INFECTION AND IMMUNITY, Dec. 1981, p. 1056-1058
0019-9567/81/121056-03$02.00/0

Vol. 34, No. 3

NOTES

Host Species-Specific Damage to Oviduct Mucosa by *Neisseria gonorrhoeae* Lipopolysaccharide

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Received 1 June 1981/Accepted 17 August 1981

The selective toxicity of gonococcal lipopolysaccharide for the mucosa of human fallopian tubes, which is demonstrated in these studies, may be responsible in part for the specificity of naturally occurring gonococcal infections for humans.

In contrast to the rapid loss of ciliary activity in organ cultures of human fallopian tubes infected with colony type 1 gonococci, similarly infected organ cultures of oviducts from rabbits, pigs, and cows maintain both their ciliary activity and their histological architecture (6, 15). The observation that gonococci attach to and damage the mucosa of the human fallopian tube, but not the mucosa of oviducts from the other species, suggested that the ability of gonococci to adhere selectively to human genital tissue might be partly responsible for the host specificity of naturally occurring infections with *Neisseria gonorrhoeae* (6). Further studies have demonstrated that part of the damage caused by gonococcal infection in human fallopian tube organ cultures is mediated by a subcellular toxic factor or factors released by gonococci (10-12). This toxic activity results in damage to human but not porcine or bovine oviduct mucosa in organ culture (5). A majority of this toxic activity appears to be mediated by gonococcal lipopolysaccharide (LPS) (12), and purified gonococcal LPS is capable of damaging human fallopian tubes in organ culture (3, 8). The present studies were undertaken to determine whether the host specificity of damage to oviduct mucosa in organ culture, demonstrated with gonococcal infection and with gonococcal toxic factor(s), was also demonstrable with purified gonococcal LPS.

N. gonorrhoeae 2686, colony type 1 (7), transparent (Tr^+) (10, 14), was used to prepare purified LPS as previously described (3). Oviducts (fallopian tubes) from humans or animals were obtained, and organ cultures were prepared essentially as previously described (6, 9). The oviducts from one pig and from one cow were prepared and maintained for the first 24 h in the laboratory in HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-minimal essential medium (MEM) supplemented with carbenicillin (200 μg/ml; Beecham Laboratories, Pittsburgh, Pa.) and cefazolin (100 μg/ml; Eli Lilly & Co., Indianapolis, Ind.), but in experiments using oviducts from all the other animals and from humans, the organ culture medium was supplemented with vancomycin hydrochloride (5 μg/ml; Eli Lilly & Co., Indianapolis, Ind.) and colistin sulfomethate sodium (3 μg/ml; Warner-Lambert, Morris Plains, N.J.) for 24 h after preparation. At 24 h after preparation of fallopian tube or oviduct organ cultures, the tissue pieces were rinsed in HEPES-MEM without antibiotics, and they were maintained thereafter in the same medium. Purified gonococcal LPS was tested for its effects on the ciliary activity of these various oviducts in organ culture by methods previously described (3). Mucosal ciliary activity was monitored before and 24 h after the addition of gonococcal LPS, and damage was quantititated as the percent of the periphery of oviduct pieces with ciliary activity remaining, as previously described (9).

Four experiments were performed with human fallopian tubes and a final LPS concentration of 1.5 μg/ml. This concentration of gonococcal LPS was lower than that present in most of the filter-sterilized supernatant fluids of organ cultures infected for 48 h with gonococci (12). This concentration of LPS reproducibly and substantially damaged the mucosa of human fallopian tubes in organ culture (Fig. 1). By contrast, in two experiments using rabbit oviducts, one experiment using pig oviducts, and two experiments using cow oviducts in organ culture, gonococcal LPS at a final concentration

