Restriction of Cell Lysis by Homologous Complement: I. An Analysis of Membrane Attack Complex Formation on Target Membranes

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The hemolytic efficiency and binding of C9 to homologous and heterologous erythrocytes was evaluated by using a standardized passive sensitization procedure to prepare antigen- and antibody-coated erythrocytes (EA) and human serum for lysis. Heterologous bovine EA were readily lysed by human serum, whereas human EA were quite resistant to lysis. Human EA bound as many C8 and C9 molecules per cell as bovine EA when incubated under identical conditions, but four times as much bound C9 was required to lyse an equal number of human EA compared with bovine EA. The susceptibility of human erythrocytes did not increase when increased volumes of undiluted human serum were used although C9 binding increased to as much as 100,000 molecules per cell. Sodium dodecyl sulfate-resistant polymerized C9 (poly(C9)) was detected on both lysed ghosts and unlysed EA bearing complement proteins C1 through C9 (EAC1-9) after incubation with undiluted human serum; however, the ratio of poly(C9) to monomeric C9 was higher on unlysed cells than on ghosts. Although bovine and human EA bound equal amounts of human C9 at the end point, the rate of lysis and C9 uptake was slower on homologous cells. The rate-limiting step occurred before C9 binding and lysis because the rates of lysis and C9 binding were equal on homologous and heterologous EAC1-8 targets, but the extent of lysis of homologous cells was still lower than lysis of heterologous cells. Human erythrocytes lose restriction against homologous hemolysis during storage in autologous plasma or in isotonic buffers.

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Soon after the introduction of erythrocytes as target cells for complement lysis assays by Bortel, it became apparent that RBCs of one species were relatively insensitive to lysis by complement from the same species. Muir and Browning and Muir described this phenomenon in some detail, but given the available knowledge and technology they could, of course, not distinguish whether this was a property of the sensitizing antibody or the lytic complement. Similar findings were made by others, but no systematic studies were performed until Houle and Hoffmann using a passive lysis system, found that complement from six species indeed lysed heterologous antibody-sensitized erythrocytes (EA) much more effectively than homologous erythrocytes (EAC). The rate of lysis and C9 uptake was most ineffective when the target erythrocytes and C9 were from the same species, thus clearly demonstrating that sensitizing antibodies played no role in this phenomenon.

Several proteins that modulate the efficiency of complement are known to exist on erythrocytes. In 1969, Hoffmann partially purified a factor from human erythrocyte membranes that was capable of accelerating the decay of the classical-pathway C3 convertase. Decay-accelerating factors (DAFs) were also extracted from rabbit and guinea pig erythrocytes. Nicholson-Weller et al. later purified human DAF to homogeneity, and Fearon demonstrated that purified C3b receptor (CR1) had DAF activity. Because both proteins shorten the lifetime of the C3/C5 convertase, the formation of the membrane attack complex (MAC) is inhibited. Although DAF inhibits homologous convertases strongly, it is important to note that CR1 and DAF are active in decaying both heterologous and homologous convertases in contrast to another recently described factor that is thought to specifically inhibit the activity of homologous MAC. This protein was first described by Schönermark et al. as a C8-binding protein and by Zalman et al. as a C9-binding protein. The latter group coined the term human restriction protein (HRP) to signal its function in homologous restriction of complement lysis.

Additional evidence for the importance of these proteins in modulating complement efficiency derives from the observations that type III paroxysmal nocturnal hemoglobinuria (PNH-III) erythrocytes are devoid of DAF and HRP, thus explaining the exquisite sensitivity of these cells to not only heterologous but also homologous complement. Although it was known that activation of the classical pathway of complement on homologous erythrocytes does not lead to effective lysis, none of the earlier studies quantified MAC formation on cells under these conditions. For this reason we compared the extent of C9 binding to target cells, C9 lytic efficiency, and the kinetics of C9 binding to lysed and unlysed homologous and heterologous erythrocytes during incubation with excess undiluted whole human serum. We used a passive lysis system described previously that allowed comparisons to be made under conditions in which antigen and antibody remained constant; only the species of erythrocytes changed. Because homologous complement...
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restriction obviously involves recognition of one or more species-specific cell membrane factors, the passive lysis system offered an additional advantage over using erythrocyte-specific antibody made in a heterologous species in that the antibody is specific for a passively applied antigen and will not bind to and possibly block a putative membrane restriction factor. Parts of these results were presented earlier in preliminary form.22

MATERIALS AND METHODS

Erythrocytes. Bovine erythrocytes (Ebh) (from J antigen-negative animals) were obtained by venipuncture, stored in modified Alsever's solution at 5°C, and used within 3 weeks. Human blood (group O) obtained fresh from Civitan Regional Blood Center, Inc (Gainesville, FL), and containing citrate-phosphate-dextrose-adrenaline anticoagulant served as the source for human erythrocytes (Ehb). It was stored at 5°C and used within 3 weeks.

