND, not done.

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<tr>
<th>Table 6. Phagocytosis of EA a, EC3b, and Unopsonized Yeast by Neutrophils and Monocytes From Patient No. 3</th>
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<tr>
<td><strong>Ingested Particles per 100 Phagocytes</strong></td>
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<tr>
<td>EA a</td>
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<tr>
<td>Normal control</td>
</tr>
<tr>
<td>Neutrophils</td>
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<tr>
<td>Monocytes</td>
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<tr>
<td>Patient No. 3</td>
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Cells from patient No. 3 were tested two times for phagocytosis, and the slides were read by three individuals.

Assays of patients and their entire families were formed only once.
usually ingested significantly by freshly isolated peripheral blood monocytes, and no EC3b ingestion was noted with either the normal control monocytes (Table 6) or the monocytes from patients No. 1 and 2 (not shown). However, 65% of the EC3b rosetted monocytes from patient No. 3 contained ingested EC3b. The most striking abnormality was the near absent phagocytosis of unopsonized yeast. Compared with normal phagocytes, the neutrophils and monocytes from patient No. 3 ingested only 5% yeast per cell, respectively.

Absence of NK cell activity. NK activity was examined on one occasion with patients No. 2 and 3 only. The cells from patient No. 2 were tested at effector-target cell ratios ranging from 200:1 down to 10:1, and the cells from patient No. 3 were tested at ratios from 90:1 to 7:1. The normal control cells exhibited significant cytotoxicity (>30%) at effector-target cell ratios of 15:1 or greater, whereas NK-mediated cytotoxicity by patients No. 2 and 3 was 6% or less at all ratios tested (data not shown).

CR3-dependent ingestion of S. epidermidis. Both S. aureus and S. epidermidis were readily ingested by normal neutrophils without need of serum opsonization (Table 7). Ingestion of S. epidermidis was partially inhibited by both anti-CR3 and anti-LFA-1, whereas the ingestion of S. aureus was either unaffected or enhanced by anti-CR3 and anti-LFA-1. The inhibitory effects of anti-CR3 and anti-LFA-1 on S. epidermidis ingestion were additive, since a mixture of anti-CR3 and anti-LFA-1 produced more inhibition than did either antibody by itself. In addition, anti-β-chain (TS 1/18) consistently produced the greatest inhibition of S. epidermidis ingestion (Table 7).

DISCUSSION

Three patients with a history since infancy of bacterial skin infections were shown to have an apparent genetic deficiency of CR3 on their neutrophils, monocytes, and NK cells. In addition, this deficiency was shown to extend to the monocytes (Table 6), and no EC3b ingestion was noted with either the normal control monocytes or the monocytes from patients No. 1 and 2 (not shown). However, 65% of the EC3b rosetted monocytes from patient No. 3 contained ingested EC3b. The most striking abnormality was the near absent phagocytosis of unopsonized yeast. Compared with normal phagocytes, the neutrophils and monocytes from patient No. 3 ingested only 5% and 7.5% as much yeast per cell, respectively.

Absence of NK cell activity. NK activity was examined on one occasion with patients No. 2 and 3 only. The cells from patient No. 2 were tested at effector-target cell ratios ranging from 200:1 down to 10:1, and the cells from patient No. 3 were tested at ratios from 90:1 to 7:1. The normal control cells exhibited significant cytotoxicity (>30%) at effector-target cell ratios of 15:1 or greater, whereas NK-mediated cytotoxicity by patients No. 2 and 3 was 6% or less at all ratios tested (data not shown).

CR3-dependent ingestion of S. epidermidis. Both S. aureus and S. epidermidis were readily ingested by normal neutrophils without need of serum opsonization (Table 7). Ingestion of S. epidermidis was partially inhibited by both anti-CR3 and anti-LFA-1, whereas the ingestion of S. aureus was either unaffected or enhanced by anti-CR3 and anti-LFA-1. The inhibitory effects of anti-CR3 and anti-LFA-1 on S. epidermidis ingestion were additive, since a mixture of anti-CR3 and anti-LFA-1 produced more inhibition than did either antibody by itself. In addition, anti-β-chain (TS 1/18) consistently produced the greatest inhibition of S. epidermidis ingestion (Table 7).

