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J Immunol (1991) 146 (2): 643–647.
https://doi.org/10.4049/jimmunol.146.2.643

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0022-1767/91/1462-0643$02.00/0
THE JOURNAL OF IMMUNOLOGY
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Vol. 146, 643–647, No. 2, January 15, 1991
Printed in U.S.A.

MONOCLONAL ANTIBODIES AS PROBES TO INVESTIGATE THE MOLECULAR CHANGES OF C5 ASSOCIATED WITH THE DIFFERENT STABILITY OF THE MOLECULE ON SHEEP ERYTHROCYTES AND *Escherichia coli* 0111:B4[1]

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The fifth C component (C5) exhibits a different stability when bound to sheep E or *Escherichia coli* 0111:B4, being fairly stable on the bacterial intermediate sensitized *E. coli* 0111:B4 coated with C components up to C5 (BAC1-5) and extremely labile on the RBC intermediate sensitized sheep E coated with C components up to C5 (EAC1-5). We examined the possibility that molecular changes of membrane-bound C5 might be responsible for the different functional behavior of the two intermediates using mAb to C5 and sensitive immunoassays to detect bound C5. The decay of EAC1-5 over 30 min of incubation at 37°C was associated with a significant drop in the reactivity of bound C5 with three of four mAb used. These results contrasted with those obtained with BAC1-5, which showed unchanged reactivity with all mAb tested over the same period of incubation. The effect of mAb on the activity of C5 was then investigated in an attempt to relate the change of the reactivity pattern of EAC1-5 with the functional modification of bound C5. MAb 1.5 and 1.6 were the only antibodies that interfered with the functional activity of C5, although through a different mechanism. In particular, mAb 1.5 was active both on fluid-phase and on membrane-bound C5 and is therefore likely to interact with the binding site for the late components on C5. Conversely, mAb 1.6 was only effective on fluid-phase C5 and acted by promoting a decay of BAC1-5 similar to the spontaneous decay of EAC1-5. We suggest that the bacterial outer membrane may protect C5 from functional decay and that mAb 1.6 interferes with the stabilizing effect of the bacteria in an as yet unclear manner.

The fifth component of the C system (C5)[3] enters the C sequence after activation that results from the enzymatic cleavage of the molecule into a small biologically active peptide, C5a, and a larger fragment, C5b (1). The enzymes responsible for the activation of C5 are essentially the C5 convertases of the classic and the alternative pathways, C423 and C3bnB, although other proteases, such as plasmin, trypsin, and lysosomal enzymes may induce the release of C5a from C5 (2). Our previous study has shown that the functional decay of bound C5, in the absence of C6, is not a general phenomenon, because it is not observed, for instance, when C5 interacts with *Escherichia coli* 0111:B4 (3). The loss of hemolytic activity of C5 bound to sheep E, as opposed to the functional stability of C5b on this strain of bacteria, does not depend on the selective release of C5 from the SRBC, nor on the cleavage of bound C5b (4).

We addressed the question as to whether different conformational changes of C5 on the two targets may be responsible for the variation in the stability of this C component. To this purpose, the molecular changes of C5 bound to sheep E and *E. coli* 0111:B4 were analyzed with mAb to C5. In addition, functional studies of C5 treated with the various mAb were performed with the intent to relate the degree of reactivity of the mAb with the membrane bound C5 to the functional stability of this molecule.

MATERIALS AND METHODS

Buffers. KRP buffer, pH 7.4, supplemented with 0.64 mM $MgCl_2$ and 0.16 mM $CaCl_2$, was prepared as described previously (3). A buffer of lower ionic strength (0.1 μ) was obtained by mixing equal volumes of KRP buffer and a solution of 9% sucrose containing 0.64 mM $MgCl_2$ and 0.16 mM $CaCl_2$. GVBS, pH 7.4, was made according to the method of Mayer (5).

Complement reagents. Human C5 was purified after the procedure described by Hammer et al. (6). The purity of this preparation was evaluated by SDS-PAGE. Purified C5 was also purchased from Cytotech (San Diego, CA). Pooled human sera were treated with KSCN and hydrazine hydrate according to the method of Dalmasso and Müller-Eberhard (7) and used as HR6-9. Our own preparation of C5 was labeled with ^{125}I (Amity-PG, Milan, Italy) using Iodogen (Pierce Chemical Co., Rockford, IL)-coated test tubes, to a sp. act. of 4×10^6 cpm/μg protein. The labeled component retained over 90% of the hemolytic activity. C6D was obtained from a previously described patient (8). The C5D serum was a kind gift from Dr. Peter Späth (Swiss Red Cross, Bern, Switzerland).

