Defect of a Complement Receptor 3 Epitope in a Patient with Systemic Lupus Erythematosus

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Abstract

Complement receptor 3 (CR3) is expressed on cells of the reticuloendothelial system and involved in the clearance of immune complexes. In this article a patient with a deficiency of the C3bi binding site of this receptor is described. Clinically this patient exhibited predominantly cutaneous manifestations of a systemic lupus erythematosus with an immune vasculitis and panniculitis.

The deficiency of the CR3 epitope was demonstrated using flow cytometry. The functional relevance of this defect was demonstrated in a rosetting assay with C3bi-loaded erythrocytes. C3bi binding was found to be significantly decreased. Furthermore, there was an impairment of phagocytosis of opsonized *Escherichia coli*. The CR3 defect is not due to an autoantibody but is assumed to have a genetic basis. These data suggest that the defect of the CR3 may be involved in the pathogenesis of the immune vasculitis in this patient. (*J. Clin. Invest.* 1993. 92:1181–1187.) Key words: complement receptor 3 • systemic lupus erythematosus • vasculitis • immune complexes • clearance

Introduction

SLE is associated with a decreased clearance of immune complexes (1). Immune complexes appear to contribute to a major extent to the pathophysiology of systemic vasculitis by activating Fc receptor and complement receptor–positive mononuclear cells (2, 3). A decreased solubilization of immune complexes is suggested as one of the most important factors leading to pathogenic concentrations of complexes. C2 and C4 deficiencies which may be responsible for decreased immune complex solubilization have been observed in increased frequency in patients with SLE (4–6). Also, in addition to genetic and environmental factors, cell surface receptors responsible for the clearing of immune complexes are likely to represent one of the factors contributing to this autoimmune disease (7).

Clearance of immune complexes is mediated in part by complement receptors that are expressed on erythrocytes (CR1)[1] and cells of the reticuloendothelial system in liver and spleen (CR3, CR4). These receptors participate in the transport, recognition and uptake of circulating immune complexes into these cells.

In this article, we report on a patient with SLE exhibiting a complement receptor 3 (CR3) deficiency. The defect is demonstrated by a lack of expression of the critical C3bi binding epitope and by a defective function of the receptor. Our results suggest that the lack of this particular C3bi binding epitope defines a new pathogenetic entity contributing to the pathogenesis of SLE.

Case report. A 38-yr-old male Turkish patient with discoid facial lesions was first seen in the immunological outpatient clinic of the Medizinische Hochschule Hannover in 1981. A serological investigation revealed antinuclear, anti-dsDNA antibodies, anti-Sm and RNP antibodies. The disease was diagnosed as a discoid lupus erythematosus. In 1986 he developed several episodes of arthralgias, Raynaud's phenomenon, a panniculitis and a vasculitis, predominantly affecting the distal extremities. Histologically the vasculitis was characterized by pericapillary predominantly lymphocytic infiltrates. In addition a photosensitivity and a malar rash were noted. At this time the diagnosis was changed to systemic lupus erythematosus because the patient fulfilled six American Rheumatism Association criteria (8). During acute vasculitic episodes acute phase proteins were elevated, complement levels (C3, C4, CH50) were decreased, and a mild proteinuria was observed. Elevated immune complex levels were detected in a radial immunodiffusion assay. Flares of the vasculitis required treatment with high doses of steroids and azathioprine.

In 1975 the patient had suffered from tuberculosis. Antibodies against hepatitis A and B virus indicated earlier infections with both viruses. His family history did not reveal any particular susceptibility to infections.

Methods

Monoclonal antibodies. Antibodies LPM19C, 14B6.E2, VIM12, TMG6-5, and MN41, recognizing the CD11b (CR3) antigen, were taken from the antibody panel of the IVth International Workshop for Leucocyte Typing (9). Additional CD11b antibodies (mAb 94 [monocyte (Mo) 1], mAb 17, defining epitope 17, and mAb 44), directed against different epitopes of the CR3, have been described in detail previously (10). To5 binds to CR1 (CD35) (11). B2 defines the CR2 (CD21) and B-Ly6 the CR4 (CD11c) (9, 12). MHM23 (CD18) reacts with the common β-chain of CD11a, CD11b, and CD11c (9). 2F12 binds to CD11a (9, 13).

