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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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 neutrophil and monocyte CR_1 (C3b/iC3b receptor) and CR_3 (iC3b receptor) demonstrated rosettes with both EC3b and EC3bi. However, EC3bi were bound only to CR_1, and not to CR_3, because EC3bi rosettes were inhibited completely by anti-CR_1. Neutrophils, monocytes, and natural killer (NK) cells also did not fluorescence stain with monoclonal antibodies specific for the α-chain of CR_3 (anti-Mac-1, anti-Mol, OKM1, and MN-41). Quantitation of C receptors with ^{125}I monoclonal anti-CR_1 and anti-CR_3 indicated that neutrophils from each patient expressed normal amounts of CR_1 per cell but <10% of the normal amount of CR_3. 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 the α-chain of CR_3. Subsequent analysis of all three patients' cells also demonstrated a deficiency of LFA-1 α-chain and the common β-chain that is shared by the CR_3/LFA-1/p150,95 membrane antigen family. The deficiency of LFA-1 probably explained the absent NK cell function, as normal NK cell activity is inhibited by anti-LFA-1 but not by anti-CR_3. The reduced phagocytic and respiratory responses to Z were probably due to CR_3 deficiency, because treatment of normal neutrophils with anti-CR_3, 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 monoclonl antibodies to the α-chain of either CR_3 or LFA-1, and monoclonal antibody to the common β-chain inhibited ingestion by 75%. Thus, both CR_3 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.

LEUKOCYTE CR_1 (C3b/iC3b receptor) and CR_2 (iC3b/C3dg/C3d receptor) have been isolated and characterized structurally,[1-4] and tests with antibodies raised to the purified receptors have given important clues to the functions of CR_1 and CR_2.[5-7] CR_3 (iC3b receptor) has a 165,000 M_r α-chain that is linked noncovalently to a β-chain of 95,000 M_r 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_r α-chain) and p150,95 (150,000 M_r α-chain).[8] Several monoclonal antibodies are specific for the α-chain of CR_3: anti-Mac-1,[9] anti-Mol,[10] OKM1, OKM9, OKM10,[8,11] and MN-41.[12] The iC3b binding site of CR_3 is probably located in the α-chain, because some of these antibodies, but not antibodies to the β-chain, inhibit EC3bi binding to CR_3.[8,11-14] CR_3 is an important phagocyte receptor that triggers both phagocytosis and a respiratory burst.[7,14] LFA-1 is expressed on both phagocytic cells and lymphocytes.[8] Studies with monoclonal antibodies have suggested a role for LFA-1 in the cytotoxic function of T cells,[13,15] 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 CR_3, LFA-1, and the common β-chain shared also with p150,95. These patients are probably similar or identical to other patients described recently whose neutrophils have defects in either phagocytosis or adherence and chemotaxis.[16-23] The present study of the leukocytes from three of these patients suggests that CR_3 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-CR_3– and anti-LFA-1–treated normal neutrophils suggest that both CR_3 and LFA-1 might have a role in the phagocytosis of unopsonized *Staphylococcus epidermidis*.

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.[24] 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

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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[24] 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 pustular 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 pilitorti, 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.[25] For natural killer (NK) cell assays, mononuclear cells, monocytes, and B lymphocytes were removed by adherence to nylon wool and glass.[26] Before C receptor assay, portions of each cell type were washed and resuspended at 4×10^6/mL in either isotonic Hanks' buffered saline solution (HBSS)/1% bovine serum albumin (BSA) or BDVA (3.5 mmol/L 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,[27] and two steps of C3 fixation with trypsin and a nickel-stabilized C3-convertase formed with purified factors B and D.[14,28] 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.[14,28] The amount and type of fixed C3 fragments on the EC3 was determined by assays for uptake of ^{125}I-labeled monoclonal anti-C3c, -C3g, and -C3d.[14,28] EC3 were prepared to contain 2.0 to 2.5 $\times$ 10^4 molecules of C3 per E. 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.[14]

