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PRELIMINARY REPORT

LACK OF BINDING OF COMPLEMENT BY IgD

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Human immunoglobulin molecules can be divided into classes, distinct not only in structure but presumably also in biological function. It is known that IgG and IgM can bind complement, whereas IgA does not. Thus IgG and IgM but not IgA, must share a specific structure, to exhibit this character. Recently, a new class of immunoglobulin, IgD, whose function *in vivo* is unknown, has been described (Rowe and Fahey, 1965a, b). It is therefore interesting to see if IgD can bind complement.

We developed a useful technique for testing the complement binding capacity of immunoglobulin without specific antibody activity. It involves the non-specific coupling of immunoglobulin molecules to the surface of erythrocytes through chromium ion, thus rendering the cells susceptible to complement dependent lysis (Inai and Tsuyuguchi, 1968). Using this method we demonstrated that IgG, regardless of the occurrence of aggregation, as well as IgM but not IgA can sensitize erythrocytes in this non-antibody mediated lysis (Tsuyuguchi and Inai, 1968).

The globulin fraction was precipitated from λ-type IgD myeloma serum with sodium sulfate and IgD was isolated by chromatographies on DEAE-cellulose, CM-cellulose and Sephadex G-200. After rechromatography on DEAE-cellulose, the material gave a single precipitin line on immunolectrophoresis against horse anti-human serum, rabbit anti-IgD and rabbit anti-λ Bence-Jones protein. The sedimentation coefficient, $S_{20,w}$, was estimated as 6.8 at a protein concentration of 0.39%.

The complement fixing activity of IgD before and after heating at 63 C for 15 min, was measured by the method of Mayer (1961) with a slight modification. Appropriate dilutions of IgD preparations were added to 50 to 100 C'H50 units of complement and incubated at 37 C for 90 min. The complement remaining in these reaction mixtures was the measured. Only 15.4 C'H50 were fixed by 550 μgN of IgD and fixation was not greater with heated IgD, whilst 50 C'H50 were fixed by as little as 1 82 μgN of aggregated IgG.

Hemolysis of sheep erythrocytes coupled with IgD or heated IgD by the $CrCl_3$ method was examined as follows. To one volume of erythrocytes suspension in saline (1×10^9 cells per ml) were added one volume of 0.15 mM $CrCl_3$ in saline and one-tenth volume of the respective protein solution in saline to give a final concentration of 0.1%. The reaction mixture was incubated for one hour at 30 C. The erythrocytes coupled to protein were washed twice with saline and once with veronal-buffered saline. They were resuspended in veronal-buffered saline at a concentration of 5×10^8 cells per ml. Hemolysis of these erythrocytes coupled to protein with dilute

guinea pig serum was followed, using the 1/2.5 scale of Mayer's C'H50 titration method (Nagaki et al., 1965). The results are shown in Fig. 1. A low concentration of complement hemolyzed cells coupled to aggregated IgG but even a high concentration did not hemolyze cells coupled to IgD or heated IgD.

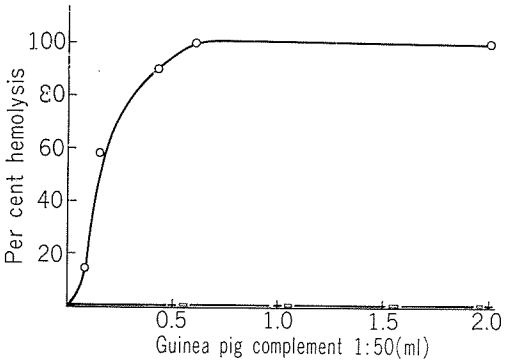

FIGURE 1. *Hemolysis of erythrocytes coupled with aggregated IgG, IgD and heated IgD by guinea pig complement*

○——○ *Erythrocytes coupled with heat aggregated IgG*

□ ····· □ *Erythrocytes coupled with IgD*

■ ····· ■ *Erythrocytes coupled with heated IgD*

IgD thus failed to show, by the methods employed, any complement binding ability.

This work was presented at the 17th Annual Meeting of the Japanese Society of Allergy in October, 1968 at Kyoto, Japan, and the U.S.-Japan Complement Seminar in November, 1968 at Tokyo, Japan. In this Seminar, T. Ishizaka presented studies on the biological activities of immunoglobulins and reported that IgD showed no complement fixing capacity.

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