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Some Antigen Similarities between Mouse Erythrocytes and Ehrlich Ascites Tumour Cells

It has been demonstrated that rabbit anti-mouse erythrocyte serum is capable of agglutinating ascites tumour cells[1]. Nothing is known, however, about the components of the erythrocyte which are responsible for producing the agglutinin; the present experiment was carried out to clarify this point, and mouse-specific ascites tumour cells and mouse erythrocytes were used.

Three female guinea-pigs were intramuscularly injected four times with 0·2 ml. erythrocyte ghosts of *CF*1 male mice with Freund's adjuvant every two weeks. Three other animals were treated in the same way with the ghosts of Ehrlich ascites tumour cells (EATC). The erythrocyte ghosts were prepared by Ponder's method[2], and EATC were subjected to Ponder's procedure after homogenization. Two weeks after the last injection the antisera (the anti-mouse erythrocyte ghost and the anti-EATC ghost) were collected from the animals, and were treated at 56° C for 30 min before use. Almost the same technique was adopted for the agglutination test as was used in the previous investigation[3]. The erythrocytes tested were obtained from various strains of mice, such as *A*, *CF*1, *C57BL*, *DDS* and *dd*—though scarcely any difference was observed between the different strains on the titres resulted. The antisera were absorbed with equal volume of each absorbent shown in Table 1. The notation "crushed . . . " means that the absorbents with which the antisera were absorbed were prepared from Ponder's ghosts by pounding them with powdered dry ice using a mortar and pestle, and then washing with saline solution (Fig. 1*a*).

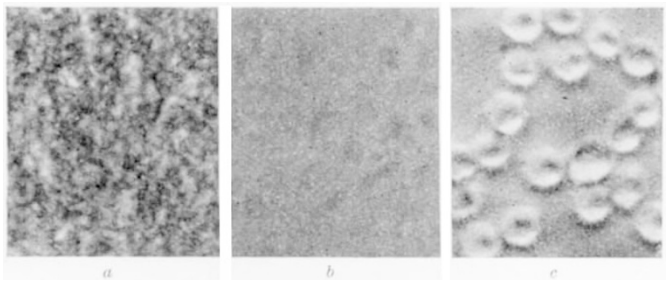

Fig. 1. Mouse erythrocytes used for the absorption (×1,000). *a*, Crushed erythrocyte ghosts; *b*, erythrocyte ghosts by Ponder's method; *c*, intact erythrocytes

Table 1. Agglutination of Mouse Erythrocytes and Ehrlich Ascites Tumour Cells with Various Absorptions

Antiserum	Absorbed* with	Maximum titre† giving agglutination of Mouse eryth.‡	EATC§
Anti-mouse eryth. ghost	No absorbent used	512	64
	Normal mouse sera	512	64
	Ascites of tumour-bearing mouse	512	64
	Intact mouse eryth.	Neg¶	64
	Ponder's mouse eryth. ghosts	Neg	64
	Crushed‖ mouse eryth. ghosts	Neg	Neg
	Crushed guinea-pig eryth. ghosts	512	64
	EATC	512	Neg
Anti-EATC ghost	No absorbent used	8	512
	Normal mouse sera	8	512
	Ascites of tumour-bearing mouse	8	512
	Intact mouse eryth.	Neg	512
	Ponder's mouse eryth. ghosts	Neg	512
	Crushed mouse eryth. ghosts	Neg	4
	Crushed guinea-pig eryth. ghosts	8	512
	EATC	Neg	Neg
Normal guinea-pig serum	No absorbent used	2	Neg

* Antisera were absorbed with equal volume of each absorbent at 37° C for 60 min.
† Equal volume of antiserum dilution and erythrocytes (2·5 per cent in phosphate buffered saline) or Ehrlich ascites tumour cells (5 per cent) incubated at 37° C for 90 min.
‡ Eryth.: erythrocyte.
§ EATC: Ehrlich ascites tumour cell.
¶ Neg: no agglutination at serum dilution 1 : 2.
‖ Crushed erythrocytes were obtained from Ponder's ghosts by pounding them with powdered dry ice in a mortar.

The observations and the findings are briefly summarized here: (1) The ghosts injected as antigens contain practically no water-soluble component. When the antisera are absorbed with normal mouse sera or ascites of tumour-bearing mouse, neither antiserum loses its agglutinating capacity—indicating that any water-soluble antigen is not concerned in these agglutination reactions.

(2) The anti-erythrocyte ghost serum is capable of agglutinating both the mouse erythrocytes and EATC, indicating that the EATC-agglutinogens are shared with erythrocyte ghosts and the cell surface of EATC. The anti-EATC ghost serum can agglutinate EATC strongly, but erythrocytes very weakly. If anti-erythrocyte serum is absorbed with EATC, its titre against erythrocyte does not decrease. When both anti-erythrocyte ghost and anti-EATC ghost sera are absorbed with intact erythrocytes, their titre against EATC never decreases; one may conclude that the haemagglutinogens are different from the EATC-agglutinogens.

(3) When both antisera are absorbed with intact mouse erythrocytes (Fig. 1*c*) or with Ponder's ghosts which keep their disk-like shape (Fig. 1*b*), they retain completely their EATC-agglutinating capacity. On the other hand, they almost lose the capacity when absorbed with crushed mouse erythrocyte ghosts. Both antisera do not, however, lose their agglutinating capacities when absorbed with crushed guinea-pig erythrocyte ghosts. In short, all the foregoing findings indicate that the mouse erythrocyte ghost and EATC contain at least common EATC-agglutinogens, and that the EATC-agglutinogens do not exist on the outer surface of the erythrocyte membrane, but rather seem to exist on the inner surface of the membrane. In other words, the outer surface of the cell membrane of EATC and the inner surface of the cell membrane of mouse erythrocyte closely resemble each other so far as their antigenicities are concerned.

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