

NOTES

Studies on Gonococcus Infection

IX. In Vitro Decreased Association of Pilated Gonococci with Mouse Peritoneal Macrophages

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Pili, in addition to enhancing attachment of gonococci to tissue culture cells, appear to reduce association (attachment/ingestion) of *Neisseria gonorrhoeae* with mouse peritoneal macrophages in vitro.

Pili have been demonstrated on organisms from those colony forms of *Neisseria gonorrhoeae* (5, 19) associated with virulence (7, 8). These pili appear to enhance the attachment of gonococci to tissue culture cells (16, 17), human sperm (4), buccal mucosal cells (13), and fallopian tubal epithelium (24). Reduction of gonococcal phagocytosis has also been attributed to pili (11, 22), but our studies suggest that human neutrophil-gonococcus interactions are primarily determined by a different, nonpilus gonococcal surface factor (18, 20, 21).

The present study suggests that pili reduce interactions (attachment/ingestion) between gonococci and mouse peritoneal macrophages in vitro. This effect is independent of the neutrophil-associating properties of the gonococci.

Isolation, characterization, and propagation of the gonococci used have been described (18). Nonactivated macrophages were obtained (15) through washing the peritoneal cavities of Black/6 mice with Dulbecco modified medium (Grand Island Biological Co.) containing NaHCO₃ (0.5 g/liter), NaCl (2.23 g/liter), *N*-2-hydroxyethyl-piperazine-*N'*-2'-ethanesulfonic acid (20 mM), and 1% heat-inactivated (56 C, 30 min) fetal calf serum (Grand Island Biological Co.) and allowing the washed, enumerated macrophages (5 × 10⁵/0.1 ml) to attach to 18-mm diameter glass cover slips for 30 min (at 36 C in a 5% CO₂ atmosphere). The macrophages were washed and overlain with similar medium which contained more NaHCO₃ (2 g/liter) and fetal calf serum (10%), but less NaCl (1.18 g/liter). Penicillin and streptomycin (10 mg/liter, Grand Island Biological Co.) were added in those experiments utilizing macro-

phages incubated for a prolonged period (24 to 48 h) in vitro, and were washed with penicillin and streptomycin-free medium before addition of gonococci. Gonococci were incubated with macrophage-laden cover slips on a rotating platform (36 C, 5% CO₂). Gonococcus-macrophage association was usually assessed by light microscopic (Zeiss Photomicroscope II with ×63 phase-contrast oil immersion objective) examination of air-dried, safranin (0.5%)-stained cover slips. In addition, on several occasions cover slips were incubated with fluorescein-conjugated antigenococcus antiserum (obtained from Tom Buchanan, Rockefeller University) used at a dilution (1:20) allowing visualization of both pilated and non-pilated gonococci. At least 400 macrophages were assessed for the percentage displaying attached or ingested gonococci. Mouse peripheral blood leukocytes were obtained through cardiac puncture. Both these leukocytes and human peripheral blood leukocytes were prepared, incubated, and evaluated as previously described (18).

Gonococci are readily visualized in the safranin-stained specimens (Fig. 1a and b). Organisms attached to macrophages, as well as gonococci which may have been ingested by the phagocytes, are found. Both of these interactions were scored as gonococcus-associated macrophages.

In 10 separate experiments utilizing macrophages tested 1 to 2 h after attachment to glass, the association with macrophages was studied for both pilated (T2) and the two previously differentiated forms of non-pilated organisms (T4 and T4*). Although these nonpilated organisms show distinct different levels of associa-

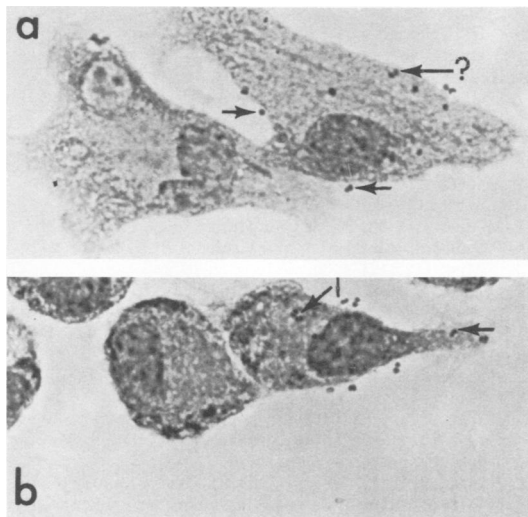


FIG. 1. Mouse peritoneal macrophages in monolayer culture (1 h after plating) incubated with gonococci exhibit organisms clearly attached (arrows), as well as bacteria which clearly have been ingested (i) by the phagocytes. Other gonococci (?) have an ambiguous relationship with the macrophages by light microscopy and may be either attached or ingested. Safranin strain, phase contrast, $\times 1400$.