E intermediates and hemolytic assay. EAC1-3 were prepared by incubating 1% IgM-sensitized RBC (EA) with C5D serum to a final dilution of 1/10 in GVBS for 70 s at 37°C and then blocking the reaction with Suramin (Bayer, FRG) as described by Harrison and Lachmann (9). EAC1-5 were obtained by mixing 1% EA with 1/10 C6D serum for Tmax at 37°C in GVBS, followed by three washes with cold GVBS. The lytic assay for EAC1-5 was performed by incubating 50 μl of the RBC intermediate (1.5×10^7 cells) in 200 μl of GVBS containing 5 μl of HR6-9 for 30 min at 37°C, and then

Received for publication March 28, 1990.
Accepted for publication October 17, 1990.
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[1] This work was supported by the 40% and 60% grants of the Italian MPI to G.D.R. and F.T. and was partially presented in abstract form at the 13th International Complement Workshop, San Diego, CA, September 1989.
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[3] Abbreviations used in this paper: C5, fifth component of C; KRP, Krebs-Ringer phosphate; GVBS, glucose veronal buffered saline; HR6-9, human reagent containing the late C components from C6 to C9; C5D and C6D, human sera with selective deficiencies of C5 or C6; EA, sensitized sheep E; EAC1-3 and EAC1-5, EA coated with C components up to C3 or C5; BAC1-3 and BAC1-5, sensitized *E. coli* 0111:B4 coated with C components up to C3 or C5; Tmax, 2 min; RT, room temperature; T30, 30 min.

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reading the supernatant at 415 nm. A similar assay was used to test EAC1-3 except that the reaction mixture also included 0.2 μg of purified C5 (Cytotech).

Bacterial intermediates and bactericidal assay. C5D and C6D sera were used to prepare BAC1-3 and BAC1-5, respectively, from IgM-sensitized *Escherichia coli* 0111:B4 obtained from the stock collection of the Institute of Microbiology, University of Trieste. The procedure employed for the preparation of BAC1-3 and BAC1-5 at Tmax has previously been described in detail (3). Selective bactericidal assays for BAC1-3 and BAC1-5 were carried out by mixing 10^5 bacterial intermediates with 5 μl of HR6-9, for BAC1-5, and the same reagent supplemented with 0.2 μg of purified C5 (Cytotech) for BAC1-3, and 0.1 μ ionic strength KRP to a final volume of 200 μl. The suspension was incubated for 30 min at 37°C and the number of killed bacteria was evaluated by the method of dilution and counting of CFU as reported by Rottini et al. (3).

mAb to C5. Purified C5 was used to immunize BALB/c mice for the production of mAb after an established procedure (10). Hybridoma cells were cloned by limiting dilutions, and ascitic fluid was obtained by injecting positive clones in pristane-primed BALB/c mice.

Antisera. A goat antiserum against human C5 was purchased from Cytotech (San Diego, CA) and was extensively absorbed with the bacteria before use in the assay with BAC1-5. Biotin-labeled donkey IgG to goat IgG and affinity purified rabbit IgG to mouse IgG were obtained from Jackson Immunoresearch (West Grove, PA). Biotin-labeled rabbit IgG to mouse IgG were provided by Zymed Laboratories (San Francisco, CA).

Labeling of antibodies. The IgG fraction of goat antiserum to human C5 was obtained by ion-exchange chromatography through DE-52 (Whatman Biochemicals Ltd., Kent, England) equilibrated with 15 mM phosphate buffer, pH 7. Labeling of the IgG with D-biotin-*N*-hydroxysuccinimide ester (Sigma Chemical Co, St. Louis, MO) was carried out following a procedure described previously (11). The rabbit IgG to mouse IgG were labeled with ^{125}I (Amity-PG, Milan, Italy) by the chloramine-T method (12) to a sp. act. of 4×10^6 cpm/μg protein.

