Chemotactic activity of Neisseria gonorrhoeae

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SUMMARY Sonicated whole cells of one of three tested gonococcal strains stimulated neutrophil migration in the agarose gel model. The activity was retained by the material pelleted by high-speed centrifugation of the sonicate. This supports the theory that certain strains possess a cytotaxin bound to the outer membrane.

Introduction

Opposing views have recently been expressed concerning the capacity of the gonococcus to produce chemotactic factors (cytotaxins) in the absence of complement. James and Williams, using the agarose gel method, found that gonococci could produce cytotaxins in liquid medium but that the response depended on the growth medium and the particular strain used. The chemotactic factor was stable to 100°C for 30 minutes. Watt and Medlen, using Boyden chambers, could not find cytotaxins in the supernatants of 24-hour broth cultures nor in the butanol-acetic extracts of these supernatants. Outer envelope preparations at a concentration of 1 mg/ml were, however, chemotactic. They concluded that chemotaxis is probably a consequence of an interaction of natural antibodies with the gonococcus causing complement activation.

In the present study we showed that whole gonococci contained chemotactic substances, which are part of high-molecular weight complexes.

Materials and methods

Strain 15057, an isolate from the male urethra, has been described. Strain F62 was kindly obtained from Dr T M Buchanan, Seattle, Washington, USA, and strain 5556 was a local isolate from the male urethra.

Gonococci were grown in liquid culture medium according to Wolf-Watz (proteose peptone No 3 (Difco), 15 g; corn starch, 1 g; K$_2$HPO$_4$, 4 g; KH$_2$PO$_4$, 1 g; and NaCl, 1 g to 1 l of medium and one ampoule of IsoVitalex and 20 ml 0.5 mol/l NaHCO$_3$ added before use) at 37°C on a rotary shaker until late log phase (about 15 hours) from small inocula of T2 colonies. The cells were harvested at 10 000 x g for 15 minutes and washed in PBS; the wet weight was determined and they were frozen at a concentration of 250 mg/ml. The sample was sonicated in 30-second bursts on ice in a 100 W MSE apparatus to more than 95% lysis. In one experiment the culture fluid was centrifuged at 100 000 x g for one hour after precipitation of whole organisms as described above, the pellet washed once in Parker 199 medium, resedimented at 100 000 x g, and suspended in Parker 199 medium.

Spontaneous and stimulated neutrophil migration was assessed as described. Isolated leucocytes (1 x 10⁴ neutrophils/μl) which had been washed twice and obtained after dextran sedimentation of erythrocytes were added to a 10 μl well in an agarose plate. Opposing this well were two other wells; one contained the different gonococcal preparations or an Escherichia coli supernatant bacterial factor (BF) and the other control media (to assess spontaneous migration).

Statistical analyses were performed with Student’s t test.

Results

The sonicated whole 15057 gonococci (at a concentration of 250 mg/ml wet weight) were as active as BF as a cytotaxin (figure). Both significantly stimulated neutrophil migration compared with spontaneous migration (p<0.001). The culture medium in which the gonococci had been grown, however, exerted a stimulatory effect, which was about half that of the gonococcal preparation. Two different preparations of 15057 were tried and found to be equally active whereas two other strains (F62 and 5556) caused only a migration similar to the medium’s (data not shown). The washed pellet, produced by centrifugation at 100 000 x g for one
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![Graph showing distance to the leading front cells migrating under agarose, either spontaneously (A) or after stimulation with the sonicated whole gonococcal strain 15057 (B), the bacterial factor (BF) from E coli (C), the culture medium (D), and the washed pellet obtained after centrifugation (E). (** = p < 0.001 and *** = p < 0.001 when compared with migration elicited by the medium alone.) The numbers at the bottom of the bars denote the number of experiments. The broken line represents the mean ± 2 SD limit for spontaneously migrating neutrophils determined from the analyses of 75 healthy volunteers.](http://sti.bmj.com/)

Discussion

This finding supports those of James and Williams that certain strains can produce substances capable of stimulating migration of neutrophils. Since the present experiments were performed in the absence of plasma or serum this factor evidently does not belong to the complement system. The finding that the activity could be pelleted from the culture fluid supernatant supports that of Watt and Medlen that the gonococcal outer envelope also contains active substance. Future research is needed, however, to determine its chemical nature—that is, whether it belongs to those formylated peptides or lipids which have previously been suggested as the main source of bacterial chemotactic factors. Finally, the excretion of the present chemotactic substance by certain gonococcal strains may contribute to the classical gonococcal discharge.

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References

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