Deficiency of prostacyclin production in meningococcal shock

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Abstract
A deficiency of prostacyclin (PGI₂) production by the vascular endothelium might underline the severe vasoconstriction and intravascular thrombosis that characterise meningococcal shock. The effect on PGI_2 synthesis by human umbilical vein endothelial cells (HUVEC) in culture was examined in sera from children with meningococcal shock, healthy adults, and children with other febrile illnesses. In comparision with adult controls, PGI_2 synthesis was reduced when HUVEC were incubated with the sera from 10 of 13 patients with meningococcal shock. A similar defect was observed with only four of 20 sera from children with other febrile illnesses. The effect of sera from patients with meningococcal shock on HUVEC was reversible with normal serum, and seems to be due to the absence of a factor necessary for PGI_2 production rather than an inhibitor.

These findings suggest that a deficiency of PGI_2 may have a role in the pathogenesis of meningococcal shock and that exogenous PGI_2 may be of therapeutic benefit.

Despite early diagnosis and appropriate treatment the mortality from meningococcal infection has not decreased over the last 30 years. The case mortality overall is 10% and rises to an unacceptable 40% for those with septicaemia and shock.[1] Attempts to improve the prognosis have so far been hampered by a lack of understanding of the pathophysiology of the disorder.

Clinical observation suggests that there are three major processes involved in the pathogenesis of meningococcal shock. Firstly, an increase in vascular permeability allows albumin to leak from the circulation, resulting in profound hypovolaemia and oedema.[2] Secondly, intense vasoconstriction of some vascular beds and dilatation of others leads to impaired organ perfusion.[3] Thirdly, intravascular thrombosis of both large and small vessels associated with platelet and clotting factor consumption intensifies tissue ischaemia.[4] These three features suggest that in meningococcal sepsis there is disruption of the homoeostatic function of the endothelium that normally maintains vascular permeability and thromboresistance.

Disseminated intravascular coagulation, with activation of clotting factors and deposition of fibrin within the microvasculature, has been considered to be an essential component of meningococcal disease. Several studies have documented both thrombocytopenia and derangement of coagulation.[4 5] However, the mechanism responsible for the thrombocytopenia and the role of the disordered endothelial-platelet interactions in the pathophysiology of meningococcal infection have not been well studied.

Prostacyclin (PGI₂) is a potent inhibitor of platelet aggregation and a powerful vasodilator.[6] It is released from membrane bound arachidonic acid and is actively produced by the vascular endothelium. Together with other important endothelium derived platelet inhibitory factors, such as nitric oxide,[7] PGI_2 has an important role in thromboresistance. PGI_2 production increases appreciably in response to hypoxia and stress,[8] suggesting that under these circumstances it may be particularly important in preventing intravascular platelet activation and tissue ischaemia.

We postulated that a deficiency of PGI_2 synthesis by the endothelium might be involved in the intravascular thrombosis and vasoconstriction seen in meningococcal disease. We suggest that this defect may be due to the absence of a factor necessary for PGI_2 production or the presence of an inhibitor in the serum of patients with the disorder. We therefore investigated the effect of sera from patients with meningococcal shock on endothelial PGI_2 production, and have compared this with that seen from sera from children with other febrile illnesses.

Patients and methods
PATIENTS WITH MENINGOCOCCAL DISEASE
Twenty three children (mean age 4·6 years; range 0·3 to 12·7 years) with severe meningococcal disease, referred to the Hospital for Sick Children, Great Ormond Street over the last six years, were studied. The diagnosis was made clinically on the basis of a characteristic purpuric rash in all cases and was confirmed by culture and/or serum antigen test in 18 patients. The haemophilus rapid antigen test was negative in the remaining patients. Shock was detected in 18 patients, this was defined by at least two of the following: impaired peripheral perfusion, as signified by poor capillary return, oliguria (less than 1 ml/kg/hour) or hypotension (at least 2 SD below the mean for age). Serum was available, in the acute phase, from only 13 of the patients with meningococcal shock.