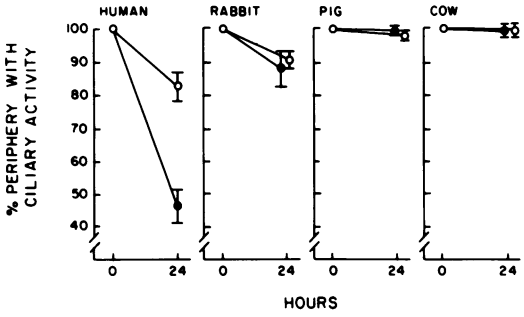

FIG. 1. *Effect of purified gonococcal LPS on the ciliary activity of human, rabbit, pig, and cow oviducts in organ culture. Organ cultures were incubated for 24 h in a medium of either HEPES-MEM* (○-----○) *or HEPES-MEM containing gonococcal LPS* (○-----●) *in a concentration of 1.5 μg/ml (four humans and one cow) or 5.0 μg/ml (two rabbits, one pig, one cow). Results are expressed as the mean ± standard error of the percentage of the periphery of oviduct pieces with ciliary activity relative to the zero-time values for the same pieces. LPS damaged the human fallopian tube mucosa as compared to HEPES-MEM control cultures ($P < 0.005$), but did not produce detectable damage to rabbit, pig, or cow oviducts.*

of 1.5 μg/ml (one cow) or 5.0 μg/ml (both rabbits, the pig, one cow) failed to damage the mucosa as compared to HEPES-MEM control oviduct organ cultures (Fig. 1).

Colistin, like polymyxin B, has been shown to neutralize toxic effects of LPS (3, 13). In the experiments reported here with human fallopian tubes, gonococcal LPS damaged the mucosa, suggesting that multiple rinses with antibiotic-free medium before the addition of LPS had effectively removed colistin from the organ cultures. We assume that similar rinsing remove colistin from those animal oviduct organ cultures in which it was used for the initial 24 h, and, therefore, the neutralizing effects of residual colistin did not account for the failure of gonococcal LPS to damage these tissues. This assumption is further supported by the observation that gonococcal LPS also did not damage those animal oviducts which had been maintained in medium without colistin.

Studies of the mechanisms of gonococcal pathogenicity have been hampered because of the specificity of naturally occurring gonococcal infection for humans and because of the lack of an established animal model (1, 4). Organ cultures of human fallopian tubes have provided an experimental model in which gonococcal infection and damage to genital mucosa can be studied without the participation of host factors such as leukocytes, complement, or antibodies (9–12, 16). The selectivity of attachment of gonococci to and damage of human fallopian tubes in organ culture parallels the host specificity of naturally occurring gonococcal disease (6). Because this damage may in large part be accounted for by gonococcal LPS (12), and because purified gonococcal LPS damages the mucosa of human fallopian tubes in organ culture (3, 8), it was of interest to determine whether LPS was capable of mediating the host specificity of gonococcal toxic damage to oviduct mucosa. Such a role for LPS is not without precedent, since LPS appears responsible for the highly specific host-parasite interactions of certain legumes and the *Rhizobium* species with which they are infected (17).

The present studies demonstrated that purified gonococcal LPS, in concentrations less than those usually detected in the supernatant fluid of human fallopian tube organ cultures infected with gonococci, reproducibly damaged the mucosa of human fallopian tubes in organ culture. However, the same or higher concentrations of gonococcal LPS failed to damage the mucosa of lapine, porcine, or bovine oviducts. It is known that there are substantial variations among host species in their reactivity to bacterial endotoxins, and humans, rabbits, and dogs are generally considered the most susceptible to the biological effects of LPS (2). The damage by gonococcal LPS to oviduct mucosa of the human host but not to that of the other animals tested may represent another manifestation of hyperresponsiveness of humans to bacterial endotoxins. It is also possible that the human fallopian tube mucosa contains receptors for gonococcal LPS which are not present in the other animal species. The toxic action of gonococcal LPS, which is selective for the genital mucosa of the human host, may be responsible in part for the specificity of gonococcal infections for humans.

These studies were supported in part by Public Health Service research grant AI-13488 from the National Institute of Allergy and Infectious Diseases. C.R.G. participated in these studies while a recipient of National Research Service Award AI-05533 from the National Institute of Allergy and Infectious Diseases.

We thank E. Sylvie Cousar for technical assistance and R. T. Birmingham, A. M. G. Crook, J. M. Edwards, J. W. Ellis, and N. E. Witthauer for their interest and help in providing fallopian tubes for these studies. We also are grateful to Laura Mooneyham and Jeanne Woodall for their help in preparing the manuscript.

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