Antiserum. Bovine antibodies specific for Brucella abortus were prepared as previously described.4

Lipopolysaccharide antigen. Lipopolysaccharide (LPS), which does not activate either the alternative or classical complement pathways,23 was prepared by dimethyl sulfoxide extraction of B abortus, strain 11193-3, and was alkali treated.24 The material contained 17.2% carbohydrate, 0.6% 2-keto-3-deoxyoctanoic acid, and 20% protein.4

Isotonic buffer solution. The preparation of isotonic veronal-buffered sodium chloride containing 0.15 mmol/L CaCl2, 0.5 mmol/L MgCl2, and 0.1% gelatin (Fisher Scientific, Pittsburgh) (GVB) has been described.4 GVB containing 0.5% human serum albumin (GVB; Sigma Chemical Co, St Louis) was used where indicated.

Sensitization of erythrocytes. For passive sensitization Ebh or Ebh were coated with optimal amounts of B abortus LPS and sensitized with optimal concentrations of antibody to LPS (EA) by using procedures reported previously.4 Ebh were coated with antigen and sensitized on the same day that Ehm (bovine erythrocytes sensitized with antibodies to LPS) were prepared to ensure that both cell preparations were of equal age. Ehm were also prepared by direct sensitization with rabbit antibodies (Cappel Laboratories, West Chester, PA) against Ehm by following standard procedures.

Complement components and C9-depleted serum. Fresh bovine blood was obtained by venipuncture and clotted, and the serum was stored at -70°C. Whole human serum was prepared by clotting fresh frozen human plasma (Civitan Regional Blood Center) as previously described25 and stored at -70°C. Human C8 and C9 were purified to homogeneity as described.25 Human serum depleted of C9 was prepared by immunofluorescence techniques.25 All sera were adsorbed three times at 0°C with Ehm to remove natural antibody.

Radioiodination of C8 and C9. C8 and C9 were radioiodinated to specific activities of 1 to 4 x 106 cpm/mol, respectively, by using the Iodobead (Pierce Chemical Co, Rockford, IL) technique as described by the manufacturer. Both proteins retained greater than 90% of their hemolytic activities.

Quantification of cell-bound complement components. EA (4 x 106 cells/mL) were incubated for 60 minutes at 37°C with equal volumes of human serum containing 10 µg/mL 125I-C8 or 111I-C9, or EA bearing complement proteins C1 through C8 (EAC1-8) were incubated for 30 minutes at 37°C with GVB containing 3 µg/mL 125I-C9 and 9 µg/mL C9 unless otherwise specified. After the addition of cold isotonic buffer, the cell suspension was centrifuged for ten minutes at 13,800 g. The amount of cell lysis was determined by measuring hemoglobin release. The cell pellet was washed three times, and then the radioactivity associated with the pellet was measured in an LKB 1270 Rack-gamma II gamma radiation counter (LKB Instruments, Stockholm). Nonspecific binding was determined either by incubating EA with heat-inactivated human serum containing 125I-C8 or 111I-C9 rather than fresh human serum or by incubating EA rather than EAC1-8 with GVB containing 125I-C9 under the conditions described earlier. Nonspecific binding was less than 5% of the total counts bound. All assays used duplicate samples and were repeated at least twice.

Determination of amounts of C8 and C9 bound to lysed and unlysed EA. EhmA (4 x 106 cells/mL) were mixed with equal volumes of whole human serum containing 10 µg/mL 125I-C8 or 10 µg/mL 111I-C9 and were incubated, centrifuged, and washed according to the procedure described earlier. Half of the treated cell sample was centrifuged for ten minutes at 13,800 g in a microfuge to pellet both lysed and unlysed cells, and the associated radioactivity was determined. The remaining half of the treated cell sample was resuspended in 1 mL GVB and centrifuged for five minutes at 1,000 g to pellet only the unlysed cells, which represented 40% to 65% of the cell population. The supernates were transferred to fresh tubes and centrifuged for ten minutes at 13,800 g to pellet the lysed cells. Both ghosts and unlysed cells were washed one additional time before the radioactivity was measured. Corrections for nonspecific binding were made by subtracting counts associated with EA or water-lysed ghosts that had been incubated with heat-inactivated whole human serum containing 10 µg/mL 125I-C8 or 10 µg/mL 111I-C9. Nonspecific binding to cells and ghosts was found to be equal.