Triton-acetone extracts of erythrocyte and bacterial lysate. Membrane-enriched erythrocyte lysate was obtained by suspending the RBC in 5 mM phosphate buffer, pH 8, followed by centrifugation in microfuge at 15,000 × *g* for 30 min at 4°C. The pellet was resuspended in PBS containing 2% Triton X-100 and 2 mM EDTA and kept at RT for 15 min. Five volumes of ice-cold acetone were then added and the proteins of the sample were allowed to precipitate for 30 min at −70°C. After centrifugation at 15,000 × *g* for 30 min at 4°C, the precipitate was dried out by flushing the test tube with nitrogen; resuspended in 50 mM Tris-HCl (pH 7.6) containing 20% glycerol, 0.64 mM $MgCl_2$, and 0.16 mM $CaCl_2$; and incubated for 2 h at RT before use. A similar procedure was followed to obtain the bacterial extract except that intact bacteria were directly resuspended in PBS supplemented with Triton X-100 and EDTA to prepare the bacterial lysate.

Determination of antibody binding to C5. Screening of the mAb for their reactivity with C5 was performed by a solid phase ELISA. To this end, the wells of microtiter plates (Dynatech, PBI, Milan, Italy) were coated with 200 μl of purified C5 (0.3 μg/well) in 0.1 M sodium bicarbonate buffer, pH 9.6, overnight at 4°C. After blocking unreacted sites with PBS containing 1% BSA, the mAb were allowed to react with C5 for 1 h at 4°C at the dilution of 1/100 and their binding to C5 was evaluated by incubating first with 1/1000 biotin-labeled rabbit secondary antibody (Zymed Laboratories) for 1 h at 37°C, and then with alkaline phosphatase conjugated to streptavidin (Jackson Immunoresearch, West Grove, PA) for 30 min at 37°C. The enzymatic reaction was developed using para-nitrophenyl-phosphate (Merck, Milan, Italy) at the concentration of 1 mg/ml in 0.1 M glycine buffer, pH 10.4, containing 1 mM $MgCl_2$ as substrate. After incubation for 1 h at 37°C, reading was performed in a Multiskan 340 apparatus (Flow Laboratories Inc., Milan, Italy) at 405 nm.

The amount of mAb binding to cell-associated C5 was evaluated after two distinct procedures. When intact cells were used, 50 μl of either EAC1-5 or BAC1-5 (about 10^6 cells) were incubated with equal volumes of each mAb at the dilution of 1/50 for 1 h at 4°C and, after three washes, the cell pellet was mixed with 25 μl of ^{125}I-labeled antibodies to mouse IgG (300,000 cpm) and additionally incubated for 30 min at RT. Triplicate samples from each reaction mixture were layered over 1 ml of silicone oil 550 (Merck, Milan, Italy) in 1.5-ml polypropylene tubes and centrifuged at 10,000 × *g* for 5 min at 4°C. The tubes were then cut to isolate the pellet and the cell-bound radioactivity was measured. Binding of mAb to C5 extracted from EAC1-5 and BAC1-5 was also evaluated by ELISA. Amounts of 2 μl of membrane extracts, corresponding to about 10^6 cells, were applied to 8-mm nitrocellulose disks (Schleicher-Schuell, Dassel, FRG). The disks were transferred into the individual wells of a 24-well plate (Costar, Cambridge, MA) and incubated with 50 mM Tris-HCl, pH 7.6, containing 0.5 M NaCl (Tris-buffered saline), 2% BSA, and 0.5% Tween 20 for 1 h at 37°C and subsequently with 1/50 mAb in Tris-buffered saline-0.05% Tween 20-0.1% BSA overnight at 4°C. The procedure for the evaluation of the bound mAb was similar to the ELISA described above, except that at the end of the incubation after the addition of the substrate, 150 μl of the reaction mixture were transferred into the wells of the Dynatech plates for reading. The Triton-acetone extracts were assayed in parallel with unrelated mAb and the results were expressed as ELISA index:

$$\frac{(\text{OD mAb to C5} - \text{OD unrelated mAb})}{\text{OD unrelated mAb}}$$

SDS-PAGE and autoradiography. ^{125}I-C5 bound to EAC1-5 and BAC1-5 was analyzed by SDS-PAGE on a 8.5% gel made under reducing conditions (13) followed by autoradiography. The intermediates were prepared as indicated above, by incubating 10^8 EA or sensitized *E. coli* 0111:B4 first in 5% C6D serum with 50 ng ^{125}I-C5 and then solubilized in 100 μl of a solution containing 10 mM *n*-octyl-α-D-glucopyranoside (Sigma) for 1 h at RT followed by the addition of the sample buffer for the SDS-PAGE analysis.