Cell preparation and phenotypic studies. PBMC were isolated from the interphase of a Ficoll-Isopaque gradient (Biochrom, Berlin, Germany) (14). Granulocytes (PMN) were purified from the pellet by

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1. *Abbreviations used in this paper:* CR, complement receptor; IC, immune complexes; Mo, monocyte.

incubation in 5% hydroxyethylstarch for 60 min. The supernatant was centrifuged and erythrocytes lysed by incubation in distilled water for 60 s.

Cell surface antigens were determined using indirect immunofluorescence as previously described in detail (15). 100 μl of primary antibodies were applied as ascites in dilutions of 1:200 in PBS. Analysis was performed by flow cytometry on a FACScan® (Becton Dickinson and Co., Heidelberg, Germany) counting 10,000 cells per sample. PBL and monocytes were separately investigated by gating on the different cell populations.

To block the epitope 17 with a putative autoantibody 10^6 granulocytes of two healthy donors were incubated for 30 min at 4°C with either 1 ml serum or 1 ml immunoglobulin (5 μg/ml) obtained from sera of the patient or a control SLE patient using a protein G column. Expression of epitope 17 and the CD11b control epitope 44 was determined as described above.

Binding of patient's serum to recombinant CR3 expressed in COS cells was kindly performed by Dr. M. A. Arnaout (Massachusetts General Hospital, Boston) (16). For the activation studies monocytes isolated from the interphase of a Ficoll-Hypaque gradient (Biochrom) were incubated for 15 min at 37°C in PBS supplemented with PMA at a concentration of 0.1 μg/ml or in PBS alone. Cell surface expression of CD11b epitopes was determined as described above.

Furthermore, PMN of the patient were washed three times in 0.9% NaCl acidified with HCl to pH 4.0 or in 0.9% NaCl alone. After washing the viability of the cells was determined using trypan blue staining. Viability was > 70% in three assays performed. Subsequently the expression of epitope 17 and epitope 44 as a control were examined using flow cytometry.

Generation of cell lines. Lines of peripheral blood lymphocytes containing 30% CD16+ natural killer cells were cultured as previously described (17). Briefly, peripheral blood cells were isolated using a Ficoll-Hypaque gradient. 10^5 of these cells were plated in 96-well V-bottomed plates on a feeder layer of 5×10^4 EBV-transformed B cells and 5×10^4 peripheral blood lymphocytes per well. Culture medium was RPMI 1640 supplemented with 10% IL-2 containing lymphocyte-conditioned medium (18).

Rosetting assays. C3b-loaded sheep erythrocytes were prepared as previously described (19, 20). Erythrocytes loaded with C3bi were obtained by incubating 10^8 C3b-loaded sheep erythrocytes with 50 μg of complement proteins Factor H and 4 μg Factor I in 1 ml of dextrose-gelatin-veronal buffer supplemented with magnesium and calcium for 1 h at 37°C. For rosette assays, 10^6 PMN in 100 μl PBS-BSA were incubated for 30 min at 4°C with 10^7 erythrocytes loaded with C3bi in an equal volume. In parallel PMN were preincubated for 30 min with mAb 17 in ascitic fluid diluted 1:50 at 4°C and used in the assay as described above. The percentage of PMN binding three or more erythrocytes was blindly assessed by two independent observers using a microscope at a magnification of 640.

Phagocytosis. Freshly isolated PMN were either incubated for 30 min in PBS alone, in PBS containing 2E1 (CDw32) and 3G8 (CD16) antibodies in a dilution of 1:50, or in PBS containing mAb 17 in ascitic fluid in a dilution of 1:50. Phagocytosis was determined using the commercially available Phagotest® (Orpegen, Heidelberg, Germany). Briefly, PMN were washed three times in RPMI 1640, then 2×10^5 cells were incubated with 20×10^6 FITC-labeled *Escherichia coli* in a total volume of 120 μl RPMI 1640 for 10 min at 37°C in a shaking water bath. *E. coli* that were not phagocytosed were quenched at 0°C. The percentage of PMN with FITC-labeled *E. coli* ingested was determined using a FACScan® (Becton Dickinson and Co.).

Granulocyte adherence. Adherence was tested according to a previously published method (21). 1 ml of heparinized blood was filtered through nylon wool in pasteur pipette at room temperature. The percentage of adherent PMN was calculated as follows: (PMN in original sample − PMN in effluent sample/PMN in original sample) × 100.

