HAEMATOLOGY

Role of Properdin, 0° Factor, Polyinosinic Acid, and Thrombin in the Haemolysis of Paroxysmal Nocturnal Haemoglobinuria Erythrocytes

ABSORPTION of serum at 15° C with zymosan renders the serum deficient in properdin and destroys the capacity of such RP serum to haemolyse paroxysmal nocturnal haemoglobinuria erythrocytes (PNHE)¹. Absorption of serum with zymosan at 0° C does not remove properdin, but does remove a zymosan specific fraction ('0° factor'), so that on incubation with fresh zymosan at 15° C properdin is no longer affected². Recent work in this laboratory has defined the PNHE haemolytic system as one dependent on fluid phase complement (C') activation resulting in red cell membrane destruction as a result of direct attack by C'3 sub-components³. C'1 esterase or C'1 activators have been shown to enhance PNHE lysis and in addition certain C'1 activators such as polyinosinic acid (poly I) may initiate the haemolysis of normal human erythrocytes (NHE), presumably by similar fluid phase complement reactions⁴. Thrombin has also been shown to enhance PNHE lysis, but the mechanism of this enhancement has been the subject of considerable controversy. The observations shown in Table I were designed to assess the relative role of properdin and '0° factor' in native and poly I enhanced PNHE lysis, and to compare the stimulating effect of poly I on PNHE haemolysis with the ability of thrombin ('Thrombin', bovine, Upjohn Co., Kalamazoo, Mich.), both absorbed with NHE and unabsorbed, to enhance PNHE lysis.

Table I. EFFECT OF PROPERDIN AND '0° FACTOR' DEPLETION ON POLY I-ENHANCED HAEMOLYSIS OF PNHE RED CELLS; COMPARISON OF POLY I EFFECT WITH THROMBIN EFFECT

Addition to test serum	Percentage haemolysis					
	Normal serum	pH 7.6 0° super-natant serum	RP serum	Normal serum	pH 6.5 0° super-natant serum	RP serum
Saline (0.1 ml.)	14	14	1	51	54	2
Poly I—1 μ mole P	58	56	15	81	81	64
Thrombin—50 U	66	65	63	73	73	68
Absorbed thrombin —50 U	27	24	1	53	53	28

The haemolytic test system consisted of 1 ml. of the appropriate serum, acidified where indicated with 0.3 N HCl, 0.05 ml. of a 20 per cent suspension of PNHE in 0.15 M NaCl, and 0.1 ml. of 0.15 M NaCl or 0.1 ml. of the appropriate material dissolved in 0.15 M NaCl, added in the order mentioned. After incubation at 37° for 30 min under oil, the tubes were centrifuged and read at 540 m μ in a Zeiss PMQ II spectrophotometer against the appropriate serum blank.

Removal of '0° factor' from serum does not affect the ability of serum to support PNHE haemolysis under any of the experimental conditions cited. Removal of properdin, on the other hand, does inhibit the capacity of serum to support PNHE haemolysis at both pH 7.6 and 6.5. The ability to haemolyse PNHE can be restored to RP serum by the addition of poly I; the latter stimulates PNHE haemolysis in RP serum much more effectively at pH 6.5 than at pH 7.6, but at neither pH does poly I-enhanced haemolysis proceed as well as in normal serum. Absorption of thrombin with NHE diminishes greatly its ability to stimulate PNHE cell lysis. Addition of NHE-absorbed thrombin to normal serum at pH 6.5 does not result in significant enhancement of PNHE haemolysis. The latter observation agrees with those of Hinz⁵, but is at variance with the experience of two other groups^{6,7}, who have found residual capacity for PNHE acid haemolysis stimulation in thrombin which has been exhaustively absorbed by NHE. More in keeping with these latter reports, however, we have found that absorbed thrombin can provoke moderate enhancement of PNHE haemolysis in normal serum at pH 7.6, and can partially restore haemolytic capacity to RP serum at pH 6.5. Removal of

properdin from serum diminishes, but does not completely abolish, the ability of poly I to provoke haemolysis of NHE at pH 6.5 (ref. 4).

Properdin and zymosan-specific '0° factor' are not required for poly I-induced haemolysis, although poly I-induced haemolysis is reduced somewhat in RP serum. '0° factor' is also not required for native PNHE haemolysis. Viewing properdin as related intimately to the recognized components of C' (ref. 8), and recognizing the peculiar affinity of zymosan for C'3 (ref. 9), one could speculate that properdin is, in fact, a sub-component of C'3. Such a possibility has been reinforced by our recent observations that the intermediate complex, PNHE β_{12} formed by exposure of PNHE to partially purified human β_{12} globulin (C'3a), is highly susceptible to haemolysis in RP serum in the presence of Na₂HEDTA (ref. 3). Bearing in mind the concepts recently developed on the special relationship of late-acting C' components to PNHE haemolysis^{3,4}, it is conceivable that partial depletion of such a C'3 sub-component might critically affect a haemolytic system dependent on threshold amounts of activated fluid phase, late-acting C' components (native PNHE haemolysis); might partially, but not completely, inhibit a haemolytic system capable of activating more efficiently residual fluid phase late-acting C' components (poly I-induced haemolysis); and might inhibit only slightly, if at all, a haemolytic system capable of activating and utilizing the residue of such a factor with great efficiency at the cell membrane (classical immune haemolysis).

Most observers agree that much of the ability of commercial bovine thrombin to enhance PNHE haemolysis is attributable to the presence of red cell hetero-antibodies⁵⁻⁷. However, our observations, in agreement with those of Jenkins *et al.*⁶ and Crosby and Benjamin⁷, demonstrate that even after extensive absorption with human red cells, such bovine thrombin is still capable, under certain conditions, of enhancing PNHE haemolysis. Jenkins *et al.* have clearly shown that thrombin *per se* is not involved in this enhancement; since the factor in question is heat stable, they conclude that it is non-absorbable antibody⁶. On the basis of the mechanisms discussed in this communication, however, this enhancing factor could be either a substance which, like poly I, is capable of activating C'1, or the bovine counterpart of one of the heat stable C'3 sub-components of guinea-pig serum (C'3c, C'3b) (ref. 10). That at least one of the bovine C'3 sub-components can function in a heterospecific C' system has been demonstrated by Linscott and Nishioka, who used bovine plasma as a rich source of C'3d (ref. 10).

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