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Immunology

Homologous species restriction in lysis of erythrocytes by terminal complement proteins

(complement cytotoxicity/species specificity/cellular recognition)

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ABSTRACT **The cytolytic efficiency of the terminal complement protein complex, C5b-9, varies with the species of origin of C8 and C9. In the present study, we explored the susceptibility of erythrocytes from various species to lysis by C5b6,7 plus C8 and C9 from different species. EC5b6,7 intermediates were prepared on human, guinea pig, rabbit, mouse, and rat erythrocytes with human C5b6 and guinea pig C7. The degree of lysis of these intermediates by C8 and C9 was found to vary widely depending on the species of the proteins and the target cells. In all cases, lysis was least efficient when C8 and C9 were homologous with respect to the target cell species. This effect was mostly attributable to C9. The inefficient lysis in a homologous system is not due to a failure of C9 binding. Rather, the poor lysis in the homologous system may be attributable to inefficient insertion or channel formation.**

The complement (C)-mediated lysis of erythrocytes (E) is accomplished by the interaction of the late C components C5–C9 with one another and with the membrane lipid bilayer (1–7). When C5 is cleaved into C5b and C5a by the C5 convertases generated either by the classical or the alternative pathway of complement activation, it forms a stable complex with C6 that lyses cells together with C7, C8, and C9 (6–10).

The efficiency of lysis depends largely on the species of C8 and C9. It has been shown that the lysis of sheep EAC1-7 is much more efficient with guinea pig C8 than with human C8 (11). Also, the temperature requirement in the terminal stages of lysis differs with the C8 species (12). Human and guinea pig C9 differ also in their efficiency to lyse EC5b-8 intermediates formed on erythrocytes other than those of sheep (13). We and others have reported that the lysis of sheep EC5b6,7 was much more efficient with guinea pig C8 and C9 than with human C8 and C9, whereas the opposite was true for guinea pig erythrocytes or erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (14, 15).‡

This difference is not due to an inefficient interaction of the components C5b6,7,C8, and C9 with each other (13).‡ In the present study, we explored the susceptibility of erythrocytes from various species to lysis by C5b6,7 plus C8 and C9 from different species. We were particularly interested in the efficiency of C8 and C9 from homologous versus heterologous sources.

MATERIALS AND METHODS

Buffers. *VBS.* Veronal-buffered saline (pH 7.4), ionic strength = 0.15, was prepared by diluting a stock solution 1:5 with water (16). *DGVB.* Veronal-buffered saline (pH 7.4), ionic strength = 0.075, contained dextrose (2.5%), gelatin (0.1%), $CaCl_2$ (0.15 mM), and $MgCl_2$ (1.0 mM) (16). *EDTA/VBS.* This was prepared by mixing 9 vol of VBS with 1 vol of 0.1 M EDTA.

Target Cells. Freshly drawn erythrocytes from humans, guinea pigs, rabbits, rats, and mice were washed three times in EDTA/VBS and adjusted to 1×10^8/ml in DGVB.

Complement. Serum from humans, guinea pigs, rabbits, rats, and mice was obtained by clotting the blood for 30 min at room temperature and 1 hr at 0°C. As a source of C8 and C9, each serum was diluted 1:100 with EDTA/VBS (EDTA/serum). C8-deficient human serum (C8D-HS) was a gift from M. Frank (National Institutes of Health).

Components. C5b6 was prepared from human serum according to the method described by Yamamoto and Gewurz (17). Guinea pig C7, C8, and C9 were purified as described (4, 18). Human C8 and C9 were purified as described (19). All components were diluted with DGVB.

Formation of EC5b6,7 Intermediates. One volume of erythrocytes from various species was incubated with 2 vol of C5b6 in varying concentrations for 10 min at 27°C. Excess guinea pig C7 was added and the mixture was incubated for an additional 20 min at 27°C. The cells were then washed in DGVB and resuspended in EDTA/VBS to a final concentration of 1×10^8/ml.

RESULTS

Lysis of Erythrocytes from Various Species by C5b6,C7 and Serum from Various Species as the Source of C8 and C9. Human, guinea pig, rabbit, rat, or mouse erythrocytes were incubated with limiting amounts of human C5b6 and excess guinea pig C7 to form EC5b6,7 intermediates. These intermediates were suspended in EDTA/VBS and incubated with human, guinea pig, rabbit, rat, or mouse EDTA/serum for 90 min at 37°C as sources of C8 and C9. The lysis of the respective target cells is illustrated in Fig. 1. The degree of lysis of a particular target cell was found to vary widely depending on the species of C8 and C9. In all five cases, lysis was least efficient when C8 and C9 were homologous with respect to target cell species.