Immunohistology. A subcutaneously located skin nodule on the upper arm of the patient with the typical histology of lupus panniculitis and lesions of three other patients with chronic discoid lupus erythematosus as controls were biopsied after informed consent and part of the material was snap frozen. Cryostat sections (6 μm) were stained using the indirect immunoperoxidase method with 3-amino-9-ethylcarbazole as a chromogen. Primary antibodies were Mol (CD11b), mAb 44 (CD11b), mAb 17 (CD11b), B-Ly6 (CD11c), and 5E5, a control antibody of the same isotype but irrelevant specificity. All antibodies were applied in a dilution of 1:100. The secondary antibody was a peroxidase-conjugated goat anti-mouse IgG F(ab')₂ (Dianova, Hamburg, Germany).

Detection of immune complexes. Circulating immune complexes (IC) were determined in a commercially available radial immunodiffusion assay (Freka®-CIC-test; Fresenius, Bad Homburg, Germany). Briefly, IC were precipitated from serum in 2.0% polyethylene glycol, resuspended, and washed in an NaCl-barbiturate buffer with 2% polyethylene glycol and finally transferred on plates coated with antibodies against C1q, C3c, IgG, IgM, and IgA. Staining of the precipitates was performed with Coomassie blue after 48 h of incubation.

Immunoprecipitation. 2×10^7 PMN were surface labeled with 1 mCi of ^{125}J in 480 μl PBS and 20 μl lactoperoxidase (2 mg/ml). Cells were lysed in 500 μl 0.5% NP-40 plus 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, and protease inhibitors. Lysates were precleared using CNBr-activated Sepharose beads (Pharmacia Fine Chemicals, Uppsala, Sweden) coupled with an irrelevant (rabbit anti-mouse) antibody and incubated overnight at 4°C with Sepharose beads coupled with CD11b antibodies 44 and mAb 17. Immunoprecipitates were washed four times and resolved by SDS-polyacrylamide gel electrophoresis analysis using a 10% polyacrylamide gel under reducing conditions (22).

Northern blot analysis. Total cellular RNA was prepared from PBL or PMN of the patient and a healthy control. 20 μg of denatured RNA
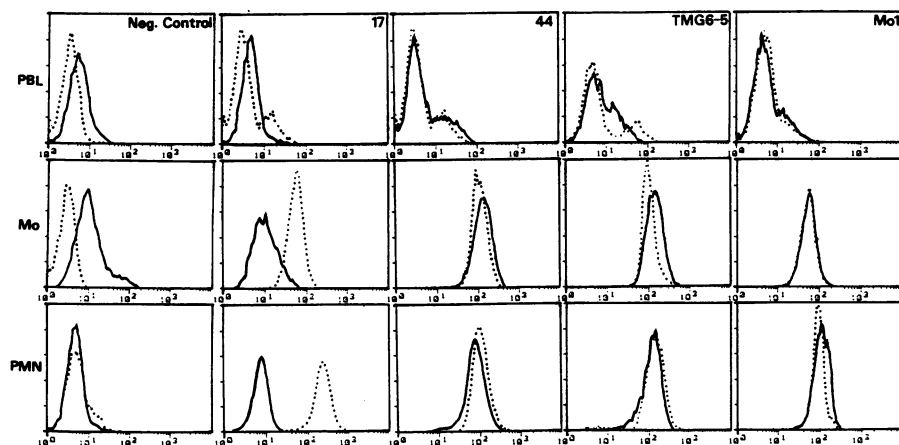