Three patients with a history since infancy of bacterial skin infections were shown to have an apparent genetic deficiency of CR3 on their neutrophils, monocytes, and NK cells. In addition, this deficiency was shown to extend to the LFA-1 α-chain and the common β-chain that is shared by CR3, LFA-1, and p150,95. The absence of CR3 activity was correlated with the absence of the 165,000 M r protein in the 110,000 to 229,000 M range and functional deficiencies in either phagocytosis and respiratory burst or adherence and chemotaxis. Not only do these other patients have a similar clinical history, but they have also been shown to be missing the same surface antigens by use of monoclonal antibodies. The variation in calculated mol wt of the missing normal glycoprotein (the α-chain of CR3) is now recognized to have been due to differences in SDS-PAGE gel conditions.21

Two years ago, when the surface glycoprotein deficiency of the patients in England was first detected, it was noted that the clinical histories and neutrophil functional deficiencies appeared to be similar to a case of "gp150" deficiency reported earlier by Arnaout et al. In both sets of patients, phagocytosis of EA, and opsonized zymosan, as well as the respiratory burst response to opsonized zymosan were reduced to 30% to 50% of normal. In addition, Fc and C3 receptors appeared to be expressed normally. SDS-PAGE gels confirmed that a 175,000 M, glycoprotein was missing from the neutrophils of patients No. 1, 2, and 3. Subsequently, SDS-PAGE analysis on the same gel of neutrophils from the original "gp150" deficiency patient and from patients No. 1 and 2 indicated that all three patients were probably deficient in the same protein (unpublished observations). A further indication of the identity of "gp150" deficiency and CR3 deficiency was the absence of surface staining with anti–Mac-1, anti-Mol, and OKM1 (Table 3). Finally, immunoblotting of SDS-PAGE gel slabs of neutrophils from patients No. 1 and 2 demonstrated that the α-chain of CR3 was undetectable, and that the position of the missing glycoprotein corresponded exactly to the position of CR3 α-chain.

Because EC3b is not bound exclusively to CR3, the CR3...
deficiency state could not be detected by simple rosette assay with EC3bi. Indeed, EC3bi rosetting with the patients’ cells appeared to be normal with all leukocyte types except monocytes. Normal neutrophils bind EC3bi to CR1 and CR3, simultaneously. Monocytes may have less CR1 per cell than do neutrophils, as EC3bi binding to monocytes is primarily CR1-dependent. B lymphocytes bind EC3bi to both CR1 and CR3, whereas NK cells bind EC3bi primarily to CR3 because they lack significant amounts of CR1 and CR2. The CR3 deficiency was demonstrated by anti-CR3 treatment of cells. This resulted in complete inhibition of neutrophil and monocyte EC3bi rosette formation and a reduction of lymphocyte EC3bi rosettes down to the same level as CR3-dependent EC3dg rosettes.

Blockade of CR1 function by anti-Mac-1 was the first suggestion that CR3 and Mac-1 were identical. However, it was subsequently reported that the same molecule recognized by anti-Mol was not CR3, but a closely associated antigen involved in Fe-mediated functions as well as iC3b-mediated functions. Neutrophils from a Mol-deficient patient formed rosettes with EC3bi that were not inhibited by saturating amounts of anti-CR1. However, subsequent analysis of this patient’s cells with Fab-anti-Mol revealed small amounts of Mol (CR3) that were not detected previously by immunofluorescence. This small number of CR3 explained the EC3bi rosettes observed in the presence of anti-CR1, as treatment of the Mol-deficient neutrophils with a mixture of anti-CR3 and anti-Mol inhibited EC3bi rosetting completely. Further evidence for the identity of Mol and CR3 came from studies that demonstrated that the isolated Mol glycoprotein bound to EC3bi but not to EC3b. Thus, it was concluded that the antigen recognized by anti-Mol, as well as by anti-Mac-1, MN-41, and OKM1, is CR3.