Antibodies to C receptors, LFA-1, and NK cells. Rabbits were immunized with purified CR_1, and IgG $F(ab')_2$ fragments were prepared.[2] E11 monoclonal anti-CR_1[29] was kindly provided by Dr Nancy Hogg of the Imperial Cancer Research Fund, London. Monoclonal anti-CR_3 α-chain antibodies used were anti–Mac-1,[9,28,30] purchased from Hybritech Inc (San Diego); OKM1,[31] purchased from Ortho Diagnostics (Raritan, NJ); anti-Mol,[10,32] kindly provided by Dr M. Amin Arnaout of Harvard Medical School, Boston; and MN-41,[12] kindly provided by Dr Allison Eddy and Dr Alfred Michael of the University of Minnesota Medical School, Minneapolis. The TS 1/12 and TS 1/22 monoclonal antibodies specific for the α-chain of LFA-1 and TS 1/18 specific for the β-chain of CR_3/LFA-1/p150,95 were described previously.[8] Anti–Leu-7 (HNK-1)[33] was purchased from Becton Dickinson Co (Sunnyvale, Calif).

C receptor assays. C receptors were assayed by EC3 rosette formation[14,25,28] in either HBSS/1% BSA or BDVA. CR_1 and CR_3 on neutrophils were quantitated with ^{125}I E11 and ^{125}I MN-41, respectively.[29] To prevent Fc receptor binding of ^{125}I 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 ^{125}I 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. EA_{IgG}, prepared with sheep E and subagglutinating dilutions of rabbit IgG anti-sheep E antibody,[34] were suspended at 2×10^8/mL in BDVA and tested for rosette formation with neutrophils suspended at 4×10^6/mL in BDVA. One hundred microliters of EA_{IgG} was mixed with 100 μL of neutrophils in a 10 $\times$ 65-mm plastic tube and incubated on a rotator for 15 minutes at 37 °C. The EA_{IgG} preparation giving the highest proportion of rosettes was used for studies of phagocytosis. After formation of EA_{IgG} 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 EA_{IgG}. Two hundred cells were scored as having either 0, 1, 2, 3, or 4+ ingested EA_{IgG}, and the number of EA_{IgG} 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.[25] Pellets of 1×10^6 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 a 10 $\times$ 65-mm tube 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.[34]

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.[35,36] 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*),[37] or unopsonized heat-killed and FITC-labeled[38] *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 (3×10^9) and neutrophils (5 $\times$

10^5) 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, 5×10^5 neutrophils were treated with 5 μg of monoclonal IgG, 96 μg of $F(ab')_2$ anti-CR_1, or 100 μg of heat-aggregated human myeloma IgG_1 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 ^{51}Cr-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 (1×10^8) 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 ^{125}I-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 CR_3.

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 2[24] 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 CR_3[24] suggested that the patients' neutrophils might be deficient in the entire CR_3 antigen. Neutrophils, monocytes, and lymphocytes were examined for surface staining with OKM1 and other antibodies known to be specific for the α-chain of CR_3 (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[33] 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 CR_3 α-chain. To determine whether the glycoprotein missing from gels of the patients' neutrophils[24] was the α-chain of CR_3, SDS-PAGE gels of neutrophils were blotted onto nitrocellulose and tested for reaction with ^{125}I-MN-41 (Fig 1). A radioactive band with 150,000 estimated M_r 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 CR_3.

Assay of C receptors by EC3 rosette formation. It had been postulated that the Mac-1/OKM1/Mol/MN-41 antigen was CR_3 because anti-Mac-1,[9] anti-Mol,[10] and MN-41[12] each inhibited CR_3 activity and the isolated OKM1 antigen bound to EC3bi but not to EC3b.[11] Thus, the patients' neutrophils should have been deficient in CR_3 activity, and demonstration of this would provide additional confirmation of the identity of CR_3 with the Mac-1/OKM1/Mol/MN-41 antigen.