Figure 1. Expression of CD11b antigens 17, 44, TMG6-5, and Mol on cells of the patient (——) and a healthy control (· · ·). The phenotype of PBL, monocytes, and PMN is demonstrated. The patient's cells were negative using mAb 17, whereas seven other antibodies revealed normal expression of their respective CD11b epitopes. mAb 44, Mol, and TMG 6-5 are shown here as representative examples for a large panel of CD11b antibodies tested. FACS® histograms of SLE control patients were identical to the healthy control. Histograms are shown after FACScan® analysis of 10,000 cells. Fluorescence intensity is shown in a logarithmic scale on the x axis, cell number in a linear scale on the y axis.
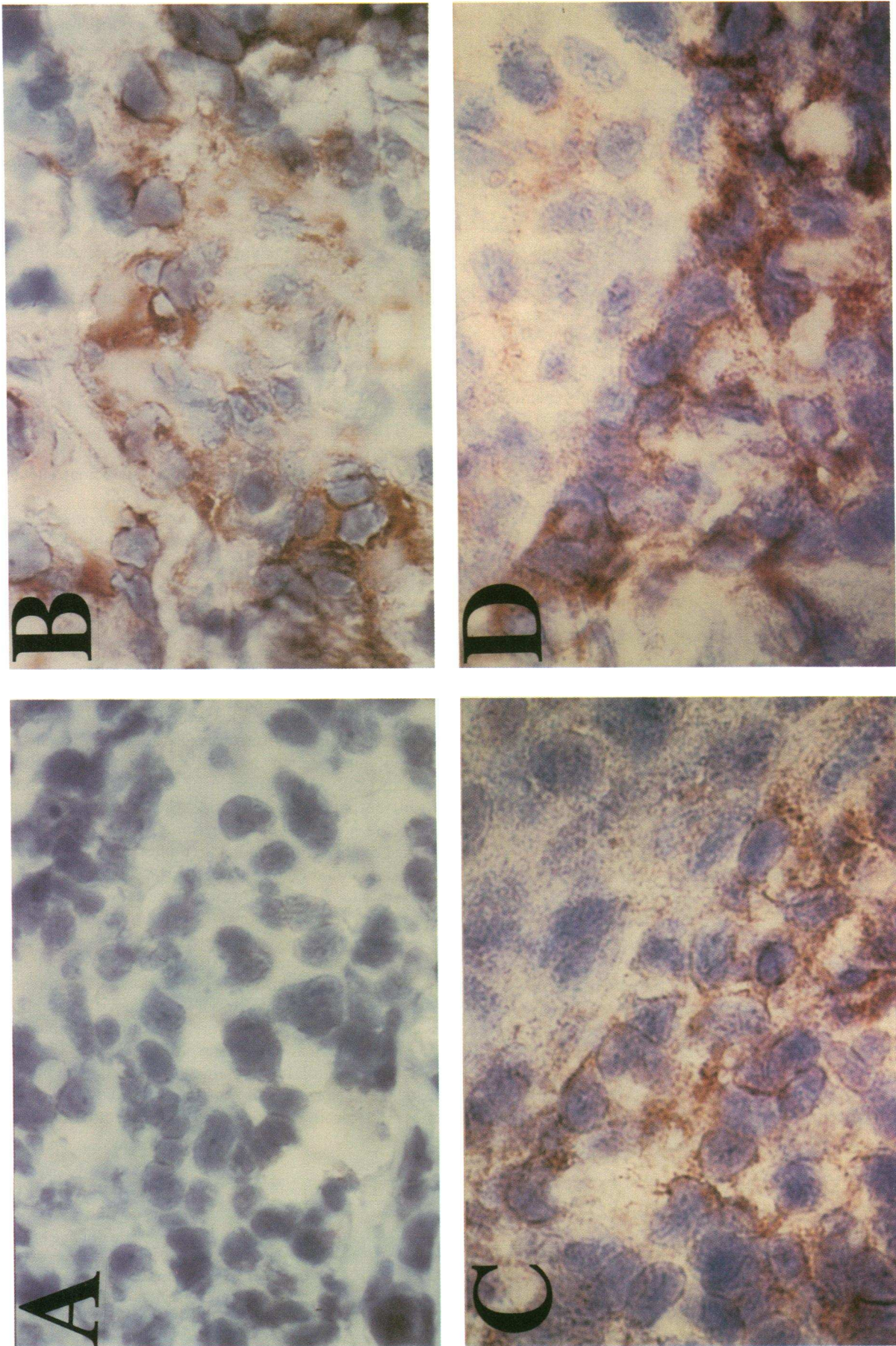
Figure 2. Immunohistology of the skin. Macrophages of skin lesions of the patient did not react with mAb 17 (*A*); in contrast CD11b antibody 44 (*B*) revealed a positive staining. Normal expression of both epitope 17 (*C*) and 44 (*D*) on macrophages close to a sweat duct is shown in one representative control patient with chronic discoid lupus erythematosus.
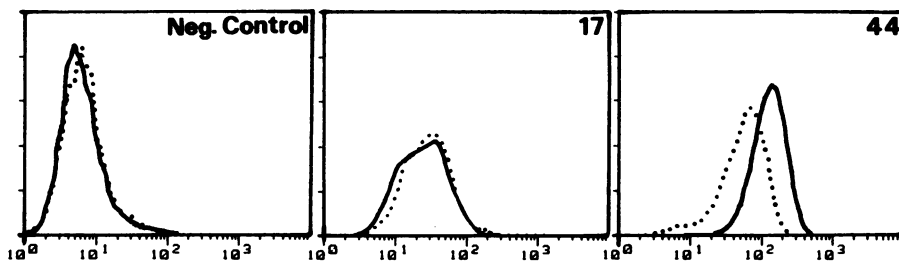
Figure 3. Activation of monocytes of the patient with PMA. Increased expression of the CD11b epitope 44 after incubation in PBS + PMA 0.1 μg/ml (——) compared with incubation in PBS alone (· · ·) revealed mobilization of intracellular CR3 stores that were expressed on the cell surface after activation. In contrast, epitope 17 was still not expressed.