Abbreviations: C, complement (individual C components are designated by number—i.e., C8, C9, etc.; the letter b, as in C5b, refers to a fragment of a complement molecule); C5b6, stable complex of C5b and C6; EC5b6,7, erythrocytes carrying C5b, C6, and C7 (erythrocyte species is indicated in each experiment, but the C5b6,7 species is not because all experiments were done with human C5b6 and guinea pig C7).

‡ Hänsch, G., Hammer, C. H., Mayer, M. M. & Shin, M. L. (1980) *Fourth International Congress of Immunology*, Paris, July 1980, Vol. 15, p. 206.

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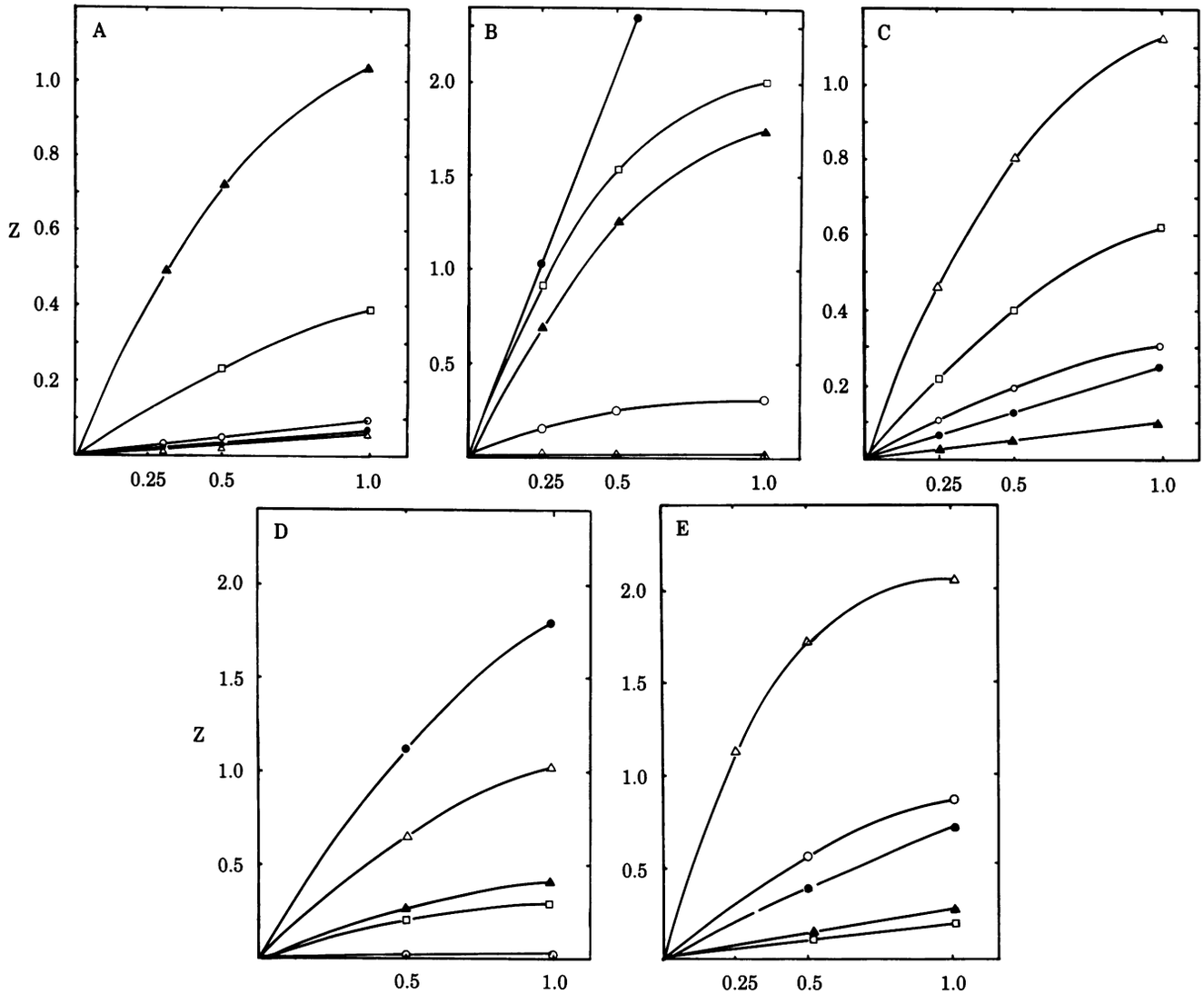

FIG. 1. Lysis of EC5b6,7 with human serum (●), guinea pig serum (△), mouse serum (○), rat serum (□), or rabbit serum (▲). Sources of erythrocytes: *A*, human; *B*, guinea pig; *C*, rabbit; *D*, mouse; *E*, rat.