As described previously,[28] CR_3 cannot be specifically assayed by EC3bi rosette formation unless steps are taken to avoid EC3bi binding to CR_1 and CR_2. With normal neutrophils or monocytes, EC3bi were bound simultaneously by way of both CR_1 and CR_3; 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			
	Anti-Mac-1 (%)	Anti-Mol (%)	OKM1 (%)	Anti-Leu-7 (%)
Normal controls				
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.

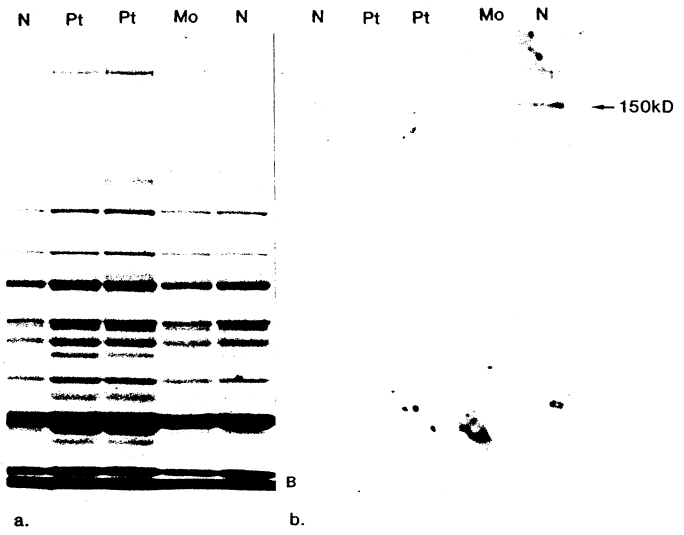

Fig 1. Immunoblotting and autoradiography of an SDS-PAGE gel slab with ^{125}I-MN-41 (anti-CR_3 α-chain). Panel A is a gel stained with Coomassie blue, and panel B is the autoradiogram of an immunoblot of a duplicate gel run in parallel. From left to right, the gel tracks contained normal neutrophils (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.[24]

rosettes required treatment with both anti-CR_1 and anti-CR_3 (Table 2). With lymphocytes, EC3bi were bound to CR_1 and CR_2 on B cells and to CR_3 on NK cells, so that anti-CR_1 and anti-CR_3 only inhibited EC3bi rosetting partially. The 10% of lymphocyte EC3bi rosetting in the presence of anti-CR_1 and anti-CR_3 was probably CR_2 dependent because assay of CR_2 with EC3dg[28] 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.[28]

With neutrophils from all three patients, the percentage of EC3bi rosettes was in the low normal range when assayed in isotonic HBSS and nearly all cells rosetted with EC3bi in 4 mS BDVA (Table 3). Abnormally, however, neutrophil EC3bi rosettes were inhibited completely by anti-CR_1 alone, demonstrating that EC3bi were bound only to CR_1 and that CR_3 activity was absent.

Monocytes from two of the three patients rosetted poorly with EC3bi in HBSS (Table 3) and resembled anti-CR_3–treated normal monocytes (Table 2). Monocytes from patient No. 3 did rosette normally with EC3bi in HBSS. As with neutrophils, all of the patients' monocyte rosettes with EC3bi 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 EC3b, EC3bi, and EC3dg. Patient No. 3 had an elevated proportion of CR_1-bearing lymphocytes (EC3b rosettes). Treatment of the patients' lymphocytes with anti-CR_1 alone reduced the proportion of EC3bi rosetting cells down to the same level as the EC3dg

Table 2. EC3 Rosette Formation With Isolated Normal Neutrophils, Monocytes, and Lymphocytes Tested at the Same Time as the Patients' Cells

Leukocyte Type	Rosette Formation With EC3b (%)	EC3bi (%)	EC3dg (%)
Neutrophils			
HBSS/BSA (14 mS)	85	90	ND
+ anti-CR_1†	0	81	ND
+ anti-CR_3‡	86	79	ND
+ anti-CR_1 and -CR_3	0	0	ND
BDVA (4 mS)	91	98	ND
+ anti-CR_1	0	96	ND
Monocytes			
HBSS/BSA (14 mS)	78	88	ND
+ anti-CR_1	0	83	ND
+ anti-CR_3	77	28	ND
+ anti-CR_1 and -CR_3	0	0	ND
BDVA (4 mS)	90	98	ND
+ anti-CR_1	0	96	ND
Lymphocytes			
BDVA (4 mS)	26	23	10
+ anti-CR_1	0	18	10
+ anti-CR_1 and -CR_3	0	10	10

ND, not done.

