SELECTIVITY FOR HUMAN GENITAL MUCOSA OF A TOXIC FACTOR ELABORATED BY NEISSERIA GONORRHOEAE

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1. Introduction

Human fallopian tube organ cultures (FTOC) have provided a valuable tool for the study of the pathogenicity of Neisseria gonorrhoeae [1-8]. It has been shown that after inoculation into FTOC, gonococci attach to and invade the mucosa of tissue pieces, producing loss of ciliary activity and disruption of the epithelium [1,2,5-8]. The observation that gonococci failed to attach to or invade the ciliated cells of fallopian tube mucosa but nevertheless inhibited ciliary activity, led to the suggestion that the organisms might elaborate a factor which is toxic for these cells [6-8]. This hypothesis was supported by the demonstration that filter-sterilized media from gonococcal-infected FTOC produced loss of ciliary activity in recipient human organ cultures [3,4,6]. As a preliminary step in the characterization of the toxic factor(s) present in the media of donor FTOC infected with gonococci, samples of such media have been tested in recipient oviduct organ cultures of human and non-human origin to assess the species specificity of the toxic factor(s).

2. Materials and Methods

2.1. Organ cultures

Fallopian tubes were obtained from non-pregnant, pre-menopausal women undergoing hystero-salpingo-ectomy for surgical indications. Oviducts were obtained from pigs and cows at slaughter. Organ cultures were prepared as described previously [1,2,5] and maintained in HEPES-buffered Eagle's minimal essential medium (HEPES-MEM) [5] supplemented with cefazolin (Eli Lilly & Co.) (100 µg/ml) and carbenicillin (Beecham Laboratories) (200 µg/ml). As the porcine and bovine oviducts were not obtained under aseptic conditions, they were transported from the slaughter house in the above medium supplemented with nystatin (E.R. Squibb & Sons) (50 units/ml) and then transferred to medium free of nystatin upon receipt in the laboratory.

2.2. Collection of sterile filtrates from gonococcal-infected fallopian tube organ cultures

In the experiments described here, all media components were prepared using sterile, pyrogen-free water and laboratory glassware that had been rendered pyrogen-free by heating at 180°C for 3 h. Media in human FTOC infected with a pilated, transparent colony type clone of Neisseria gonorrhoeae, strain 2686, were collected and sterilized by passage through a 0.45 µm membrane filter (Millipore Corp.) as described previously [3,6]. The filter-sterilized organ culture supernatant fluids were then lyophilized in pyrogen-free vials and stored at room temperature or 4°C.
2.3. Transfer of filtrates to recipient organ cultures, and assessment of tissue damage

The content of each vial was restored to its original volume with sterile, pyrogen-free water, vortexed briefly, and then agitated in a sonicating water bath for 15 min. Each sample was then diluted 1:4 in fresh HEPES-MEM supplemented with antibiotics as described above, passed through a 0.45 μm membrane filter (Millipore Corp.) and the fluid mixture used as the medium for oviduct organ cultures of human, porcine or bovine origin. In each experiment, organ cultures maintained in HEPES-MEM alone served as controls. Damage to oviduct organ cultures was assessed by monitoring the percentage of the periphery of tissue pieces with ciliary activity as described previously [5].

3. Results and Discussion

In each of three experiments, one of which is shown in Fig. 1, the decrease in ciliary activity of FTOC maintained in diluted, filtered media from gonococcal-infected donor FTOC was significantly greater (P < 0.001) than that of control FTOC maintained in normal medium. The difference between the two groups of organ cultures was manifest within 24 h. In contrast, in three experiments with porcine oviducts, and two experiments with bovine oviducts, the ciliary activity of oviduct organ cultures maintained in diluted, filtered media from gonococcal-infected donor FTOC was not significantly different from that of control organ cultures during at least 2 days of observation. The results of representative experiments with porcine and bovine tissues are shown in Fig. 1. In all these experiments, media from uninfected donor FTOC were not tested, since earlier experiments have shown that such media do not adversely affect recipient FTOC [3,6].

In previous studies of the species-specificity of gonococcal infection, it was found that gonococci attached to and damaged human genital tissue, but not porcine, porcine or bovine tissue [2]. It was subsequently shown that a factor was present in filter-sterilized media from gonococcal-infected FTOC which was toxic for fallopian tube mucosa [3]. Although the nature of the toxic factor(s) is unknown at present, preliminary data suggest that highly purified gonococcal endotoxin is capable of damaging fallopian tube mucosa [4,6,9]. Whatever the nature of the toxic factor(s), the data presented here indicate that it is preferentially active against human genital tissue. Thus, the host specificity of N. gonorrhoeae for humans may be accounted for, at least in part, by both the ability of gonococci to selectively attach to human genital mucosal tissue, and the selective activity against human genital tissue of a toxic factor elaborated by gonococci.

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