Inhibitory Effect of Homologous Serum on Lysis of Erythrocytes by C5b6,7 and Heterologous C8 and C9. To evaluate whether the markedly decreased lysis of erythrocytes carrying C5b6,7 with C8 and C9 from the same species is due to their differential binding to cell-bound C5b6,7 or to the incompatibility of C8 or C9 with the target membranes, the following experiment was performed. Mouse EC5b6,7 were mixed with mouse serum in various concentrations and excess human C8 and C9 and incubated for 90 min at 37°C. Human C8 and C9 were chosen because mouse EC5b6,7 lysed well with human serum (Fig. 1*D*). The lysis was inhibited, and the degree of inhibition depended on the concentration of mouse serum (Fig. 2 *Left*). The results indicate that mouse C8 and C9 bind to mouse EC5b6,7 thus competitively inhibiting the binding of human C8 and C9. When guinea pig EC5b6,7 were incubated with various amounts of guinea pig C8 and C9 and excess human C8 and C9 (since human serum was known to lyse guinea pig erythrocytes efficiently), the homologous C8 and C9 inhibited the lysis by human C8 and C9 (Fig. 2 *Right*). When guinea pig EC5b6,7 or mouse EC5b6,7 preincubated with guinea pig C8 and C9 or with mouse EDTA/serum, respectively, were washed and then treated with human C8 and C9, marked inhibition of lysis was observed (results not shown).

Role of Homologous C8 and C9 in Restricting Lysis of Erythrocytes by C5b-9. The lysis of EC5b6,7 is least efficient when homologous C8 and C9 are used. In order to study whether C8 or C9 or both are responsible for this phenomenon the following experiments were performed. Guinea pig EC5b6,7 were made with varying amounts of C5b6. These intermediates were incubated with human C8 plus human C9, human C8 plus guinea pig C9, guinea pig C8 plus human C9, or guinea pig C8 plus guinea pig C9. The homologous C8 and C9 were not effective in lysing guinea pig EC5b6,7; however, human C8 and C9 were very effective (Fig. 3). When homologous C9 was used with human C8, lysis was as inefficient as with homologous C8 plus C9. Guinea pig C8 plus human C9, however, lysed guinea pig erythrocytes almost as much as human C8 plus human C9.

A similar experiment was done with human erythrocytes. To evaluate the role of C9 in this system, human EC5b6,7 were first incubated with excess human C8, with excess human C8 and C9, or with VBS for 10 min at 37°C. The cells were washed and incubated with rabbit EDTA/serum as the source of heterologous C8 and C9 (see Fig. 1). Human EC5b6,7 treated with buffer lysed about 8 times better with C8 and C9 from rabbit than did EC5b6,7 that had been preincubated with human C8 and C9. Preincubation with human C8 alone reduced the lysis

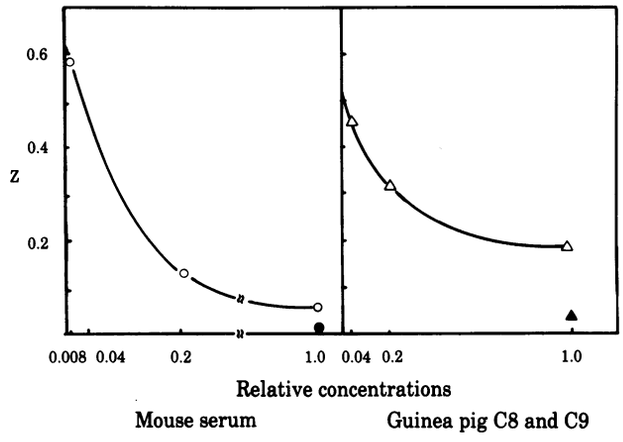

FIG. 2. The inhibitory effect of homologous serum on the lysis of EC5b6,7 by heterologous, effective serum. Mouse (*Left*) or guinea pig (*Right*) EC5b6,7 were incubated with various amounts of mouse serum and excess human C8 and C9 (100 units each) or with various amounts of guinea pig C8 and C9 and excess human C8 and C9 (100 units each), respectively. Relative concentration of guinea pig C8 and C9 = 1.0 is equal to 200 units of each. ●, Mouse serum alone; ▲, guinea pig serum alone.