*Anti-CR_1: rabbit F(ab')$_2$-anti-CR_1; 82 μg per 5 × 10^5 cells.

†Anti-CR_3: anti-Mac-1 or Mn-41; 1 μg per 5 × 10^5 cells.

Table 3. EC3 Rosette Formation With Isolated Neutrophils, Monocytes, and Lymphocytes From Patients No. 1, 2, and 3

Leukocyte Type	Rosette Formation With EC3b (%)	EC3bi (%)	EC3dg (%)
Neutrophils			
HBSS/BSA (14 mS)	85	82	ND
+ anti-CR_1*	0	0	ND
BDVA (4 mS)	98	98	ND
+ anti-CR_1	0	0	ND
Monocytes			
HBSS/BSA (14 mS)	77	(16, 15, 98)	ND
+ anti-CR_1	0	0	ND
BDVA (4 mS)	90	89	ND
+ anti-CR_1	0	0	ND
Lymphocytes			
BDVA (4 mS)	(23, 18, 34)	(12, 14, 38)	11
+ anti-CR_1	0	11	11

The results from all three patients were averaged unless they were significantly different. In these cases, the results from patients No. 1, 2, and 3 are listed separately in parentheses. Each assay was performed at least two times. ND, not done.

*Anti-CR_1: rabbit F(ab')$_2$-anti-CR_1; 82 μg per 5 × 10^5 cells.

rosetting cells (11%), suggesting that EC3bi were bound only to CR_1 and CR_2 and that CR_3 was missing from the patients' lymphocytes.

Quantitation of neutrophil CR_1 and CR_3. CR_1 was quantitated with ^{125}I E11 monoclonal anti-CR_1 (Table 4). Neutrophils from normal volunteers and normal family members expressed 2.5 to 4.5 $\times$ 10^4 CR_1 per cell, whereas neutrophils from the three patients expressed somewhat higher amounts of CR_1 per cell (Table 4). Even though IgG, rather than $F(ab')_2$, E11 was used, binding of the ^{125}I E11 to Fc receptors was negligible, as there was no inhibition of ^{125}I E11 uptake by amounts of heat-aggregated IgG.

Neutrophil CR_3 was quantitated with ^{125}I-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 $\times$ 10^4 ^{125}I MN-41 molecules per cell, but this was reduced to 6.5 $\times$ 10^4 molecules per cell after incubation with heat-aggregated IgG, suggesting that some binding of the ^{125}I MN-41 was Fc receptor dependent. When neutrophils from the three patients were examined in the presence of heat-aggreated IgG, only 5,500, 8,500, and 7,800 molecules of ^{125}I 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 CR_3 than did normal individuals. However, in family No. 2, normal family members had 48% to 75% (average 63%) of the normal amount of CR_3 per neutrophil.

Deficiency of LFA-1 α-chain and common β-chain. The expression of LFA-1 α-chain and the β-chain shared by the CR_3/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 CR_3 α-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 α-chain and common β-chain. With lymphocytes from patients No. 1 and 2, <10% of the normal amounts of LFA-1 or β-chain was detectable. Likewise, the B lymphoblasts from patient No. 3 had <10% of the normal amount of LFA-1 and β-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_{IgG}, EC3b, and unopsonized yeast (Table 6). Phagocytosis of EA_{IgG} by the neutrophils from patient No. 3 was equivalent to the normal control, whereas monocyte phagocytosis of EA_{IgG} was 65% of normal. EC3b are not

