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

**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* K 12 were kindly provided by Dr E. Gotschlich (The Rockefeller University, New York) and Dr Alf Rytel (Stockholm). They were dissolved in water and thermostated at 37°C before use.

**Platelet-rich-plasma (PRP) was obtained by centrifuging anticoagulated blood for 20 minutes at 100 g.** Platelet-poor-plasma (PPP) was obtained by centrifuging blood for 20 minutes at 18 000 g. The PPP 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 PPP 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 concentration 1 mg/ml) did not induce platelet aggregation in either PPP or PRP. The aggregation rate of citrated PPP in the control experiment with ADP was 0.645 ± 0.014 arbitrary units per min (mean ± SEM of 5 experiments).

**Discussion**

These results could be explained by the suggestion that the circulating capsular antigens described by Rytel *et al* (1974) are possibly bound by other antigens in the body fluids. It could also be that the bacterial polysaccharides are not involved in the pathogenesis of DIC in meningococcal infection.
Effects of meningococcal and E.-coli capsular polysaccharides on platelet aggregation

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<th>Aggregation Rate (Δ OD) (%) min</th>
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<td>Human platelets</td>
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<td>Dog platelets</td>
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<td>Heparinized plasma</td>
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<td>Citrated plasma</td>
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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.

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 septicemia. 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).

References

Hjort, P. F. and Rapaport, S. I. (1965). The Shwartzman...


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