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IMMUNOLOGY

Hæmolytic Complement and Serum Bactericidin

In a previous communication[1], serum bactericidin was related to a clotting factor extracted from fibrin. Several properties of this bactericidin are known to be shared by hæmolytic complement, such as inactivation by heating at 56° C for 30 min and removal by filtration through Seitz filter pads. Complement, like bactericidin, is absent from uncoagulated cell-free plasma[2]. Further investigations comparing the two serum activities were undertaken.

Both guinea pig and human sera served as sources of complement. Experiments were set up as follows. To fresh serum dilutions made up in 0·2 ml. of 0·9 per cent sodium chloride were added 0·2 ml. of 0·75 per cent sheep erythrocyte suspensions. Such cells had to be sensitized with immune hæmolysin when guinea pig serum was used, whereas with human serum this was not necessary. Tubes were incubated in a 37° C water bath for 1 h and read for complete hæmolysis. The smallest amount for this reaction or unit of complement was 0·005–0·01 ml. of guinea pig serum or 0·01–0·02 ml. of human serum. The difference between the action of guinea pig and human serum was explained by the finding that human serum normally contained both complement and sheep hæmolysin. Heating at 56° C for 30 min destroyed complement but left behind antibody which would bring about sheep cell lysis with guinea pig serum. The antibody could not be removed by repeated washings of the sensitized cells with saline. Its titre was determined by lysis with guinea pig serum (2 units of complement) as well as agglutination with anti-human globulin. Identical results were obtained. With most sera the minimal reactive dose was 0·005 ml. Normal human serum also contains sheep red blood cell agglutinins but of a much lower titre. A high titre of such agglutinins, as present in the sera of patients suffering from infectious mononucleosis, did not affect the hæmolysin titre.

No difference in the action of human and guinea pig complement was observed. Neither one was able to bring about the lysis of human Rh-positive cells sensitized with low-titred antibody, which strongly reacted with anti-human globulin. Like bactericidin, both were inactivated by lytic factor and heparin[1]. Only 0·01 ml. of potent lytic factor preparation was required to block the action of 2 units of complement. The reaction was immediate. It could be reversed by the addition of excess complement. Barium sulphate in 10 or 25 mg quantities absorbed in 10 min all complement from 0·1 ml. of human or guinea pig serum but did not affect serum bactericidin. Both complements also differed from bactericidin in thermostability at temperatures below 56° C, as reported previously[1].

Sera of a number of patients suffering from a variety of infectious and organic diseases were analysed for complement and sheep hæmolysin. Considerable reduction in either one factor or both was noted in many instances, but no consistent pattern emerged. High bactericidin-levels often coincided with reduced complement activity. Guinea pig serum, though rich in complement, had no bactericidin.

From the results of this investigation as well as previous studies[1,3] it would seem that clotting factor extracted from fibrin, *C* reactive protein, serum bactericidin, and hæmolytic complement share a chemical grouping that arises immediately prior to or during fibrin formation. Indirect evidence[4] points to a steroid glucuronide. It would also appear most likely that their differences in physico-chemical and physiological properties are due to association with different proteins.

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Specificity of Isoantisera against Leukæmic and Thymic Lymphocytes

This communication concerns the specificity of isoantisera prepared against leukæmic and against thymic lymphocytes of *AKR* mice, as determined by immune cytolysis[1–3]. Discovery of a strong antigen associated with the thymic lymphocytes of *AKR* mice is also reported. A preliminary report has been made[4].

For immune cytolysis of *L*4946 leukæmic lymphocytes of *AKR* mice, the small-scale assay system previously described in detail[2] was used. The only change was use of 100,000 cells per assay tube. Essentially the same cytolysis system was used for thymic lymphocytes, except that absorbed hamster serum at a final concentration of 10 per cent was used as source of complement[3].

Isoantisera were prepared by injection of leukæmic and of thymic lymphocytes of *AKR* mice into mice of strains *C57BL*/6 and *C3HeB*/*Fe* (ref. 3). The cytolytic potencies of each of the four isoantisera were determined against *L*4946 leukæmic cells and against thymic lymphocytes (Table 1). Cytolytic potency is expressed as 100/cytolytic titre, where the cytolytic titre is the final concentration of isoantiserum (per cent) giving 50 per cent cytolysis of cells present in an assay tube. Specificity is expressed as the ratio of cytolytic potencies[5]; it refers to specificity against cell types, and has no direct bearing on the presence or absence of specific tumour antigens.

The relatively high potency of *C57BL*/6 isoantisera for cytolysis of *L*4946 leukæmia (Table 1, *A*) correlates well with the complete resistance of *C57BL*/6 mice to intraperitoneal transplantation of *L*4946 cells. The low potency of *C3HeB*/*Fe* isoantisera for cytolysis of *L*4946

Table 1. Specificity of Isoantisera against Leukæmic and Thymic Lymphocytes

Isoantisera prepared against	Cytolytic potency for cytolysis of *L*4946 leukæmia (*AKR*)	Thymic lymphocytes (*AKR*)	Specificity against *L*4946 leukæmia
A. Isoantisera prepared in *C57BL*/6 mice.			
*L*4946 leukæmia	78	4	19·5
Thymic lymphocytes	24	450	0·053
Specificity against *L*4946 leukæmia	3·2	0·0089	366
B. Isoantisera prepared in *C3HeB*/*Fe* mice.			
*L*4946 leukæmia	4	340	0·012
Thymic lymphocytes	3	450	0·0067
Specificity against *L*4946 leukæmia	1·33	0·76	1·8