Table 4. Quantitation of CR_1 and CR_3 on Neutrophils From Patients and Their Families With ^{125}I Monoclonal Anti-CR_1 and Anti-CR_3

	Molecules per Neutrophil of ^{125}I	
	Anti-CR_1 (E11)	Anti-CR_3 (MN-41)
Family No. 1		
Patient No. 1	59,700	5,487
Patient No. 2	50,200	8,500
Normal brother	33,000	57,500
Mother	28,100	51,500
Father	34,900	65,500
Normal control	35,000	67,000
Family No. 2		
Patient No. 3	72,000	7,800
Normal sister	60,300	34,600
Normal sister	53,200	31,300
Normal brother	65,600	42,000
Mother	58,900	48,000
Father	56,400	49,100
Normal control	45,000	63,000

Assays of patients and their entire families were formed only once.

Table 5. Immunofluorescence Cytofluorographic Analysis of CR_3 and LFA-1 on Neutrophils, Monocytes, and Lymphocytes from Patients No. 1 and 2

	Median Fluorescence Channel		
	Anti-CR_3 α-Chain	Anti-LFA-1 α-Chain	Anti-β-Chain
Cell Type	OKM1	TS 1/12	TS 1/18
Neutrophils			
Patient No. 1	0	4	60
Patient No. 2	8	40	52
Normal brother	184	144	400
Father	172	132	408
Mother	212	128	432
Normal control	408	104	544
Monocytes			
Patient No. 1	0	0	0
Patient No. 2	8	28	20
Normal brother	256	412	688
Father	212	356	564
Mother	312	536	800
Normal control	312	420	740
Lymphocytes			
Patient No. 1	ND	20	12
Patient No. 2	ND	12	24
Normal control	ND	924	924

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.

Table 6. Phagocytosis of EA_{IgG}, EC3b, and Unopsonized Yeast by Neutrophils and Monocytes From Patient No. 3

	Ingested Particles per 100 Phagocytes		
	EA_{IgG}	EC3b	Yeast
Normal control			
Neutrophils	285	Not ingested	387
Monocytes	370	Not ingested	376
Patient No. 3			
Neutrophils	286	Not ingested	10
Monocytes	242	195	27

Cells from patient No. 3 were tested two times for phagocytosis, and the slides were read by three individuals.

usually ingested significantly by freshly isolated peripheral blood monocytes,[40] 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% 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).

CR_3-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-CR_3 and anti–LFA-1, whereas the ingestion of *S aureus* was either unaffected or enhanced by anti-CR_3 and anti–LFA-1. The inhibitory effects of anti-CR_3 and anti–LFA-1 on *S epidermidis* ingestion were additive, since a mixture of anti-CR_3 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).

Table 7. Inhibition of Normal Neutrophil Phagocytosis of *S Epidermidis* but not *S Aureus* by Treatment With Monoclonal Anti-CR_3 and Anti–LFA-1

Antibody Treatment of Neutrophils	PMN Ingesting (%)	Inhibition of Ingestion
S epidermidis		
None	55 ± 7*	—
Anti-CR_3		
MN-41	44 ± 6	20
Anti–Mac-1	40 ± 7	27
Anti–LFA-1: TS 1/22	35 ± 9	36
Anti-CR_3 + anti–LFA-1		
MN-41 + TS 1/22	22 ± 6	60
Anti–β-chain: TS 1/18	14 ± 5	75
Anti-CR_1: E11	55 ± 9	0
Heat-aggregated IgG	68 ± 15	0
S aureus		
None	67 ± 21	—
Anti-CR_3		
MN-41	76 ± 8	0
Anti–LFA-1: TS 1/22	70 ± 9	0
Anti-CR_3 + anti–LFA-1		
MN-41 + TS 1/22	81 ± 9	0
Heat-aggregated IgG	82 ± 14	0

*Results represent the means ± SD of four or more tests.