Comparison of monomeric and polymeric C9 on lysed and unlysed EA. A modification of the method described by Tschopp et al26 was used to compare ratios of cell-bound monomeric and sodium dodecyl sulfate (SDS)-resistant polymeric C9 (poly C9). Briefly, radioiodinated C9 (5 µg/mL) was added to whole human serum, and 100, 250, or 500 µL of the whole human serum was added to 50-µL samples of EhmA (2 x 106 cells/mL), and the mixtures were incubated for 60 minutes at 37°C. After the cells were centrifuged and washed, lysed and unlysed cells were separated as previously described. Both ghosts and unlysed cells were washed two times in 5 mmol/L phosphate buffer, pH 7.6. The pellets were solubilized in 4% SDS in 50% glycerol at 37°C for 15 minutes. Solubilized cell pellets were applied to a linear 2% to 20% acrylamide gel and electrophoresed according to Laemmli27 under nonreducing conditions. The gel was fixed in 50% methanol/10% acetic acid and processed for autoradiography by using Kodak XAR-5 x-ray film. The relative proportions of poly(C9) and monomeric C9 were determined by scanning the autoradiographic films on a laser densitometer (Model SL-504-XL, Biomed Instruments, Inc, Fullerton, CA) and determining the area under each peak.

RESULTS

Comparisons of amounts of C8 and C9 bound to homologous EA and heterologous EA. As shown in Table 1, EhmA or EhmA incubated with a volume of whole human serum sufficient to cause complete lysis of the heterologous cells resulted in binding of similar amounts of C8 or C9 on both cell types, provided whole human serum had been extensively adsorbed with Ehm to remove natural antibody. However, only approximately 50% of the homologous EA lysed. Furthermore, after separating unlysed cells and ghosts in a second incubation it was found that similar amounts of both C8 and C9 were bound to both populations. The amounts of C8 and C9 bound were less than in the first experiments, probably because of the use of a different source of whole human serum. These data suggest that the ability of EA to resist lysis by excess homologous complement is not due to failure of homologous EA to bind amounts of C9 that are sufficient
for lysis of heterologous EA. The ratios of bound C9 to bound C8 were about the same for each target and were within the ratios reported earlier by others for heterologous immune lysis.

Comparisons of amounts of cell-bound C9 required to lyse 50% of homologous EA or 50% of heterologous EA. Incubating E$_{hA}$ or E$_{bA}$ with dilutions of $^{125}$I-C9- or $^{125}$I-C8 for 60 minutes at 37°C. Lysis and binding of C8 and C9 were determined as described in Materials and Methods.

Hemolytic efficiency of C9 on homologous and heterologous EA. During the course of these studies, it was noticed that incubating fresh E$_{hA}$ with increasing volumes of whole human serum caused an increase in lysis until a plateau of about 40% to 60% was reached. Therefore, the amounts of C9 bound under these conditions were determined. E$_{hA}$ (50 µL at 2 x 10$^9$ cells/mL) were incubated with 50, 100, 250, or 500 µL whole human serum containing 5 µg/mL $^{125}$I-C9 as described previously. As shown in Fig 2A, 1 x 10$^7$ E$_{hA}$ incubated with 500 µL whole human serum bound approximately 110,000 C9 molecules per cell, although only about 40% of the cells lysed. Lysis did not increase over that achieved when 1 x 10$^7$ EA had been incubated with 50 µL whole human serum, which resulted in binding of <20,000 C9 molecules per cell. Such a high uptake of C9 in the absence of increased lysis was not a consequence of the passive sensitization procedure used to prepare EA. E$_{bA}$ prepared by the conventional method of sensitizing with antibodies directed against surface antigens displayed similar behavior (Fig 2B). The reason for the lower total lysis achieved in this system is probably related to the smaller number of antibodies that can be attached to such cells before strong agglutination becomes an experimental problem.