from each sample was separated using a 1% agarose gel electrophoresis and subsequently bound onto a nylon membrane. Hybridization of filters was performed using cDNA probes for CD11b and CD11a (Mac-1 and pLar, kind gift of Dr. Timothy Springer, Harvard Medical School [24]) labeled with ^{32}P to a sp act of 10^9 cpm/μg. After extensive washing the filters were autoradiographed.

Sequencing of the L-domain of CD11b. Total cytoplasmic RNA was extracted from PBL or PMN of the patient and a healthy control by the guanidium/cesium chloride method (23). Oligo dT primed reverse transcription was performed with 1 μg of total cytoplasmic RNA in a 20-μl vol using 200 U reverse transcriptase (Moloney murine leukemia virus reverse transcriptase; BRL, Eggenstein, FRG) in a standard procedure (23).

A 0.7-kb fragment corresponding to bases 474–1,200 of published CD11b cDNA (24) which spans the "L-domain" was amplified by PCR. PCR conditions were as follows: 20 pmol of each primer (upstream: 5'-ccagaagttcccagaggccc-3', downstream: 5'-ccaccgcccagtca-tagctc-3'), 200 μM dNTP, 2 U Vent polymerase, and 1 μl of the reverse transcriptase reaction in 100 μl 1× Vent buffer (New England Biolabs, Schwalbach, FRG) were denatured (300 s/95°C) followed by 40 cycles 60 s/95°C, 80 s/54°C, 90 s/75°C, and a final extension of 120 s/75°C. PCR fragments were then gel purified on a 1.5% agarose gel, phosphorylated using 8 U polynucleotide kinase (Boehringer, Mannheim, FRG) under standard conditions (23) and cloned in both orientations into the EcoRV site of the pBluescript KS (+) vector (Stratagene, Heidelberg, FRG). Six clones derived from two different PCRs were sequenced in both strands using a sequencing kit (Amersham, Braunschweig, FRG).

Results

A CR3 defect is present on all CD11b+ cells. Granulocytes (PMN), Mo, and PBL of the patient and 20 controls with systemic lupus erythematosus were tested for the expression of the C3bi receptor using various CD11b antibodies. The C3bi binding site of CR3, defined by monoclonal antibody 17, was not expressed on PBL, Mo, and PMN of the patient (Fig. 1). In contrast, seven other CD11b epitopes tested were present in normal density (Fig. 1). Control cells exhibited normal expression of all CR3 antigens including epitope 17 (Fig. 1). This suggested a defect of the C3bi receptor on cells of this patient involving the C3bi binding site. CD11a and CD11c, sharing a common β-chain (CD18) with CD11b, and CD18 itself were

normally expressed (data not shown). The increased intensity of autofluorescence of the patient's PBL and monocytes shown by the negative control antibody may reflect an activated state of these cells in comparison to the normal control.

The same receptor defect could also be demonstrated on tissue sections of a skin biopsy. CD11b+ cells in a skin lesion of the patient did not bind mAb 17 (Fig. 2 *A*). In contrast, the CD11b antibody 44 showed a normal staining pattern (Fig. 2 *B*). CD11b+ cells in skin lesions of three control patients with chronic discoid lupus erythematosus (Fig. 2, *C* and *D*) and four control patients with systemic lupus erythematosus (not shown) expressed both the epitope 17 and 44.