DISCUSSION

Three patients with a history since infancy of bacterial skin infections were shown to have an apparent genetic deficiency of CR_3 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 CR_3, LFA-1, and p150,95. The absence of CR_3 activity was correlated with the absence of the 165,000 M_r surface glycoprotein recognized by monoclonal antibodies that inhibit CR_3 activity (anti–Mac-1, anti-Mol, and MN-41). The present findings with these patients' cells, considered together with previous data on the inhibition of CR_3 activity with these or similar antibodies[8–13] and the isolation of functional CR_3 with OKM1,[11] provide further evidence that CR_3 is the same as the antigen recognized by these antibodies. Tests of the isolated CR_3-deficient neutrophils and monocytes revealed nearly absent ability to phagocytose bakers' yeast, confirming other studies with anti-CR_3–treated normal phagocytes, which indicated that CR_3 was a yeast receptor as well as an iC3b receptor.[14] However, because the patients had recurrent infections with bacteria and not with yeast, it was significant that tests of normal neutrophils treated with monoclonal anti-CR_3 and anti–LFA-1 suggested that both CR_3 and LFA-1 might have important roles in the phagocytosis of *S epidermidis*.

The three patients described herein are now recognized to be similar or identical to several other patients previously described who had deficiencies of neutrophil surface glycoproteins in the 110,000 to 229,000 M_r range and functional deficiencies in either phagocytosis and respiratory burst[19,20] or adherence and chemotaxis.[16–18,21] 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 CR_3) 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[24] appeared to be similar to a case of "gp150" deficiency reported earlier by Arnaout et al.[19] In both sets of patients, phagocytosis of EA_{IgG} 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_r 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[19] 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 CR_3 deficiency was the absence of surface staining with anti–Mac-1, anti-Mol, and OKM1 (Table 3).[20] Finally, immunoblotting of SDS-PAGE gel slabs of neutrophils from patients No. 1 and 2 demonstrated that the α-chain of CR_3 was undetectable, and that the position of the missing glycoprotein corresponded exactly to the position of CR_3 α-chain.

Because EC3bi are not bound exclusively to CR_3, the CR_3

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 CR_1 and CR_3 simultaneously.[28] Monocytes may have less CR_1 per cell than do neutrophils, as EC3bi binding to monocytes is primarily CR_3 dependent. B lymphocytes bind EC3bi to both CR_1 and CR_2, whereas NK cells bind EC3bi primarily to CR_3 because they lack significant amounts of CR_1[41] and CR_2.[42] The CR_3 deficiency was demonstrated by anti-CR_1 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 CR_2-dependent EC3dg rosettes.

Blockade of CR_3 function by anti–Mac-1 was the first suggestion that CR_3 and Mac-1 were identical.[9] However, it was subsequently reported that the same molecule recognized by anti-Mol was not CR_3, but a closely associated antigen involved in Fc-mediated functions as well as iC3b-mediated functions.[10,43] Neutrophils from a Mol-deficient patient formed rosettes with EC3bi that were not inhibited by saturating amounts of anti-CR_1.[20] However, subsequent analysis of this patient's cells with ^{125}I Fab–anti-Mol revealed small amounts of Mol (CR_3) that were not detected previously by immunofluorescence.[20] This small number of CR_3 explained the EC3bi rosettes observed in the presence of anti-CR_1, as treatment of the Mol-deficient neutrophils with a mixture of anti-CR_1 and anti-Mol inhibited EC3bi rosetting completely.[20] Further evidence for the identity of Mol and CR_3 came from studies that demonstrated that the isolated Mol glycoprotein bound to EC3bi but not to EC3b.[11] Thus, it was concluded that the antigen recognized by anti-Mol, as well as by anti–Mac-1, MN-41, and OKM1, is CR_3.