Other studies excluded a direct role of CR3 in Fe receptor-mediated functions. The reduced ingestion of EA105 or EA106,24 observed in these patients required use of small amounts of sensitizing anti-E antibody, and with larger amounts of IgG, no deficiency of ingestion was noted.20 No deficiency in Fe-mediated ingestion was noted in the patients identified by Anderson et al. Further insights came from studies of normal neutrophils treated with monoclonal anti-CR3. Inhibition of EA105 ingestion was observed only with EA106 bearing small amounts of IgG14,20 and did not occur in buffers containing either EDTA or N-acetyl-d-glucosamine that inhibit the iC3b binding site of CR3. The data suggested the possibility that CR3 might bind weakly to some component of the sheep erythrocyte membrane and thereby synergistically enhance the Fe receptor-mediated ingestion of EA105.

Quantitation of neutrophil CR1 and CR3 demonstrated that the three patients had amounts of CR1 in the high normal range and 8% to 12% of the normal amount of CR3. In the healthy members of family No. 1, the CR3 number appeared to be normal by radioimmunoassay, but only half-normal amounts of CR3 were detected by cytofluorographic analysis. In family No. 2, neutrophil CR3 of healthy members was 47% to 75% (average 63%) of normal by radioimmunoassay, suggesting that this deficiency is genetic and that perhaps the parents are heterozygotes. Other recent studies of three families of similar patients in Texas have been more definitive.22 In these studies it was shown that heterozygous parents and siblings could be identified by half-normal expression of CR3 when neutrophils were assayed after stimulation with F-Met-Leu-Phe that causes full expression of the latent cytoplasmic pool of CR3. Biosynthesis studies in this latter group of patients suggested that the deficiency might result from an inability to synthesize a normal β-chain precursor required for processing of the α-chain precursor and subsequent incorporation of αβ₂ complexes into the membrane.22 One major finding was that CR3-deficient neutrophils and monocytes were virtually unable to phagocyte unopsonized yeast. This did not result from some generalized phagocytic defect, as neutrophils from patient No. 3 exhibited avid Fe receptor-mediated ingestion of EA105, and his monocytes exhibited CR1-mediated ingestion of EC3b. This latter finding presumably indicates in vivo activation of monocytes, as unactivated monocytes do not ingest EC3b. Thus, with the assays used, CR3 deficiency had little or no effect on ingestion mediated by Fe receptors or CR3, and was selective for the absence of yeast ingestion. Recent studies have confirmed the role of CR3 in the phagocytosis of yeast by demonstration that treatment of normal neutrophils with anti-CR3 (anti-Mac-1, anti-Mol, OKM1, or MN-41) produced 70% to 90% inhibition of yeast binding, ingestion, and respiratory bursts. Thus, CR3 functions as a yeast receptor as well as an iC3b receptor.