The formation of functional EAC1-^{125}I-C5 or BAC1-^{125}I-C5 was checked with HR6-9. Autoradiography was performed on the dried gel with X-OMAT AR film (Eastman Kodak, Milan, Italy).

RESULTS

Characterization of mAb to C5. Four different mAb to C5 (1.2, 1.3, 1.5, and 1.6) were selected for this study because they provided useful information on the molecular rearrangement of membrane-bound C5. All of these mAb reacted with the α-chain of C5 when examined by Western blot (14), and were found to be IgG2b (1.3 and 1.5), IgG2a (1.2), and IgG1 (1.6). All four mAb recognized C5 bound to BAC1-5 and EAC1-5, but did not react with the intermediates BAC1-3 and EAC1-3.

Study of the molecular changes of C5 bound to EAC1-5 and BAC1-5. Our previous observation that BAC1-5, unlike EAC1-5, carry functionally stable C5 (3) raised the question of whether membrane-bound C5 undergoes different molecular rearrangement on the two targets.

First, we considered the possibility that the active fragment C5b is cleaved on sheep erythrocytes, thus leading to reduced functional stability of EAC1-5, but not on bacteria. This issue was examined by subjecting to SDS-PAGE analysis the C5 bound to EAC1-5 and BAC1-5 at Tmax and at the time of decay of EAC1-5 (T30). The electrophoretic patterns of C5 on the two targets, presented in Fig. 1, are consistent with the cleavage of C5 into C5b and C5a and do not reveal further degradation products of C5b on EAC1-5 at T30.

In the absence of major structural alterations of cell-bound C5, we searched for an alternative explanation for the different stability of EAC1-5 and BAC1-5 in distinct conformational changes of C5 on the two targets. These changes occur on C5 upon activation in the fluid phase by cobra venom factor, as shown by Di Scipio et al. (15) using circular dichroism. To obtain similar information on cell-bound C5, EAC1-5 and BAC1-5 were incubated with various mAb to C5 for 1 h at 4°C and subsequently with rabbit ^{125}I-IgG anti-mouse IgG for 30 min at RT. Differences in the amount of antibody binding to the same intermediate prepared at Tmax and T30 can be interpreted as evidence for molecular rearrangement of cell-bound C5 occurring over half-hour incubation. As shown in Figure 2, the only informative antibody in this respect was mAb 1.6, that exhibited a substantially decreased reactivity with EAC1-5 at T30 concomitant with the functional decay of bound C5. Conversely, the reac-

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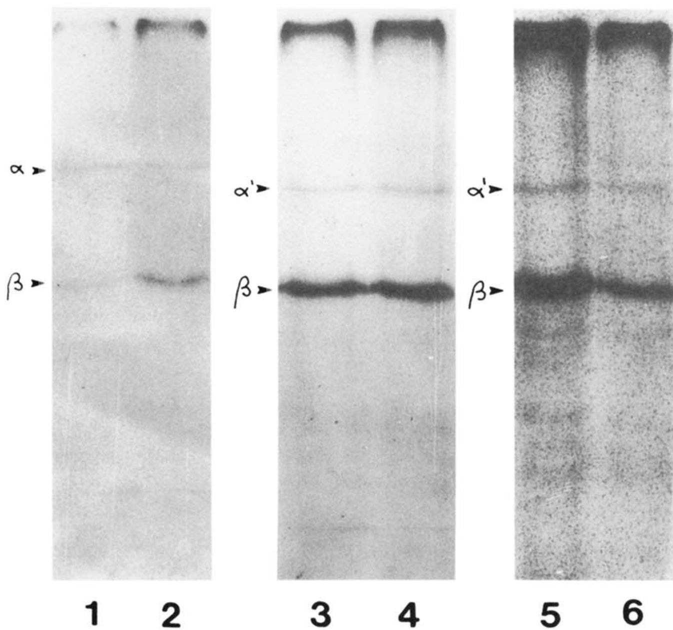

Figure 1. SDS-PAGE analysis of soluble and cell-bound ^{125}I-C5. Soluble ^{125}I-C5 (0.25 ng) was analyzed either untreated (*lane 2*) or treated with Triton-EDTA and acetone (*lane 1*), as control for C5 extraction from the cell membranes. Cell-bound C5 was obtained from EAC1-5 at Tmax (*lane 3*) and T30 (*lane 4*), and from BAC1-5 at Tmax (*lane 5*) and T30 (*lane 6*). The intermediates carrying ^{125}I-C5 and the solubilized pellets were prepared as described in *Materials and Methods.* The samples of cell-bound C5 (5×10^7 cells) and of soluble ^{125}I-C5 were analyzed under reducing conditions on a 8.5% gel and autoradiographed.

