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D. T. Fearon

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Dependence of Complement Rosettes on a Nonenzymatic and an Enzymatic Step. M. P. Dierich and B. Landen: Institute f. Med. Microbiology, Augustusplatz, D65, Mainz/Germany.

Rosette formation between typical EAC14^{oxy}23 (human complement components; 1.2 hemolytically active C2 sites and 1×10^4 C3 molecules) and Raji cells can be reduced by 20 to 30%, of 0.5 mM diisopropylfluorophosphate (DFP) are present during the rosette assay. Reduction of rosette formation is only slightly less pronounced, if EAC14^{oxy}23 are pretreated for 10 min at 23°C with 0.5 mM DFP. To elucidate the possible contribution of the $C\bar{4}\bar{2}$ enzyme to the formation of rosettes, EAC1423 were prepared with nonoxidized C2. These EAC1423 were kept at 37°C for 30 min to decay the C2 and to obtain EAC143. Such cells formed about 10 to 15% rosettes. By treating the EAC143 with increasing amounts of oxidized C2 to form EAC14^{oxy}23, rosette formation was increased up to 40%. This C2 dependent increase in rosette formation could be suppressed by DFP or by performing the rosette assay at 4°C. For comparison SRBC were treated with tannic acid and coated with purified C3 (E^{tan}-C3) to exlcude any enzymatic activity. These E^{tan}-C3 formed about 20% rosettes with Raji cells (E^{tan}-BSA were negative), which could not be inhibited by DFP and which reacted equally well at 4°C as at 37°C. Treatment of Raji cells with glutaraldehyde (0.01 to 0.1%) left their interaction with E^{tan}-C3 unaltered but blocked their rosette formation with EAC14^{oxy}23. The same reaction pattern resulted from pretreatment of the lymphoid cells with IgG anti-C3. These results suggest the participation of an enzymatic action in complement dependent rosette formation. In conjunction with the report about C4 in Raji cell membranes (Ferrone, S. Pellegrino, M. A. and N. R. Cooper, Science *193*, 53, 1976) and our own data, indicating the presence of C3 in the lymphoid cell plasma membrane, it is tempting to assume the "bridge formation mechanism" as a possible explanation for this interaction: proteolytic cleavage of a Raji cell membrane component (C4 or C3?) by the $\overline{C42}$ enzyme → liberation of new binding sites (from C4 or C3?) → interlinkage of the Raji cell and the EAC14^{oxy}23.

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Activation of the Alternative Complement Pathway by *Escherichia Coli*: Resistance of Bound C3b to Inactivation by C3bINA and β1H. D. T. Fearon, Departments of Medicine, Harvard Medical School and Robert B. Brigham Hospital, Boston, Mass.

The surfaces of activators of the alternative pathway, such as zymosan and rabbit erythrocytes, provide sites which protect bound C3b from inactivation by C3b inactivator (C3bINA) and β1H, and P,C3b,Bb from decay-dissociation by β1H. This circumstance permits surface-associated amplified generation and deposition of C3b. The capacity of a bacterial surface, if analogous to zymosan and rabbit erythrocytes, to protect and thereby accumulate increasing amounts of C3b by local circumvention of the regulatory proteins would be compatible with a unique role of the alternative pathway in host defense. The susceptibility of C3b bound to *Escherichia coli* to C3bINA was compared with that of C3b bound to the nonactivator, sheep erythrocytes, in the state, EAC4b,3b. *E. coli* W3110, killed by heating at 60°C for 60 min, activated the alternative pathway in human serum diluted 1:5 in GVB containing 2 mM Mg^{++} and 8 mM EGTA as demonstrated by 82% C3 and 87% B consumption. The killed *E. coli* were coated with C3b by incubation with purified C3, B and $\bar{D}$, washed in GVB containing 0.04 M EDTA (GVB-EDTA) and resuspended in GVB with 0.15 mM Ca^{++} and 0.5 M Mg^{++} (GVB^{++}). The dose responses of particle-bound C3b were established by incubating dilutions of EAC4b,3b and *E. coli*-C3b, respectively, with B and $\bar{D}$ in GVB^{++} for 60 min at 37°C, followed by centrifugation and hemolytic assay of residual fluid phase B. The relative susceptibilities of C3b bound to sheep erythrocytes and *E. coli* to inactivation by purified C3bINA in the presence of β1H were studied in a time-dependent fashion with amounts of particle-bound C3b that gave comparable inactivation of B by $\bar{D}$. The purified control proteins inactivated 76% C3b on EAC4b,3b in 60 min at 37°C but reduced C3b activity on *E. coli* by only 10%. Similarly, when human serum diluted in GVB-EDTA was used as the source of control proteins, 96% of C3b activity on sheep erythrocytes was abolished in 60 min, whereas only 18% of C3b on *E. coli* was inactivated in the same time interval. Thus, the surface of *E. coli*, like that of two other activators of the alternative pathway, zymosan and rabbit erythrocytes, protects bound C3b from inactivation and promotes alternative pathway-dependent opsonic recognition.

Purification and Characterization of a Mouse Serum Protein with Specific Binding Affinity for C4 (Ss protein). Arturo Ferreira, Paul Weisz-Carrington, Irma Gigli and Victor Nussenzweig. Department of Pathology; Irvington House and Department of Dermatology, New York University School of Medicine, New York NY 10016.

A new protein named Ss-bp, or C4-bp, with specific binding affinity for activated C4 has been found in mouse serum. Ss-bp is a heat-stable (60° C, 1 hr) β-globulin with a sedimentation coefficient of 10S. Ss and Ss-bp are separated by filtration of EDTA-plasma in Sephadex G-200. However, in serum, Ss-bp binds tightly to Ss and the complex is found in the excluded volume of the column. After electrophoresis in agarose at pH 8.6, the Ss-bp/Ss complex moves faster toward the anode than either protein alone. For this reason Ss-bp may appear to be polymorphic in serum of mice with high levels of C4 (Ss-H) versus those with low levels of C4 (Ss-L) (Ferreira, Takahashi and Nussenzweig, J. Exp. Med., in press).

Ss-bp levels are higher in serum of males (160 μg/ml) than in female mice (60 μg/ml). As in the case of other mouse C components (C4, C5), the concentration of Ss-bp in serum is markedly influenced by testosterone. Castration of males reduces the Ss-bp levels, and injections of testosterone in females or in castrated males raise the concentrations to those of normal males.

Ss-bp from mouse also interacts with human C4b as shown by the incorporation of ^{125}I-Ss-bp to EAC1,4b or EAC4b, but not to EAC$\bar{1}$ prepared with human C components. The amount of ^{125}I-Ss-bp bound to the red cell intermediates is directly proportional to the number of C4b sites. C4b, and to a lesser extent native C4, in the fluid phase, inhibit the binding of ^{125}I-Ss-bp to EAC4.

The incorporation of Ss-bp onto EAC4 is time and temperature dependent, and does not occur at 0°C. Maximum uptake is observed at 37°C after 60 min of incubation. The formation of the Ss-bp-EAC4b complex is not inhibited by chelation of divalent cations or by DFP, PMFS, SBTI or ovomucoid. Virtually no decay or dissociation of Ss-bp from EAC4 is observed after incubation at 37°C for many hours.

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