The defect is apparently not due to autoantibodies. Since in SLE and in autoimmune mice various autoantibodies against cell surface antigens have been described, the presence of autoantibodies blocking epitope 17 had to be ruled out (25–27). In the presence of an autoantibody in the serum, decreased expression of the epitope 17 should have been observed. Therefore normal granulocytes were incubated with both serum and purified immunoglobulin (5 μg/ml) of the SLE patient and four healthy controls for 30 min. Expression of epitope 17, however, was not altered after either incubation (data not shown). The patient's serum was also tested for its effect on binding of fluoresceinated EC3bi to recombinant CR3 expressed in COS cells, but again no difference was observed between the patient's serum and a heat-inactivated control serum (data not shown). Furthermore, PMN of the patient were washed three times in acidic buffer (pH 4.0) to elute a putative autoantibody from the cell surface. However, flow cytometry revealed still no expression of the epitope 17 even after this procedure (data not shown).

In addition, monocytes of the SLE patient were activated using PMA. Expression of the CR3 epitope 44 was enhanced after activation by mobilization of intracellular CR3 stores (10, 28, 29). In contrast, monocytes of the patient did still not express epitope 17 (Fig. 3). Therefore, the patient did not appear to have intracellular CR3 stores expressing epitope 17.

As a final control, lines obtained from peripheral blood of the patient and a healthy control person were cultured for four months. Here again epitope 17 was not expressed on the pa-
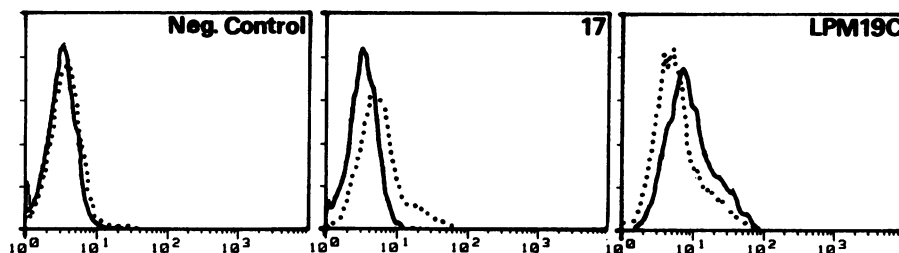

Figure 4. Phenotype of a cell line of the CR3-deficient patient after culture of 4 mo. There was no expression of epitope 17 in the patient (——); in contrast the CD11b control antibody LPM19C detected 30% CR3+ cells. A control line (· · ·) expressed both epitope 17 and LPM19C.
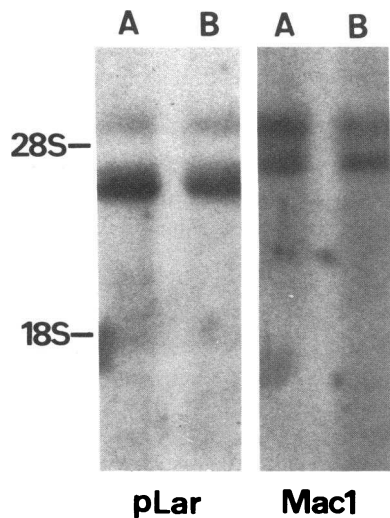
Figure 5. Northern blot analysis of PMN of the patient (*A*) and a healthy control (*B*) using a CD11b (Mac1) and CD11a (pLar) cDNA (24). No gross alteration of the patient's CD11b message was observed.

pLar   Mac1

Figure 7. PMN rosetting with C3bi- and C3b-loaded erythrocytes. A representative example of three independent assays is shown. Normal control cells formed 69% rosettes with C3b (■) and 45% with C3bi (▨). Preincubation with mAb 17 blocked the formation of rosettes with C3bi almost completely (□). In contrast, the patients' PMN also formed 65% rosettes with C3b (■), but only 2% with C3bi (▨). Preincubation with mAb 17 (□) did not further influence rosette formation with C3bi. Rosetting was performed as described in Methods.

tient's PBL although 30% of the cells were CD11b+ (Fig. 4). Thus, the presence of autoantibodies was considered unlikely.

Northern blot analysis and immunoprecipitation with mAb44 as primary antibody revealed no gross alteration of the patient's CR3 and the encoding RNA in comparison to normal controls (Figs. 5 and 6). In the immunoprecipitation with mAb 17 no signal was obtained (Fig. 6).