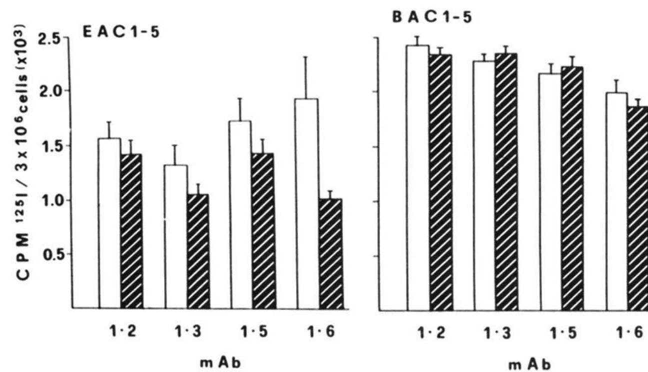

Figure 2. Quantitative evaluation of binding of various mAb to EAC1-5 and BAC1-5 prepared at Tmax (□) and T30 (▨). EAC1-5 or BAC1-5 (3×10^6 cells) were incubated with each mAb diluted 1/50 for 1 h at 4°C, washed and subsequently mixed with ^{125}I-IgG anti-mouse Ig (300,000 cpm) to measure bound antibodies. The results represent mean values of three experiments (± SEM).

tivity of this mAb with the two preparations of BAC1-5, obtained over the same time interval as EAC1-5, was virtually unchanged. The other three mAb showed only slight, but not significant, differences in their binding to the intermediate examined at Tmax and T30.

Next, we explored the possibility that the conformational changes of bound C5 be more clearly recognized on the molecule extracted from the membrane. To this end, EAC1-5 and BAC1-5 were treated with Triton X-100 and EDTA and the extracted proteins were precipitated with cold acetone. SDS-PAGE analysis of the C5 recovered in the precipitate did not reveal evident structural modifications, thus excluding that the treatment itself altered the molecule (data not shown). The results of the ELISA employed to measure the binding of mAb to the extracted C5 confirmed the finding of the previous experiment that mAb 1.6 reacts differently with EAC1-5 at Tmax and T30 and showed also that mAb 1.3 and 1.5 behaved in a similar manner (Fig. 3). By contrast, none of the mAb revealed changes in their reactivity with C5 extracted from BAC1-5 at Tmax and T30.

Effect of mAb on the formation of BAC1-5. Our observation that three mAb exhibited decreased reactivity with EAC1-5 at T30 as compared with EAC1-5 at Tmax, raised the question of whether the sites of the molecule recognized by these mAb were somehow involved in the biologic activity and stability of bound C5. To investigate whether these mAb had any effect on the formation of functionally active BAC1-5, C5 was incubated with the mAb for 2 h at 4°C before the addition to BAC1-3 and the killing of the resulting bacterial intermediate was then assayed by means of the remaining late components. The data presented in Figure 4 clearly indicate that treating C5 with mAb 1.5 inhibited the formation of BAC1-5, which instead could be regularly obtained in the presence of the other three mAb. However, mAb 1.5 did not prevent the binding of C5 to BAC1-3, as proved by the positive reaction of the formed intermediate with antibodies to C5 or mouse IgG (Fig. 5). The finding that mAb 1.5 interfered with the function of C5, while still allowing

