Section: Lectures

Interaction between Antigen, Antibody and Complement

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The first component of complement (C1) is a complex of the binding protein C1q and two zymogens, C1r and C1s. The zymogens are activated on binding of immune complexes, IgG aggregates or chemically crosslinked IgG protomers to the heads of C1q. In the classical pathway the activation of C1 is the first step in a sequence of activation steps of other components of the complement system. This sequence finally leads to the lysis of cells recognized by the antibodies. The structure of C1q has been elucidated by chemical, electron microscopic and spectroscopic studies. It consists of six globular heads held together by collagen stems, in a structure resembling a flower bouquet.

In our work we approached three problems. Concerning the structure of the C1 complex we found that monomeric C1s (M_r, 85,000; $s_{20,w}$, 4.3 S), the dimer (M_r, 170,000; $s_{20,w}$, 6.7 S) of C1r, and the tetrameric complex (C1r, C1s)$_2$ (M_r, 340,000; $s_{20,w}$, 8.7 S) are elongated molecules. Hydrodynamic equivalents of cylindrical shape have a diameter of 3.3 nm and lengths of 20 nm for C1s, 36 nm for (C1r)$_2$ and 64 nm for (C1r, C1s)$_2$. In electron micrographs the C1r, C1s complex appears as a chain composed of six to eight globular domains with a contour length of 51 nm. A structure is proposed in which (C1r)$_2$ forms a core to which C1s protomers are associated at both ends. The C1 complex ($s_{20,w}$, 16.3 S) reconstituted from C1q, C1r and C1s dissociates under the conditions used for electron microscopy. Some features of the C1 complex are revealed in the dissociation products.

In order to study the mode of activation by IgG antibodies rabbit anti-SII pneumococcal polysaccharide IgG antibody was cross-linked by dithiobis-(succinimidyl propionate). The IgG dimers were visualized by electron microscopy and resembled two Y-shaped structures connected in random orientations. The three arms of the Y's were of equal length. The six globular domains of the IgG protomers could be seen, but the Fab arms and Fc stems could not be distinguished from each other. Electron microscopy revealed that the dimers were bound to the globular heads of C1q. From the dependence of the weight

average molecular weight on dimer concentration, an equilibrium constant of about $10^6 M^{-1}$ was derived for the binding of dimers to Clq. The number of IgG dimers which could be accommodated at a single Clq molecule was derived to be three. The data do not allow a clear distinction between noncooperative and cooperative binding.

The binding equilibrium was independent of whether the IgG dimers were liganded with an SII nonasaccharide hapten or not. The results are at variance with an allosteric mechanism of the action of antigen in Dl activation. They lend support to the association hypothesis of complement activation. The data suggest that clusters of about six IgG molecules, connected by a multivalent antigen or arranged at the cell surface, are recognized by Clq with a binding constant of about $10^{10} M^{-1}$.

We learned more details on the activation mechanism from the dependence of the kinetics of Cls activation and of the concentrations of $(ClrCls)_2$ and of Clq. A large concentration dependence of the sigmoidal kinetics was observed in the 2 to 180 nM concentration range. This was explained by association-dissociation equilibria between the antibody saturated Clq and various forms of the $(ClrCls)_2$ complex (unactivated to activated). The establishment of these equilibria (binding constant $2x10^7 M^{-1}$) was assumed to be fast as compared to the rates of activation steps (rate constants $10^{-3} s^{-1}$ and $10^{-2} s^{-1}$ at $30^{o}C$). The fast re-quilibration of the Cl complex explains the finding that small amounts of antibody saturated Clq catalyzed the activation of large amounts of Cls. The interpretation of the kinetic results was supported by a direct demonstration of the dissociation of Cl into Clq and $(ClrCls)_2$ by analytical and density gradient centrifugation. No difference was found between the rates of activation and the dissociation properties of reconstituted Cl and Cl isolated from serum.

References:

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