Sequencing of cDNAs spanning the putative C3bi binding region showed no mutation in the patient in comparison to a healthy control and published data (data not shown) (24).

Family studies. To obtain a family study, expression of epitope 17 and CD11b control epitopes was examined in the patient's brother and daughter. Expression of epitope 17 was normal on PMN of the brother but reduced to ~ 50% per cell on PMN of the daughter in comparison to 30 normal controls as determined by flow cytometry (data not shown). The parents could not be examined.

Functional consequences of the CR3 defect. To test whether the defect in the C3bi binding site leads to functional consequences binding of the ligand C3bi to its receptor was examined in a rosetting assay. PMN of the patient and controls were incubated with erythrocytes loaded with C3bi as described above. Only 2% of the patient's PMN formed rosettes compared with 45% of a control (Fig. 7). C3bi rosetting could be decreased to 2% in controls by preincubation of the cells with mAb 17, demonstrating that mAb 17 interferes with the C3bi binding site. In contrast, rosette formation of the patient's PMN was not altered by addition of mAb 17 (Fig. 7).
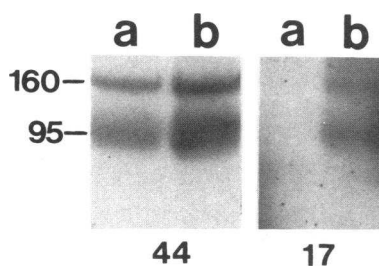

Figure 6. Immunoprecipitation from PMN of the patient (*a*) and a normal control (*b*) using mAb44 and mAb17 as precipitating antibodies. Precipitation with mAb 44 revealed a normal size of the patient's CD11b (160 kD), whereas mAb17 did not precipitate the patient's CD11b.
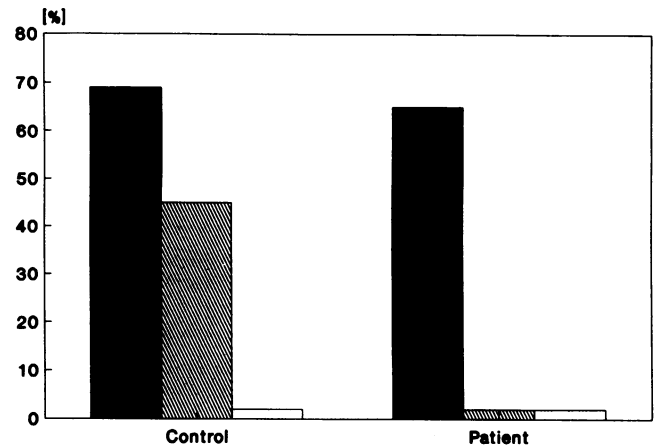
Granulocyte phagocytosis of immune complex–loaded bacteria is mediated via Fc and complement receptors. To test for the functional impact of the C3bi receptor defect on phagocytosis, opsonized FITC-labeled *E. coli* were incubated with PMN and phagocytosis of opsonized *E. coli* was compared to that of healthy controls (Fig. 8). Only 60% of patients' PMN compared to 85% of controls ingested *E. coli.* After blocking the contribution of the low affinity Fc receptors to phagocytosis by preincubation with CD16 and CDw32 antibodies the defect was even more pronounced: Only 30% of PMN compared with 60% of controls phagocytosed *E. coli* under these conditions, thus demonstrating a defect of C3bi mediated phagocytosis. In contrast, adherence of PMN was not impaired in comparison to three normal controls as has been shown for patients with leukocyte adhesion deficiency (data not shown).

Discussion

While the etiology of SLE is still unclear, decreased IC clearance is considered as one major factor in the pathogenesis of this disorder (30, 31). However, it is not known whether such decreased clearance is one of the prerequisites or merely a secondary phenomenon following production of large amounts of IC after the onset of disease. A high percentage of patients lacking complement components C2 or C4 has been described to be associated with SLE or at least SLE-like syndromes (4, 5, 32, 33), showing that deficient complement dependent solubilization and clearance of IC does contribute to the emergence of disease manifestations in this disorder.