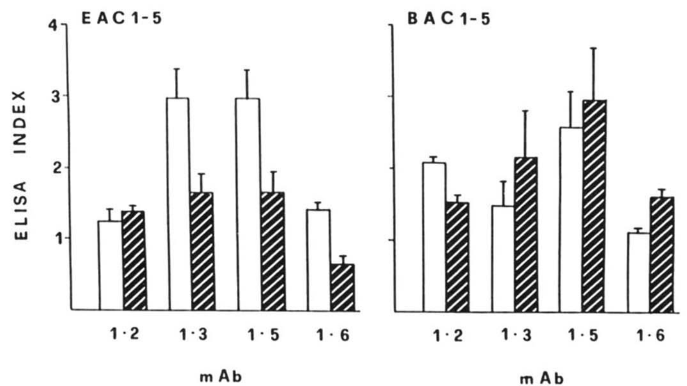

Figure 3. Quantitative immunoenzymatic evaluation of binding of mAb to C5 extracted from EAC1-5 and BAC1-5 at Tmax (□) and T30 (▨) by Triton X-100 and EDTA and then precipitated by cold acetone. Details of the assay are reported in *Materials and Methods.* The results represent mean values of three experiments (± SEM) and are expressed as ELISA index (see *Materials and Methods*).

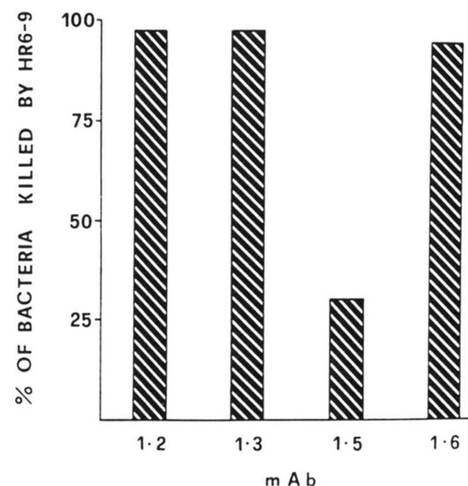

Figure 4. Effect of mAb on the functional activity of C5. Purified C5 (0.2 μg) was incubated with the various mAb at 1/50 final dilution for 2 h at 4°C and then added to BAC1-3 and HR6-9. The mixture was incubated for 30 min at 37°C and the number of bacteria killed was evaluated as described in *Materials and Methods.*

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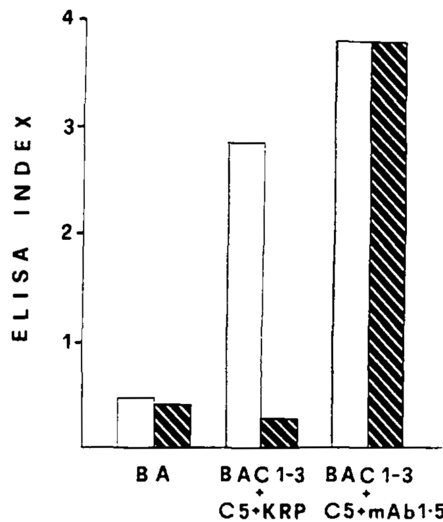

Figure 5. Demonstration of binding of C5 treated with mAb 1.5 to BAC1-3. This bacterial intermediate was exposed to C5 preincubated with either mAb 1.5 or buffer. At Tmax the bacteria were washed and further incubated for 30 min at 37°C. The presence of C5 or mAb on the intermediate was then revealed by biotin-labeled antibodies to C5 (□) or to mouse IgG (▨) followed by alkaline-phosphatase streptavidin as described in *Materials and Methods.* BA were used as control to test the specificity of the antibodies. The results are expressed as ELISA index.

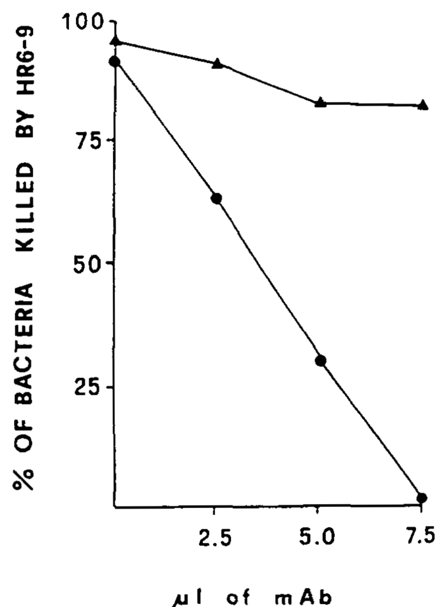

Figure 6. Effect of mAb 1.5 (●) and 1.6 (▲) on C5 bound to the bacteria. BAC1-5 (10^5) formed at Tmax were exposed to increasing volumes of each of the two mAb to a final volume of 150 µl for 2 h at 4°C, and then washed. The residual activity of bound C5 was measured by adding HR6-9.

the formation of BAC1-5, suggests that this mAb may inhibit the assembly of the terminal components on bound C5. This possibility was explored by incubating BAC1-5 at Tmax with mAb 1.5 or mAb 1.6, as control, and then testing their susceptibility to killing by the remaining terminal components. Under these experimental conditions, mAb 1.5 inhibited the killing of BAC1-5 in a dose-dependent manner, whereas mAb 1.6 had no effect even at very high concentration (Fig. 6).