CONTROLS
(a) Healthy adults
Serum was collected from 12 hospital workers

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Accepted 17 July 1991

who did not have a history of significant disease. This department has previously compared normal childhood and adult sera in the bioassay employed in this study and has not found any difference between the groups. Adult sera were therefore used to standardise the assay because of the ethical and logistic difficulties involved in obtaining samples from normal children.

(b) Children with other febrile illnesses

Serum was obtained from 20 children admitted to this hospital. Seven had non-meningococcal meningitis or encephalitis, one septicaemia, two Kawasaki disease, four toxic shock syndrome, two polyarteritis, and four had mild febrile illnesses. Shock, as defined by the criteria above, was diagnosed in six of these patients: four with toxic shock syndrome, one with haemophilus meningitis, and one with bacteroides septicaemia.

COLLECTION OF SAMPLES

Blood was collected within 24 hours of admission and serum separated within three hours. The samples were either used immediately or stored at $-70°C$ until required.

CELL CULTURE

Human umbilical vein endothelial cells (HUVEC) were isolated by established methods[9] and cultured in Dulbecco's modified Eagle's medium containing 3·5 g/l sodium bicarbonate to which 1% L-glutamine, 20% heat inactivated fetal calf serum, and 1% penicillin and streptomycin were added. Cells in first passage were grown to confluence in 24 well, flat bottom plates (Flow; 2·0 cm² per well).

BIOASSAY FOR PGI₂ PRODUCTION BY HUVEC IN CULTURE

Previously described methods were adapted to detect PGI_2 in the supernatant of HUVEC in culture.[10] All experiments were performed in duplicate. Twenty four well plates with confluent HUVEC were maintained at 37°C in a heating block. The wells were aspirated to near dryness and then incubated, in duplicate, with 500 µl of culture medium (without fetal calf serum) containing 20% patient or control serum. After 10 minutes, 100 µl of culture supernatant was aspirated and added to 100 µl of normal platelet rich plasma (platelet count 300 to 500×10⁹/l). The mixture was stirred for one minute, in a PAP-4 (BIO/Data) aggregometer, the platelets were then challenged with ADP (2–4 µmol/l), and aggregation recorded. The inhibition of platelet aggregation by the culture supernatants was expressed as a percentage of the maximal aggregation obtained with platelet rich plasma and fresh medium that had not been incubated with HUVEC (percentage full aggregation). Neither patient nor control serum was found to affect platelet aggregation without prior incubation with HUVEC, excluding the possibility that the sera used contained a factor or factors that stimulate platelet aggregation.

Where supernatants were found not to inhibit

platelet aggregation, crossover experiments were performed. The culture supernatants used for the initial assay were removed and the cells were reincubated with fresh medium containing 20% control serum for 10 minutes. The culture supernatants were then assayed for recovery of PGI_2 activity.

MEASUREMENT OF 6-KETO PGF₁α

In order to confirm the results of the bioassay, the stable metabolite 6-keto $PGF_{1\alpha}$ was measured using a radioimmunoassay kit (Amersham). 6-Keto $PGF_{1\alpha}$ was measured in culture supernatants after incubation of HUVEC with the sera from seven children with meningococcal shock, which had been found not to induce PGI_2 production employing the bioassay method and eight normal adult controls.

STATISTICS

For the PGI_2 bioassay, a median and the 95% confidence intervals were calculated. The difference between the three patient groups was assessed using a Kruskal-Wallis analysis of variance. A rank sum test was employed to show the recovery of PGI_2 production by HUVEC and to express the results from the 6-keto $PGF_{1\alpha}$ assay.