We have routinely observed that the maximum lysis obtainable with E$_{hA}$ prepared from freshly drawn cells was less than 35% and that the degree of lysis increased to approximately 65% after about 3 weeks of storage of erythrocytes in autologous plasma or after three to four days of storage of EA in isotonic buffer. Furthermore, homologous EA could be lysed completely by whole human serum after storage times longer than approximately 6 to 7 weeks (manuscript in preparation). This phenomenon probably accounted for the slight variations in maximum lysis of homologous EA that occurred when experiments were performed on different days. However, as shown in Fig 2, the maximum amount of lysis achieved by incubating EA with increasing volumes of homologous complement did not rise. Instead, it plateaued with increased C9 binding. In fact, E$_{hA}$ that bound ten times as much C9 as was required for 100% lysis of heterologous EA. Complete lysis of homologous EA could not be achieved under our conditions.

Homologous EAC1-9 resist lysis even when very large amounts of C9 are cell bound. During the course of these studies, it was noticed that incubating fresh E$_{bA}$ with increasing volumes of whole human serum caused an increase in lysis until a plateau of about 40% to 60% was reached. Therefore, the amounts of C9 bound under these conditions were determined. E$_{bA}$ (50 µL at 2 x 10$^9$ cells/mL) were incubated with 50, 100, 250, or 500 µL whole human serum containing 5 µg/mL $^{125}$I-C9 as described previously. As shown in Fig 2A, 1 x 10$^7$ E$_{hA}$ incubated with 500 µL whole human serum bound approximately 110,000 C9 molecules per cell, although only about 40% of the cells lysed. Lysis did not increase over that achieved when 1 x 10$^7$ EA had been incubated with 50 µL whole human serum, which resulted in binding of <20,000 C9 molecules per cell. Such a high uptake of C9 in the absence of increased lysis was not a consequence of the passive sensitization procedure used to prepare EA. E$_{bA}$ prepared by the conventional method of sensitizing with antibodies directed against surface antigens displayed similar behavior (Fig 2B). The reason for the lower total lysis achieved in this system is probably related to the smaller number of antibodies that can be attached to such cells before strong agglutination becomes an experimental problem.

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Polymerized C9 is formed on both lysed and unlysed EA during incubation with homologous complement. Very little poly(C9) was detectable on either E$_{hA}$ or E$_{bA}$ when 2 x 10$^7$ cells were incubated for 60 minutes at 37°C with 100 µL $^{125}$I-C9--augmented human serum (data not shown). However, when the serum volume–to–cell number ratio was increased, poly(C9) was easily detected on polyacrylamide slab gels by autoradiography (Fig 3). Because facilitated C9 polymerization has been implicated as a factor in enhanced lysis of PNH-erythrocytes (PNH-E) compared with normal erythrocytes, $^{14}$ we used differential centrifugation to separate ghosts from unlysed cells after incubation with homologous complement and examined the two populations for formation of polymerized C9. Interestingly, at the highest serum volume used, the ratio of poly(C9) to monomeric C9 was sixfold higher on unlysed than on lysed EAC1-9 as...
Kinetics of C9 binding and lysis of homologous and heterologous target cells. EA, prewarmed to 37°C, were mixed with prewarmed whole human serum, and aliquots were withdrawn at the time points shown in Fig 4. Both the rate of lysis and the rate of C9 uptake were greater on E₅₈A than on E₆₅₈A during incubation with whole human serum, and as expected, maximum lysis of homologous EA was less. After five minutes, 85% of E₅₈A were lysed by whole human serum, but only 6% of E₆₈A were lysed in the same time period, and the maximum lysis of E₅₈A was 40% after 90 minutes (Fig 4, upper panel). Although there was little increase in C9 binding after 60 minutes on either E₅₈A or E₆₈A, the uptake of C9 was considerably slower on E₆₈A, especially during the first 15 minutes of incubation with human serum. To determine whether this slower rate of C9 uptake on homologous cells was a property of C9 or a reflection of a slower rate of MAC formation due to restriction at an earlier step, C9 uptake and lysis were determined by using preformed human and bovine EAC1-8 target cells (antibody-sensitized-erythrocytes bearing C-components 1 to 8) carrying the same number of C5b-8 sites (≈1,800 per cell). No differences in the rates of C9 uptake by human or bovine preformed EAC1-8 could be detected (Fig 4, lower panel), yet the extent of homologous lysis was significantly less. Thus, restricted lysis cannot be explained by a reduced rate of C9 binding.