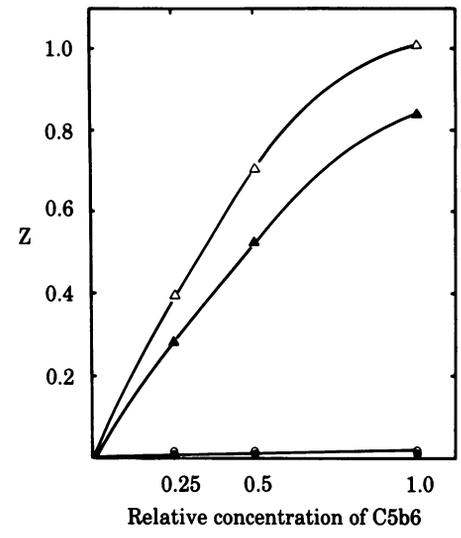

FIG. 4. Lysis of human EC5b6,7 or EC5b6,7,8 with human C8, C9, or human C9 and rabbit serum. Human EC5b6,7 intermediates were preincubated with human C8 (△), human C8 and C9 (●) or EDTA/VBS (△) for 10 min at 37°C. Then excess rabbit serum was added.

by rabbit EDTA/serum only slightly (Fig. 4). These findings suggest that C9 plays the major role in the species specificity and that C8 has only a minor effect.

DISCUSSION

We studied the species compatibility between target erythrocytes and the terminal C components in lysis mediated by C5b-9. We found that human, guinea pig, rabbit, rat, and mouse erythrocytes all bind human C5b6 together with guinea pig C7 to form EC5b6,7 intermediates. The degree of lysis, however, was found to vary widely with the species of origin of C8 and C9. This differential lysis varied up to 10-fold even though the amount of C5b6,7 was the same on a particular target cell.

The different susceptibility to lysis of a target cell carrying C5b6,7 by sera of various species does not appear to be due to an incompatible interaction of the C components with one another, as also suggested by others (11, 13). Evidence for this interpretation is provided by two different experiments. (*i*) Serum from each species is able to lyse at least one target cell efficiently (Fig. 1), and (*ii*) the homologous serum competes with an efficient heterologous serum for the C5b6,7 binding site (Figs. 2 and 4).

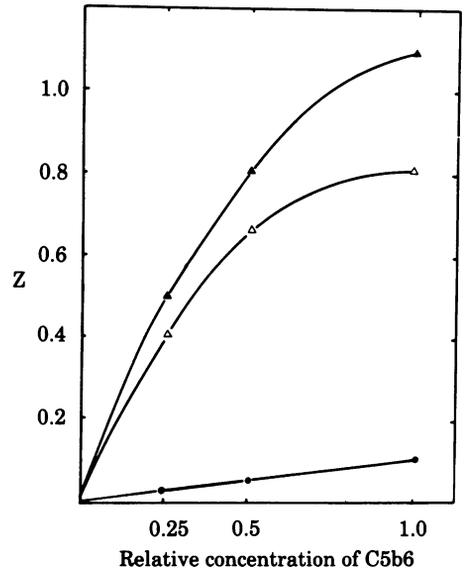

FIG. 3. Lysis of guinea pig EC5b6,7 with guinea pig C8 plus guinea pig C9 (●), human C8 plus guinea pig C9 (○), guinea pig C8 and human C9 (▲), or human C8 and C9 (△).

It is of special interest that serum homologous with regard to the target cell is the least efficient source of C8 and C9. The homologous C8 and C9, however, bind to the C5b6,7 intermediate because they inhibited the lysis by an efficient source of C8 and C9 (Figs. 2 and 4). Therefore, the restriction of lysis by homologous serum resides in the C8 or C9 protein, or both.

Studies with the individual components C8 and C9 showed that the effect of homologous species restriction is mostly attributable to C9 (Figs. 3 and 4). Thus, in guinea pig and human erythrocytes the species of C8 influences the lysis only minimally, whereas the species of C9 is critical. A similar result was also observed by Yamamoto (13) with guinea pig erythrocytes and guinea pig C9.

Accordingly, the interaction between C proteins and target cells that would lead to lysis is markedly reduced when the C9 and the target cell are from the same species.

The biological importance of such species restriction is evident. It minimizes self-inflicted cell damage by the membrane attack components during specific or nonspecific inflammatory processes in which the activation of the C cascade, with the generation of biologically active mediators, occurs. The destruction of invading foreign cells by terminal C components, however, is not affected by the homologous restriction.

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