Results

PGI₂ SYNTHESIS BY HUVEC

The sera from 12 normal adults induced PGI_2 synthesis by HUVEC (fig 1), as measured by inhibition of platelet aggregation by culture supernatants (median percentage full aggregation 21·5; 95% confidence interval 7 to 36). In contrast, the serum from 10 of 13 patients with meningococcal shock failed to support endothelial PGI_2 production (median percentage full aggregation 89; 95% confidence interval 36 to 98). Similar PGI_2 stimulating activity to the controls was observed with the sera from 16 of
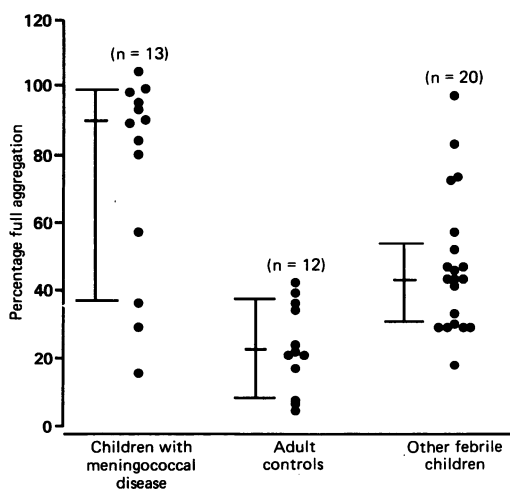

Figure 1   Human umbilical vein endothelial cell PGI_2 production after incubation with sera from patients with meningococcal shock, adult controls, and children with other febrile illnesses (Kruskal-Wallis analysis of variance, $p<0.05$).

20 children with other febrile illnesses (median percentage full aggregation 43; 95% confidence interval 30 to 52). In four children, three with severe illness (pneumococcal meningitis, toxic shock syndrome, and bacteroides septicaemia respectively) and one with Lyme disease, PGI_2 production was diminished. The three patient groups were shown to be different by Kruskal-Wallis analysis of variance ($p<0.05$).

The difference between the sera from the patients with meningococcal shock and the adult controls demonstrated by bioassay was confirmed using the 6-keto $PGF_{1\alpha}$ radio-immunoassay. On incubation of HUVEC with sera from meningococcal shock patients, the median 6-keto $PGF_{1\alpha}$ detected was 4·2 ng/ml (range 2·7–6·6) in comparison to 11·3 ng/ml (range 2·2–31) with HUVEC incubated with control sera (rank sum test, $p<0.03$).

CROSSOVER EXPERIMENTS

When HUVEC previously cultured with sera from patients with meningococcal shock were reincubated with normal serum there was a significant increase in PGI_2 production (rank sum test, $p<0.03$) (fig 2).

CHARACTERISATION OF THE DEFECT

Heating sera from patients with meningococcal shock to 56°C for either 10 or 30 minutes did not affect its activity. Storage of these sera for more than two weeks at 4°C did not change their effect on PGI_2 synthesis. Mixing experiments, employing the bioassay, have shown that addition of as little as one part normal serum to three parts serum from patients with meningococcal shock, restores the ability to induce endothelial PGI_2 production (results not shown), suggesting that normal serum is augmenting the meningococcal serum rather than reversing an inhibitor.

PLATELET AND CLOTTING STUDIES
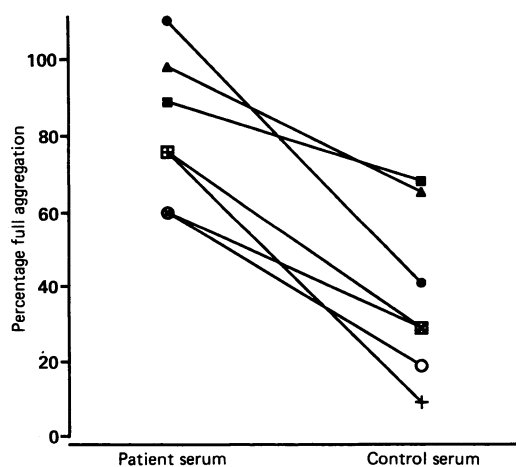
Platelet and clotting studies were available from