Other studies excluded a direct role of CR_3 in Fc receptor-mediated functions. The reduced ingestion of EA_{IgG}[19] or $EA_{IgG}C$[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 Fc-mediated ingestion was noted in the patients identified by Anderson et al.[21] Further insights came from studies of normal neutrophils treated with monoclonal anti-CR_3. Inhibition of EA_{IgG} ingestion was observed only with EA_{IgG} bearing small amounts of IgG[14,20] and did not occur in buffers containing either EDTA or *N*-acetyl-D-glucosamine that inhibit the iC3b binding site of CR_3.[14] The data suggested the possibility that CR_3 might bind weakly to some component of the sheep erythrocyte membrane and thereby synergistically enhance the Fc receptor-mediated ingestion of EA_{IgG}.[14]

Quantitation of neutrophil CR_1 and CR_3 demonstrated that the three patients had amounts of CR_1 in the high normal range and 8% to 12% of the normal amount of CR_3. In the healthy members of family No. 1, the CR_3 number appeared to be normal by radioimmunoassay, but only half-normal amounts of CR_3 were detected by cytofluorographic analysis. In family No. 2, neutrophil CR_3 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.[23] In those studies it was shown that heterozygous parents and siblings could be identified by half-normal expression of CR_3 when neutrophils were assayed after stimulation with F-Met-Leu-Phe that causes full expression of the latent cytoplasmic pool of CR_3. 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 $\alpha_1\beta_1$ complexes into the membrane.[23]

One major finding was that CR_3-deficient neutrophils and monocytes were virtually unable to phagocytose unopsonized yeast. This did not result from some generalized phagocytic defect, as neutrophils from patient No. 3 exhibited avid Fc receptor-mediated ingestion of EA_{IgG}, and his monocytes exhibited CR_1-mediated ingestion of EC3b. This latter finding presumably indicates in vivo activation of monocytes, as unactivated monocytes do not ingest EC3b.[40] Thus, with the assays used, CR_3 deficiency had little or no effect on ingestion mediated by Fc receptors or CR_1 and was selective for the absence of yeast ingestion. Recent studies have confirmed the role of CR_3 in the phagocytosis of yeast by demonstration that treatment of normal neutrophils with anti-CR_3 (anti–Mac-1, anti-Mol, OKM1, or MN-41) produced 70% to 90% inhibition of yeast binding, ingestion, and respiratory bursts.[14] Thus, CR_3 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 CR_3 or p150,95. This is because antibodies to the α-chain of LFA-1 inhibit normal NK activity,[13,15] whereas antibodies to the α-chain of CR_3 have no effect on NK activity.[30] Although NK activity appeared normal in the Mol-deficient patient,[20] the patients in Texas were deficient in NK activity.[22]

CR_3 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.[25] However, the function of LFA-1 on phagocytic cells is unknown. Because the CR_3/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 CR_3 and LFA-1 subunit-specific monoclonal antibodies have demonstrated inhibition of adherence functions[44] as well as phagocytic and respiratory functions.[10,14] The present study showed that both anti-CR_3 and anti–LFA-1 inhibited normal neutrophil phagocytosis of unopsonized *S epidermidis*. By contrast, previous studies[14] showed that the same TS 1/22 anti–LFA-1 did not inhibit yeast ingestion. The finding that a mixture of anti-CR_3 and anti–LFA-1 produced more inhibition than did either antibody by itself suggests that CR_3 and LFA-1 may each participate in binding to different surface components of *S*

epidermidis. In addition, monoclonal anti–β-chain (TS 1/18) consistently produced more inhibition of *S epidermidis* ingestion than did either anti-CR_3 or anti–LFA-1 alone. This was unexpected, as TS 1/18 does not inhibit either EC3bi rosettes or yeast binding to CR_3.[14] It was subsequently found, however, that TS 1/18 did inhibit CR_3-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.[15] 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-CR_3 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.[45] Recently, however, when a $F(ab')_2$ fragment of MN-41 anti-CR_3 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 CR_3 and LFA-1 to bind to bacteria. However, the data suggest a restricted specificity for CR_3 in adhesive reactions that includes EC3bi, bakers' yeast, and *S epidermidis* but not EC3b or *S aureus*. Finally, even though CR_3 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 CR_3 deficiency will be facilitated in the future with recognition that several available monoclonal antibodies are specific for CR_3 (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.

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