Here a patient with SLE is described exhibiting a defect of a complement receptor 3 epitope. The C3bi receptor CR3 is considered to play the major role in phagocytosis and thereby in clearing of IC (34, 35). The receptor is also important in the activation of CD11b-positive cells, such as lymphocytes, natural killer cells, macrophages, and granulocytes (36–38). Our patient suffering from a particular form of SLE with panniculi-
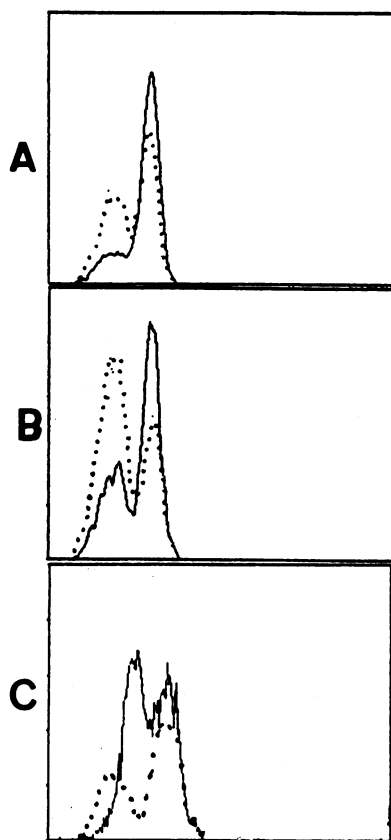
Figure 8. Phagocytosis of opsonized FITC-labeled *E. coli.* (*A*) Phagocytosis of *E. coli* was 85% for control PMN (——) compared with only 60% for the patient (· · ·). (*B*) After preincubation of PMN with CD16 (FcγRIII) and CDw32 (FcγRII) antibodies 60% of control PMN compared to only 30% of the patient's cells phagocytosed *E. coli.* (*C*) After preincubation with mAb 17 phagocytosis of a healthy control is inhibited to 55%. In contrast, phagocytosis of the patient was not further reduced.

tis lacks the expression of the CD11b epitope 17, one important functional epitope of the C3bi receptor. Two alternatives may explain this deficient expression. First, an autoantibody against the C3bi receptor could be responsible for the missing expression. Careful analysis of the effect of the patient's serum on normal cells and CR3 transfectants as well as examination of long-term cultured cell lines not expressing the C3bi receptor epitope 17 has revealed no evidence for the presence of such autoantibody, rendering the possibility unlikely. Second, the lack of this epitope can be explained by a primary gene defect or an acquired gene mutation. To test this possibility the putative C3bi binding region was sequenced. A mutation was not found in this region and may therefore be localized at a different site of the CR3 or be due to a posttranslational regulatory defect for this molecule. Lack of expression of epitope 17 might also reflect a polymorphism. However, 40 control SLE patients and normal controls did not exhibit the defect, rendering a polymorphism rather unlikely.

This is the first description of a selective defect localized in the α-chain of CR3. However, several individuals with leukocyte adhesion deficiency, a rare defect of the common β-chain of CD11a (leukocyte function antigen-1), CD11b (Mo1), and CD11c (CR4), have been reported (39, 40). These patients lack the expression of all three receptors and are clinically characterized by recurrent bacterial infections. In contrast, our patient exhibited no increased susceptibility to bacterial infections. The symptoms of leukocyte adhesion deficiency therefore appear to be primarily caused by the leukocyte function antigen-1 adhesion deficiency or by epitopes on Mo1 distinct from the epitope 17 (41).

The defect of epitope 17 on CR3 is also responsible for other functional disturbances. There is no rosetting of the pa-

tient's PMN with C3bi-loaded sheep erythrocytes when compared with normal controls, and phagocytosis of opsonized *E. coli* appears to be significantly inhibited. The defect in complement receptor 3 function demonstrated in this patient's granulocytes thus permitted an analysis of the relative contribution of Fc and complement receptor activity to the process of phagocytosis. Our results confirm earlier assumptions that IgG-Fc receptors support ~ 40% of the phagocytic function of neutrophils and monocytes (42).

In summary, this patient with a defect in the expression of the functional epitope 17 of the C3bi receptor provides further evidence that CR defects may contribute substantially to the pathogenesis of SLE once they are present. Moreover, the analysis of the patient's gene for the α-chain of CR3 may provide a tool to determine definitely the binding site of C3bi and respective monoclonal antibodies for the complement receptor 3.

Acknowledgments

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