SHORT ARTICLES

NEUTRALISATION OF IMMUNOLOGICALLY DISTINCT STAPHYLOCOCCAL DELTA HAEMOLYSINS BY ANTIBODIES

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PURIFIED δ -haemolysin from several canine strains of *Staphylococcus aureus* give reactions of only partial identity in immunodiffusion test with purified δ -haemolysin from *S. aureus* strain CN4108 (Newman) and its corresponding rabbit antibodies (Turner, 1978). This raises the question of whether these antibodies to the δ -haemolysin of strain CN4108 would neutralise the haemolytic activity of δ -haemolysin from the canine strains. Because the δ -haemolysin from both sources was neutralised by a non-immunoglobulin component of normal rabbit serum (Jackson and Little, 1958; Gladstone and Yoshida, 1967; Turner, 1978), it was essential to use only the immunoglobulin fraction of immune sera in this investigation.

MATERIALS AND METHODS

Purification of δ -haemolysin. The purification of δ -haemolysins from strains CN4108 and CN7450 has been described previously (Turner, 1978). The respective activities were 200 and 250 haemolytic units (HU)/mg.

Preparation of antisera in rabbits. The immunising schedule was similar to that of Heatley (1977) except that, to save time, the boosting doses were omitted and only the first six of the weekly doses of δ -haemolysin in Freund's complete adjuvant were administered.

Preparation of immunoglobulin. Immunoglobulin was precipitated from the rabbit sera by the addition of saturated ammonium sulphate at pH 7.0 to give a final concentration equivalent to 30% saturation (Campbell *et al.*, 1970). The precipitated immunoglobulin was centrifuged, dissolved in phosphate-buffered saline (PBS, pH 7.0), diluted to the initial volume of the serum, and precipitated twice more by the same procedure. Pre-immunisation serum from each rabbit was prepared in the same way.

Neutralisation tests. The immunoglobulin preparations were diluted by adding 0.4 ml to 0.2 ml of PBS and then serially transferring 0.4 ml of this dilution through nine more tubes. Delta haemolysin (0.2 ml), which had been assayed and diluted to 10 HU/ml, was added to each tube followed, after 20 min. at 37°C, by 0.4 ml of 1.0% (v/v) washed horse erythrocytes in PBS. After a further 30 min. at 37°C, the tubes were centrifuged and the 50% lysis endpoints determined. This gave the dilution of immunoglobulin that neutralised 1 HU because 2 HU were initially added to each tube. The activity of each immunoglobulin preparation was then expressed as the number of HU neutralised by 1.0 ml. With each immunoglobulin, the specific neutralising activity related to immunisation was determined by subtracting the value (usually about 12 HU, range <7.5–25 HU) given by the immunoglobulin from the corresponding pre-immune serum.

RESULTS AND DISCUSSION

The neutralisation tests with immunoglobulin from eight rabbits immunised with δ -haemolysin from strain CN4108 showed that about 3–5 times more δ -haemolysin CN4108 activity was

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TABLE I

Neutralisation of δ -haemolysins from Staphylococcus aureus strains CN4108 and CN7450 by anti- δ -haemolysin to strain CN4108

Immunoglobulin preparation from rabbit* no.	Number of haemolytic units from indicated strain of <i>S. aureus</i> neutralised by 1.0 ml of immunoglobulin preparation minus control value obtained with pre-immune immunoglobulin	
	δ -haemolysin from strain CN4108	δ -haemolysin from strain CN7450
2044	103	42
2045	148	40
2046	73	30
2047	39	13
2048	57	10
2049	5	< 1
2051	43	5
2052	43	14

* Immunised with δ -haemolysin from *S. aureus* strain CN4018.

neutralised than δ -haemolysin CN7450 (table). Depending on the assumptions made, these results may be explained in different ways. For example, if the affinity of each toxin for the neutralising antibodies was the same but the specific activity of δ -haemolysin from strain CN7450 was lower than that of δ -haemolysin from strain CN4108, more antibody would be needed to neutralise the greater amount of the CN7450 toxin. However, the specific activity of δ -haemolysin from strain CN4108 was about 20% higher than that of strain CN7450 which makes this assumption unlikely. The results could also be explained by assuming that the molecular weight of δ -haemolysin of strain CN4108 was several-fold higher than that of δ -haemolysin CN7450 because in these circumstances less antibody would be required to precipitate the larger molecule. In fact the results obtained on sepharose-6B chromatography (Turner, 1978) indicate that the molecular weight of δ -haemolysin from strain CN7450 is somewhat higher than the molecular weight of δ -haemolysin CN4108; consequently this argument can also be rejected.

In conclusion, the results indicate that antibodies against δ -haemolysin from strain CN4108 do not neutralise δ -haemolysin from strain CN7450 as effectively as the former toxin and further reflect the previously reported immunological relationship of partial identity of the two haemolysins (Turner, 1978).

SUMMARY

Antibodies against δ -haemolysin from *Staphylococcus* strain CN4108 (Newman) did not neutralise δ -haemolysin from the canine strain of *S. aureus* CN7450 to the same extent as δ -haemolysin prepared from *S. aureus* strain CN4108. This is additional evidence for the immunological distinctness of δ -haemolysin from these two *S. aureus* strains of human and canine origin.

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