Effects of meningococcal and *Escherichia coli* capsular polysaccharides on human and dog platelet aggregation *in vitro*

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SYNOPSIS Highly purified capsular polysaccharides from groups A, B, and C meningococci and from two strains of *Escherichia coli* did not aggregate human or dog platelets *in vitro*. Nor was there any detectable effect on platelet aggregation induced by adenosine diphosphate (ADP), epinephrine or collagen. The results do not support the hypothesis that capsular polysaccharides are involved in the pathogenesis of thrombocytopenia often seen in severe infections with these bacteria.

In recent years soluble bacterial antigens have been detected in serum and other body fluids from patients with various bacterial infections. Rytel *et al* (1974) have described two cases of fatal pneumococcal disease with disseminated intravascular coagulation (DIC) and circulating polysaccharide antigen. The serum levels of antigen were higher than in other cases of pneumococcal infections in which DIC did not develop, and the authors proposed that pneumococcal polysaccharides play a role in the pathogenesis of DIC. Similarly, Hoffman and Edwards (1972) detected circulating polysaccharide antigen only in those patients with meningococcal septicaemia who had signs of DIC.

The present experiments were performed to investigate a possible effect of purified meningococcal and *Escherichia coli* capsular polysaccharides on platelet function *in vitro*.

Material and Methods

Blood was collected from human volunteers and from mongrel dogs by peripheral venepuncture. The anticoagulants used were either heparin (5000 units per ml; Vitrum AB, Stockholm, Sweden) at a final concentration of 4 units per ml of blood, or 0.13 M (3.8%) trisodium citrate, 1 part to 9 parts of blood. Adenosine-5'-diphosphate (ADP)-sodium (Stago Laboratoire, Asnières sur Seine, France) 0.2 mg was dissolved in distilled water and further diluted in Michaelis buffer.

Collagen: Stago Laboratoire

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Epinephrine: Stago Laboratoire, 0.915 mg per ml further diluted in Michaelis buffer

Endotoxin (lipopolysaccharide): A highly purified endotoxin, extracted from *Salmonella typhi-murium* SL 4504 by the hot water method (courtesy of Dr A. Lindberg, Statens Bakteriologiska Laboratorium, Stockholm), was dissolved in isotonic saline.

Polysaccharides: Highly purified capsular polysaccharides from *Neisseria meningitidis*, groups A, B, and C, as well as from *E. coli* K 235 and *E. coli* Boston 12 were kindly provided by Dr E. Gotschlich, The Rockefeller University, New York (Gotschlich *et al*, 1969). They were dissolved in water and thereafter stored in a frozen state.

Platelet-rich-plasma (PRP) was obtained by centrifuging anticoagulated blood for 20 minutes at 200 g. Platelet-poor-plasma (PPP) was obtained by centrifuging blood for 20 minutes at 18 000 g. The PRP was diluted with its own PPP to a final concentration of 200 000-250 000 platelets per ml.

Platelet aggregation was determined at 37°C by the photometric method described by Born (1962) and modified by O'Brien (1962). The test substance, 0.1 ml, was added to 0.9 ml of PRP. Each substance was tested in heparinized as well as in citrated PRP from two humans and two dogs. The aggregation rate (decrease in optical density per minute) was calculated from the steepest slope of the initial aggregation curve (O'Brien *et al*, 1966).

Results

The five bacterial polysaccharides (final concentra-

	n	Aggregation Rate (Δ OD) (% min)			Maximal Decrease in OD		
		PS	LPS	ADP	PS	LPS	ADP
Human platelets	4						
Heparinized plasma	2	0	10	70	0	8	36
Citrated plasma	2	0	0	45	0	0	20
Dog platelets	4						
Heparinized plasma	2	0	15	65	0	10	20
Citrated plasma	2	0	0	48	0	0	15

Table Platelet aggregation by capsular polysaccharides (PS) from *Neisseria meningitidis* groups A, B, and C, and two strains of *E. coli*, and by endotoxin (LPS) from *S. typhi-murium*

OD = optical density

ADP was used as a control. Each figure represents the mean of two determinations.

tion 2 mg/ml) did not aggregate human or dog platelets in heparinized or citrated plasma. *Salmonella typhi-murium* endotoxin (final concentration 2.5 mg/ml), on the other hand, caused a weak reversible aggregation of human as well as dog platelets in heparinized, but not in citrated plasma. When lower concentrations were used no aggregation was observed. ADP (1×10^{-6} M) was used as a control of both human and dog PRP (table).

Platelet aggregation induced by ADP, epinephrine, and collagen was also studied after prior incubation of human heparinized PRP with each polysaccharide (final concentration 2 mg/ml) for 5 minutes at 37°C. Isotonic saline was run as a negative control. No potentiation or inhibition of the aggregation was observed.

Discussion

Thrombocytopenia and disseminated intravascular coagulation (DIC) are common complications of Gram-negative septicaemia (Corrigan *et al.*, 1968; Yoshikawa *et al.*, 1971). Endotoxins liberated from the Gram-negative cell wall are commonly believed to be responsible for coagulation abnormalities as well as many other clinical manifestations in humans with Gram-negative septicaemia. Proof is lacking, however (Hjort and Rapaport, 1965; Levin, 1973).

Thrombocytopenia and DIC may occur also in infections caused by Gram-positive bacteria, for instance pneumococci and streptococci, which do not elaborate endotoxin (Yoshikawa *et al.*, 1971). Recent investigations have shown an association between high serum levels of capsular polysaccharides and the presence of coagulation disorders in meningococcal and pneumococcal septicaemia, and it has been suggested that these substances are involved in the pathogenesis of DIC (Hoffman and Edwards, 1972; Rytel *et al.*, 1974).

This hypothesis was not supported by our results. We could not demonstrate any significant influence

on human or dog platelet aggregation *in vitro* by polysaccharides derived from meningococci groups A, B, and C and two strains of *E. coli*. The absence of aggregation cannot be ascribed to suboptimal concentrations of divalent cations since the results were the same in heparinized and in citrated plasma. *Salmonella typhi-murium* endotoxin aggregated human platelets only slightly, while the effect on dog platelets was more pronounced. This is in agreement with the findings of others (Mustard and Packham, 1970; Ream *et al.*, 1965; Nagayama *et al.*, 1971). The high concentrations of endotoxin, used to achieve only comparatively slight influence on platelet function *in vitro*, are discordant with the marked thrombocytopenia, which develops after injection of very small quantities of endotoxin into animals (Gilbert, 1960). Endotoxin thus seems to exert its effect on platelets by some intermediary mechanism that is not functioning *in vitro*. It can not be excluded that capsular polysaccharides have a similar indirect effect on platelets, although it seems less likely in view of the general lack of toxicity of these substances. Thus meningococcal polysaccharides have been used for immunization against meningococcal disease with few side effects, and tests in mice and guinea pigs have not revealed any significant toxicity (Gotschlich *et al.*, 1969).

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