Effect of mAb on the stability of BAC1-5. Since the major characteristic that distinguishes BAC1-5 from EAC1-5 is to be stable at 37°C, we examined the mAb for their ability to interfere with the stability of bound C5. The antibodies selected for this analysis were those that showed different reactivity with EAC1-5 at Tmax and T30 (see Fig. 3) and had no inhibitory effect on the function of C5. The experiment was carried out by preparing BAC1-5 at Tmax from BAC1-3 and C5, that had been incubated with the mAb for 2 h at 4°C, and then performing functional assays of bound C5 over 30 min of incubation at 37°C. The interesting finding was that BAC1-5, obtained in the presence of mAb 1.6, decayed with a half-life similar to that of EAC1-5, but remained stable when the C5 had been treated with mAb 1.3 (Fig. 7, *A* and *B*).

To exclude that the decay of BAC1-5 induced by mAb 1.6 was due to the release of C5 or mAb from the bacterial surface during the time course of the experiment, the bacterial intermediate was tested by ELISA with antibodies to C5 or mouse IgG showing similar values at Tmax and T30, at the time when cell-bound C5 was functionally inactive (Fig. 7*C*).

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DISCUSSION

The results of this study indicate that C5b undergoes different conformational changes on *E. coli* 0111:B4 and on sheep erythrocytes, that may be related, at least in part, to the functional stability of bound C5. The original observation made by Cooper and Müller-Eberhard (4) using ^{125}I-labeled C5, that the decay of C5 on EAC1-5 does not depend on the release of the molecule from the surface of the intermediate, has been confirmed by our

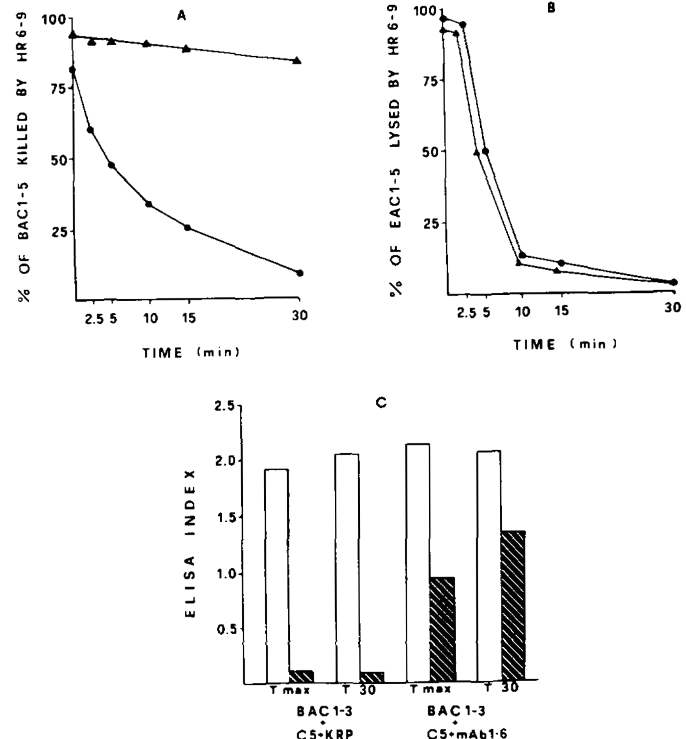

Figure 7. Antibody mediated functional decay of C5 on BAC1-5. *A*, BAC1-5 was prepared by mixing purified C5 (0.2 µg) with either mAb 1.3 (▲) or mAb 1.6 (●) to 1/50 final dilution for 2 h at 4°C before addition to BAC1-3 for the formation of BAC1-5 at Tmax. The latter intermediate was then washed and assayed for its susceptibility to killing by HR6-9 at various time intervals. *B*, Decay of EAC1-5 formed at Tmax by mixing EAC1-3 and purified C5 (0.2 µg) that had been incubated with buffer (▲) or mAb 1.6 (●) under the same experimental conditions used for BAC1-5. *C*, Demonstration of C5 (□) and mAb 1.6 (▨) on BAC1-5 formed in the presence of mAb 1.6 and examined at Tmax and T30. For details see legend to Figure 5.