Figure 2   Crossover experiments. Human umbilical vein endothelial cells initially cultured with the sera from meningococcal patients and then reincubated with adult control sera recovered the ability to produce PGI_2 (rank sum test, $p<0.03$).
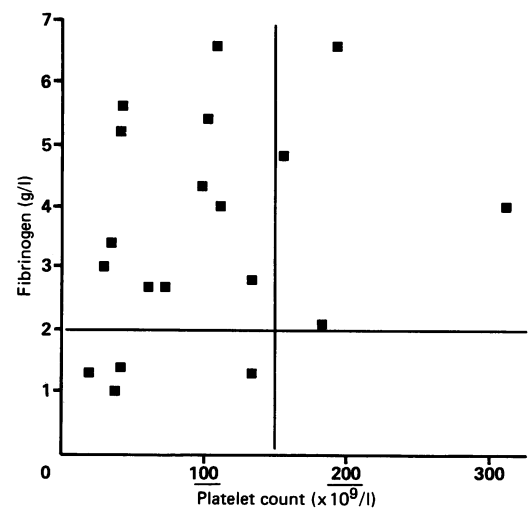
Figure 3   The platelet counts plotted against the fibrinogen concentrations from 19 children with severe meningococcal disease measured in the first 24 hours of the disease (bars indicate the lower limits of normal).

the records of 19 of the 23 children with severe meningococcal disease. In the first 24 hours after admission, 79% of these patients were thrombocytopenic but only 21% had low fibrinogen concentrations (fig 3). Platelet aggregation studies were performed on two patients with meningococcal shock. A poor response to ADP and adrenalin was observed, implying that in vivo platelet activation had occurred.

Discussion

We have shown a reduction in PGI_2 synthesis by HUVEC when incubated with the sera from 10 of 13 children with meningococcal shock when compared with normal controls. The abnormality was demonstrated both by the standard bioassay for PGI_2 and by the measurement of 6-keto $PGF_{1\alpha}$. The effect of sera from children with meningococcal shock on HUVEC was shown to be reversible, establishing that the PGI_2 deficiency was not due to a permanent alteration in endothelial cell function. The defect was not affected by heating to 56°C or by prolonged storage in the cold. Mixing experiments suggest that this phenomenon may be due to the absence of a factor necessary for PGI_2 synthesis rather than an inhibitor. The defect is not specific for meningococcal shock as it was also observed with the sera from four of 20 children with other febrile illnesses. The clinical manifestations and pathophysiology of severe infections overlap and it is therefore not surprising that similar defects may occur in the late stages of a variety of infections. None the less, if these in vitro findings reflect the in vivo situation, patients with meningococcal shock may have a defect in PGI_2 production by the vascular endothelium. This may be an important factor in the vasoconstriction, intravascular thrombosis, and platelet consumption seen in the disorder.

Impaired PGI_2 synthesis has been implicated in the pathogenesis of a number of other diseases, including sporadic haemolytic uraemic

syndrome,[11] systemic lupus erythematosis,[6] and pre-eclamptic toxaemia.[6] These conditions are all associated with endothelial damage, platelet activation, and the formation of microthrombi. We therefore looked for features of platelet activation and disseminated intravascular coagulation in 19 children with severe meningococcal sepsis in the first 24 hours of the illness. Whereas fibrinogen concentrations were normal or increased, thrombocytopenia was present in the majority of patients and was often profound and persistent. The impaired response to platelet agonists documented in the small number of patients studied by ourselves and others[5] suggest that in meningococcal disease platelets circulate in an 'exhausted' form.[12] Animal studies have also demonstrated intravascular platelet activation in endotoxaemia,[13] which together with the observed failure of heparin treatment to reduce the intravascular thrombosis of meningococcaemia,[5] adds weight to the possibility that platelet activation and consumption may be more important than the depletion of coagulation factors in this disease.

If endothelial PGI_2 synthesis is diminished in meningococcal disease, this may be an important area for therapeutic intervention. In animal models, PGI_2 infusions have been shown to be effective in reversing the consequences of endotoxaemia, improving renal perfusion, and ameliorating intravascular thrombosis.[13 14] Bihari and Tinker have found PGI_2 to be a valuable vasodilator in critically ill adults, improving oxygen delivery and so tissue oxygen consumption. Further studies to confirm the presence of defective PGI_2 production in vivo and to evaluate the potential use of exogenous PGI_2 in meningococcal shock are needed.

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