DISCUSSION

The inefficiency of lysis of erythrocytes by homologous complement is a long-known phenomenon. However, our results provide, for the first time, a quantitative assessment of the efficiency of MAC formation after activation of the classical pathway on such cells. By comparing E₅₈ and E₆₈ sensitized with the same antigen and antibody we were able to demonstrate that restriction of hemolysis does not result from a failure of MAC assembly on homologous target cells. Similar amounts of human C8 and C9 were bound to E₅₈ and E₆₈ after 60 minutes of incubation with whole human serum, and although E₅₈ were completely lysed, only 50% of E₆₈ lysed. Moreover, the surviving EAC1-9 and the lysed ghosts carried equal amounts of C8 and C9, thereby demonstrating that the differences in lysis did not result from differences in MAC assembly but from differences in MAC efficacy on individual cells. Thus, by using classical pathway–mediated lysis we extended the earlier finding of Hänisch et al who bypassed the early acting complement components by using the reactive lysis system to provide evidence for restriction of hemolysis by homologous complement at the level of MAC formation. Homologous EA, then, do not resist lysis by binding only sublytic amounts of C9 during incubation with excess serum; rather the hemolytic efficiency of C9 is decreased on homologous EA. For example, when the amount of cell-bound human C9 required to lyse 50% of E₅₈A and E₆₈A was compared, it was found that about four times more C9 was required for lysis of homologous EA. It is remarkable that homologous EA resisted lysis by even very high concentrations of cell-bound C9 (>100,000 per cell) deposited during incubation with large volumes of undiluted human serum. Although our results

Fig 2. Relationship between C9 binding and maximum hemolysis of homologous EA. (A) E₅₈A (1 × 10⁷ cells in 0.05 mL), prepared by passive sensitization with LPS and anti-LPS antibodies, were incubated with 50, 100, 250, and 500 μL of whole human serum augmented with ¹²⁵I-C9 (5 μg/mL) for 60 min at 37°C. Although the hemolysis remained constant (dashed line), the total C9 binding (open bars) to cells (lysed and unlysed) increased continuously. (B) Identical conditions as in A except that E₅₈A were prepared by sensitization with rabbit antihuman erythrocyte antibodies.

detected by scanning densitometry of the gel shown in Fig 3. Large amounts of poly(C9) were also detected on human EAC1-9 in which the sensitizing antibody was directed against membrane antigens (data not shown), again demonstrating that the method of sensitization has no effect on poly(C9) formation. Of significance is the fact that unbound radioactive C9 in the supernatant was in the form of monomeric C9 and that no poly(C9) could be detected by SDS–polyacrylamide gel electrophoresis (PAGE) and autoradiography, which indicated that poly(C9) is not shed under these conditions.
were obtained with erythrocytes that were sensitized with a foreign antigen, this procedure did not influence the results. Cells that were treated in a more conventional manner by using antibodies directed against surface antigens gave similar results.

A further significant finding was our observation that the maximum amount of lysis of fresh homologous erythrocytes was less than 35% but tended to increase with storage time.

Erythrocytes stored in autologous plasma for more than about 6 weeks or EA stored in isotonic buffer for longer than a week could be completely lysed by homologous serum. These results suggest the presence of two populations of cells, one susceptible to lysis by homologous complement and the other resistant. Because the susceptible population increases with storage time, it appears that restriction of hemolysis of homologous cells is not a stable condition but is subject to modulation.

Although our results do not yet allow us to provide a complete mechanistic explanation for homologous restriction, we have sufficient data to eliminate several possibilities. Although homologous restriction during reactive lysis could be surmounted by increasing the concentration of C5b-7 sites on target membranes,6,13 we have discovered now that the total amount of C8 and C9 bound at the end point has no effect on lysis in human serum (see earlier). Thus, we considered that it was the rate of MAC deposition and not the total number of MACs on the target membrane that determined lysis efficiency. E$_{EA}$ carry DAF that retards the efficiency of the human C3/C5 convertase, and one would expect that the rate of MAC deposition should be slower on homologous compared with heterologous cells. This prediction could be verified (Fig 4) because the rate of MAC deposition, as measured by binding of C9 contained in serum, was much slower on homologous cells. If it were the rate of MAC deposition that determines restriction, then one could also predict that homologous and heterologous EAC$_{1-8}$ carrying equal numbers of C5b-8 sites should not exhibit restriction of C9 lysis. This prediction, however, was not borne out; bovine cells carrying equal amounts of C5b-8 complexes were still lysed more than human cells by human C9, although both bound equal amounts of C9 at equal rates. Thus, we have no evidence that the rate of MAC deposition on the target is a determining factor in homologous lysis restriction.