finding that both EAC1-5 and BAC1-5 react with mAb 1.2 to the same extent when tested at Tmax and T30. Likewise, our inability to detect split products of C5 on EAC1-5 at T30 further supports the conclusion reached by the same authors (4) that a major structural alteration of bound C5 is not responsible for the decay of EAC1-5.

In the absence of either selective release or splitting of C5 on EAC1-5 at T30, a more plausible explanation for the decay of this intermediate is that C5 on EAC1-5 exhibits a conformational change different from that of C5 on BAC1-5. The procedure followed in this study to quantitate the amount of mAb binding to C5 on the two intermediates provides a reliable means to support this hypothesis. Thus, the marked difference in the reactivity of mAb 1.6 with EAC1-5 at Tmax and T30, not seen with BAC1-5, can be regarded as evidence for a different molecular rearrangement of C5 on the two targets. These results cannot be attributed to differences in the amounts of C5 bound to the two intermediates since these did not differ significantly when evaluated using ^{125}I-C5 to prepare EAC1-5 and BAC1-5 and accounted for less than 1% of the C5 offered (data not shown).

Further support in favor of different molecular changes of C5 on bacterial and RBC targets stems from the quantitative evaluation of the binding of mAb 1.3 and 1.5 to the two cell intermediates. The data obtained with these two mAb were similar to those observed with mAb 1.6 except that their reduced binding to EAC1-5 at T30 was more evident using membrane extracts rather than intact cells. An explanation for this finding is that the portion of the molecule undergoing more distinctive conformational changes involves hydrophobic domains of C5 not easily accessible to mAb on intact cells and can therefore be better recognized on C5 extracted from the membranes. The possibility that the extraction procedure may modify C5 differently depending on the target cannot be ruled out. It should be pointed out, however, that the difference in the reactivity of mAb 1.3 and 1.5 is only observed when comparing EAC1-5 at Tmax and T30, and it is unlikely that the procedure of Triton-acetone extraction may alter C5 extracted from the same intermediate. The mechanism whereby C5 becomes stable on *E. coli* 0111:B4 is unclear, but a plausible explanation for the differential behavior of the two targets is that the bacterial surface, unlike the membrane of sheep E, protects C5 from decay. The protective effect of the bacteria is exerted within a short period of time from the binding of C5 to the bacterial surface and can be counteracted by preincubating C5 with mAb 1.6, which promotes the decay of bound C5 while still allowing its binding to BAC1-3. Once bound in the absence of mAb 1.6, C5 is effectively protected and can no longer be destabilized. Since mAb 1.6 does not inhibit the killing of BAC1-5 by the late C components, it must obviously react with a site of C5 not involved in the assembly of the membrane attack complex. This, conversely, is the site of C5 recognized by mAb 1.5, which is quite effective in inhibiting the C-dependent killing of BAC1-5. On the whole, the data obtained with the two mAb 1.5 and 1.6 suggest that cell-bound C5 expresses at least two labile sites. One of these sites reacts with mAb 1.5 and is implicated in the binding of the late C components to C5, whereas the second site recognized by mAb 1.6 is critical for the bound C5 to adopt the appropriate spatial conformation necessary for its stability.

The type of interaction that C5 establishes with the two targets, and that is ultimately responsible for the difference in the functional stability of C5, is not clarified by the present data. It is interesting to note, however, that the particular structure of C5, with a relatively low number of disulfide bonds, favors the conformational change of the molecule that has already been associated with the formation of C5b from the native molecule by circular dichroism (15). The hydrophobic sites documented on C5 (16) may further contribute to the conformational change of the molecule. Evidence has now been collected indicating that the membrane-bound C4b-C3b dimer offers a high-affinity binding site for C5 (17). However, the nature of the surface components of *E. coli* 0111:B4 that protect C5 from decay remains to be defined. Whatever the reason for the prolonged susceptibility of BAC1-5 to killing by the late C components, it undoubtedly provides an additional mechanism of protection against this and possibly other strains of Gram-negative bacteria.

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