tion with both human and mouse peripheral blood neutrophils (T4 = low level, T4* = high level), they exhibit similar, higher levels of attachment/ingestion by mouse peritoneal macrophages than pilated, T2 organisms. These data, shown in Fig. 2, demonstrate that both nonpilated forms (T4 and T4*) exhibit 2 to 2.5 times higher levels of association with the macrophages than do pilated organisms. These relative levels (T4 = T4* > T2) were also observed in experiments utilizing macrophages after their culture on glass for 24 or 48 h. The association levels for all organisms tested under these conditions were slightly higher than if macrophages were utilized soon after incubation in vitro; but relative levels were the same for the different organisms (T2, T4, T4*) used. In these incubations, 2×10^7 gonococci were incubated with 5×10^5 macrophages for 30 min (multiplicity = 40 gonococci/macrophage). In other experiments, varying numbers of organisms were added to a constant number of macrophages. The results are shown in Fig. 3.

The bacterial surface may influence virulence by diverse mechanisms several of which have been reviewed recently (14). Restricting phagocytosis by polymorphonuclear leukocytes is well known for pneumococcal capsules and streptococcal M-protein (9, 25). Such phagocytosis reduction has been correlated with the charges

that specific components confer on the surfaces of bacteria (23). Some gram-negative bacteria exhibit linkage of virulence with specific side-chain cell wall antigen moieties which also appear to reduce uptake of the organisms by macrophages (3, 10). Attachment of bacteria to epithelial or epithelium-derived cells also depends on the surface characteristics of the organisms. In several instances, surface components associated with virulence have been shown to increase attachment of the organisms to these eukaryotic cells (1, 2, 6, 12). Pili on gonococci affect such increased attachment (16-18). The present study indicates that pili also reduce association of gonococci with macrophages in vitro. This dual function of gonococcal pili (enhanced attachment to epithelial cells

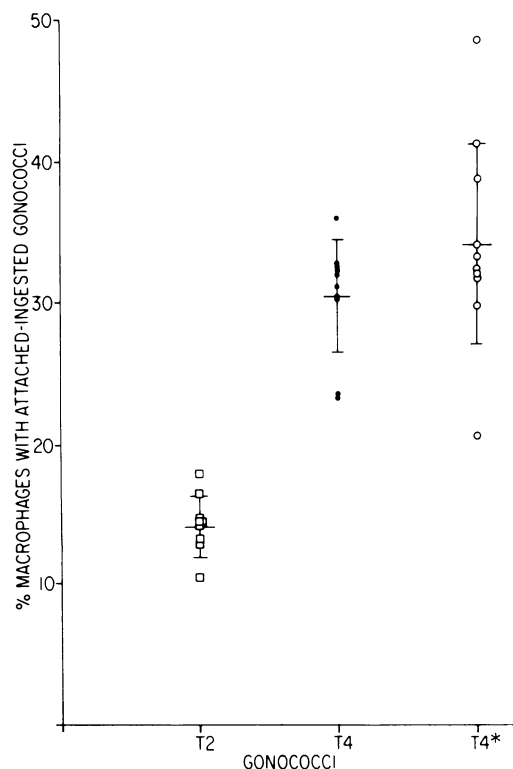


FIG. 2. Percentages of macrophages which have attached and/or ingested gonococci are shown for 10 separate experiments. In each experiment, pilated, type 2 (□) as well as two forms of nonpilated gonococci (type 4, ●; and type 4*, ○) were incubated with monolayers of macrophages derived from the same preparation. Incubations for 30 min on a shaking platform were followed by washing the macrophage monolayers which were then stained and microscopically examined. The mean and standard deviations for percentages of macrophages with which each gonococcal type was associated are shown.

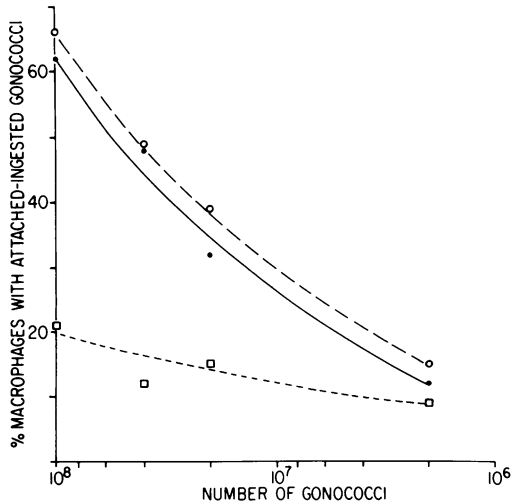


FIG. 3. Dose-response curve for macrophage association with each of the three gonococcal types (T2, \square ; T4, \bullet ; T4*, \circ) is shown. The numbers of gonococci used constitute an input multiplicity range of approximately 4 to 200 gonococci/macrophage.

and reduced association with macrophages) is similar to the recently demonstrated properties of M-protein on streptococci which appears not only to reduce phagocytosis by neutrophils, but also to increase the attachment to pharyngeal mucosa for these organisms.

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