The absent NK activity of lymphocytes from patients No. 2 and 3 probably resulted from their deficiency of LFA-1, rather than of CR3 or p150,95. This is because antibodies to the α-chain of LFA-1 inhibit normal NK activity, whereas antibodies to the α-chain of CR3 have no effect on NK activity. Although NK activity appeared normal in the Mol-deficient patient, the patients in Texas were deficient in NK activity.22 CR3 has been shown to function as a receptor for fixed iC3b and bakers’ yeast, but it is unknown whether LFA-1 and p150,95 might have similar functions as receptors and, if so, what their ligands might be. On cytotoxic T cells, LFA-1 is thought to mediate some type of adhesive function required for binding to target cells before killing reactions. However, the function of LFA-1 on phagocytic cells is unknown. Because the CR3/LFA-1/p150,95-deficiency patients had recurrent skin infections with bacteria, these surface molecules probably have important functions in host defense against bacterial infection. Studies with normal neutrophils treated with CR3 and LFA-1 subunit-specific monoclonal antibodies have demonstrated inhibition of adherence functions as well as phagocytic and respiratory functions. The present study showed that both anti-CR3 and anti-LFA-1 inhibited normal neutrophil phagocytosis of unopsonized S epidermidis. By contrast, previous studies showed that the same TS 1/22 anti-LFA-1 did not inhibit yeast ingestion. The finding that a mixture of anti-CR3 and anti-LFA-1 produced more inhibition than did either antibody by itself suggests that CR3 and LFA-1 may each participate in binding to different surface components of S
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epidermidis. In addition, monoclonal anti-β-chain (TS 1/18) consistently produced more inhibition of S epidermidis infection than did either anti-CR3 or anti-LFA-1 alone. This was unexpected, as TS 1/18 does not inhibit either EC3bi rosettes or yeast binding to CR3. It was subsequently found, however, that TS 1/18 did inhibit CR3-dependent yeast ingestion by neutrophils (unpublished observations, August 1984), and TS 1/18 was originally identified by its ability to inhibit LFA-1-dependent T cell cytotoxic reactions. The mechanism of anti-β-chain inhibition of functions is unknown, but the data suggest that the β-chain may have some common role in triggering cell functions mediated by each of the antigen family members.

No inhibition of S aureus ingestion was observed with any of the antibodies, and anti-CR3 and anti-LFA-1 usually enhanced S aureus ingestion. Others have shown that this results from "reverse opsonization," in which the Fc regions of receptor-bound antibodies bind to protein A of S aureus and thereby enhance responses. Recently, however, when a F(ab')2 fragment of MN-41 anti-CR3 was tested, no inhibition of S aureus ingestion was observed, despite the finding that the F(ab')2 fragment was active in blocking both EC3bi rosettes and yeast ingestion (G.D. Ross, October 1984, unpublished observations). Future studies will be required to characterize the ability of CR3 and LFA-1 to bind to bacteria. However, the data suggest a restricted specificity for CR3 in adhesive reactions that includes EC3bi, bakers' yeast, and S epidermidis but not EC3b or S aureus. Finally, even though CR3 may not bind directly to a particular strain of bacteria, it certainly can bind to fixed iC3b on bacteria generated by serum opsonization.

Diagnosis of other patients with CR3 deficiency will be facilitated in the future with recognition that several available monoclonal antibodies are specific for CR3 (anti-Mac-1, OKM1, OKM9, OKM10). Continued examination of these patients' cells, as well as normal cells treated with monoclonal antibodies to the individual components, should allow the functions mediated by these surface molecules to be characterized more completely.

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**NOTE ADDED IN PROOF**

We regret to report that patient No. 1 has recently died of septicemia resulting from a spreading ulcer on her left thigh despite months of hospitalization and intensive efforts with a variety of antibiotics, immunostimulants, trace metals, and white cell infusions.

**REFERENCES**

19. Ross GD, Cain JA, Lachmann PJ: Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. J Immunol 134:3307, 1985


35. Watson JV: Enzyme kinetic studies in cell populations using the fluorogenic substrates and flow cytometric techniques. Cytometry 1:143, 1980


42. Tedder TF, Clement LT, Cooper MD: Expression of C3d receptors during human B cell differentiation: Immunofluorescence analysis with the HB-5 monoclonal antibody. J Immunol 133:678, 1984

43. Dana N, Todd R, Pitt J, Colten HR, Arnaout MA: Evidence that Mol (a surface glycoprotein involved in phagocytosis) is distinct from the C3bi receptor. Immunobiology 164:205, 1983 (abstr)


Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type 3 and the related membrane antigen LFA-1


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