Polymerization of C9 is another reaction that has been implicated in determining the lytic efficiency of C9.26

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**Fig 3.** Formation of SDS-resistant poly(C9) on ghosts and unlysed homologous EAC$_{1-8}$. E$_{EA}$ were incubated with whole human serum supplemented with $^{125}$I-C9 (5 $\mu$g/mL) for 60 minutes at 37°C. Lysed and unlysed cells were separated by differential centrifugation, the membranes were collected and subjected to SDS-PAGE and autoradiography. Lane A shows a control $^{125}$I-C9 sample that had been incubated for 60 minutes at 37°C in whole human serum. Lanes B, C, and D contain identical amounts of ghost membranes derived from EA samples incubated with 0.1, 0.25, and 0.5 mL of whole human serum, respectively. Lanes E, F, and G show the corresponding membranes derived from unlysed cells.

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**Fig 4.** Lysis and kinetics of C9 binding to EA and EAC$_{1-8}$ targets. Lysis (closed symbols) of E$_{EA}$ (A) and E$_{EA}$ (B) by whole human serum (upper panel) and of E$_{EA}$AC$_{1-8}$ (C) and E$_{EA}$AC$_{1-8}$ (D) by isolated C9$_{nu}$ (lower panel) was compared with binding (open symbols) of C9$_{nu}$ present in whole human serum (upper panel) to E$_{EA}$ (O) and E$_{EA}$ (□) and binding of isolated C9$_{nu}$ (lower panel) to preformed E$_{EA}$AC$_{1-8}$ (O) and E$_{EA}$AC$_{1-8}$ (□).
Poly(C9) was detectable on both lysed and unlysed EA after incubation with larger amounts of homologous serum. In fact, the ratio of poly(C9) to monomeric C9 was greater on unlysed EAC1-9 than on lysed EAC1-9. These observations are important with respect to recent results by two research groups who reported the isolation of two proteins from Eac named C8-binding protein (C8-bp) and HRP, respectively, that they claim are involved in homologous restriction of hemolysis. It was stated that both proteins inhibit C9 polymerization. Based on our findings, we conclude that these proteins do not restrict hemolysis because of inhibition of C9 polymerization but achieve their effects through other mechanisms.

Several investigators have suggested that PNH-E are distinguished from normal erythrocytes because they lack the capacity to restrict homologous complement lysis. This concept is certainly correct as far as the role of DAF is concerned in restricting the efficiency of the C3/C5 convertase. Whether this notion is also relevant for the steps involved in MAC regulation on homologous cells because such cells apparently are devoid of C8-bp and HRP remains to be established. This cautionary note stems from the fact that some reported observations are incompatible. For example, Hu and Shin have stated that heterologous erythrocytes are lysed better by human C9 than are homologous cells because C9 inserts more efficiently into heterologous membranes. However, they could not detect differences in C9 insertion between normal erythrocytes and PNH-E but proposed instead that PNH-E are lysed more easily because poly(C9) formation was favored on PNH-E membranes. This concept is not compatible with our observation of increased C9 polymerization on unlysed cells and also with reports by Hall et al and Rosenfeld et al who found no differences in poly(C9) production or high-molecular weight C9 content on normal and PNH-E, type III. Therefore, the possible roles of both C9 insertion and C9 polymerization in homologous restriction warrant further study.

Finally, because the unlysed homologous EAC1-9 cells are also protected against lysis by both homologous and heterologous serum during a second incubation, as we will describe in the following paper, it is unlikely that the newly discovered control proteins that bind C8 and/or C9 are the only functional elements in hemolytic restriction at the MAC assembly stage. These proteins are considered to be species specific and, therefore, by definition should have no effect on heterologous complement acting during a second incubation.

In summary, our results and those of others indicate that restriction of lysis of homologous erythrocytes is a complex process that may include more than one mechanism operating at the C8/C9 step and also involves restriction at the C3/C5 convertase step. Restricting the C3/C5 convertase activity leads to a slower rate of lysis of homologous EA. However, homologous restriction of C9 activity occurs independently of a decreased rate of C9 uptake, which indicates that this protein is less active on homologous membranes because of an intrinsic characteristic of such membranes.

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