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Transfer of human membrane surface components by incorporating human cells into intact animal tissue by cell-tissue electrofusion in vivo

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Current animal models employed for the study of the obligate human pathogen *Neisseria gonorrhoeae* fail to utilize specific human gonococcal attachment receptors required to initiate pathogenesis in a clinically meaningful way. This communication presents evidence that suggests that cell-tissue electrofusion may be employed to create an animal model for this human specific pathogen. This new biotechnology was used to incorporate human membrane gonococcal receptors directly into epithelium of laboratory animals and subsequently infecting the histologically modified tissue with *N. gonorrhoeae* strain Pgh 3-2.

The creation of novel animal models which possess distinctive features that differ fundamentally from those biological properties displayed naturally by the unaltered animal species will lead to substantial broadening of our understanding of a variety of receptor-mediated processes. Gonorrhea is a human specific infectious disease of worldwide significance. The etiological bacterial agent is the obligate human pathogen *Neisseria gonorrhoeae*. The basis for this species restricted host-parasite relationship is due to pathogenesis being initiated by bacteria binding to specific gonococcal attachment receptors found exclusively on human cell plasma membranes. Because of this stringent host specificity, no suitable laboratory animal model is available for investigating gonococcal infectivity as it occurs naturally in humans.

We therefore propose that the first step in developing such a model is the transfer of human gonococcal receptors directly to epithelial tissues of anesthetized animals and subsequently exposing the histologically modified tissue to the human pathogen. In order to achieve this objective, we have recently reported that a newly devised process termed 'cell-tissue electrofusion' can be utilized to transfer functional gonococcal receptors from human cell membranes directly to rabbit corneal epithelial tissue in both in vitro and in situ experiments [1]. The ability to electrofuse individual cells to intact tissue further advances the recently developed biotechnology of electrofusing individual cells to one another in vitro [2–12]. In this communication, we present evidence that ocular inflammation due to the presence of gonococci can be elicited in rabbits histologically modified by cell-tissue electrofusion biotechniques.

Numerous attempts have been made to create laboratory animal models for gonococcal infectivity since Neisser first described the organism in 1879. Early attempts made prior to 1944 have been reviewed by Hill [13]. More recent reports of laboratory models for experimental gonococcal infections were reviewed by Kraus [14]. These modes include gonococcal infections of chimpanzees [15–20], vaginal infections of ddy mice by *N. gonorrhoeae* strain PH2 [21,22], infections of animals via subcutaneous tissue cavity chambers [23–25], and infection of the chorioallantoic membranes or other tissues of chick embryos [26]. In addition to the obvious multiplicity of scientific problems associated with these in vivo models, they all share the major disadvantage in that they all lack human cell surface gonococcal attachment receptors required to initiate pathogenesis as it occurs naturally in humans. Thus, we performed this investigation to overcome this major disadvantage in order to develop future animal models that would be more clinically relevant for studies in the pathogenesis and immunobiology of host specific infections, such as gonorrhea.

For a variety of technical and scientific reasons, we elected to transfer gonococcal attachment receptors from human cell surface membranes to intact corneal epi-

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thelium of anesthetized rabbits. This decision was based upon the following rationale: (a) an ocular model would be much simpler to establish with our new electrobiotechnology than either a vaginal, cervical or urethral model; (b) the eye is a natural site of gonococcal infections in humans (e.g., ophthalmia neonatorum); (c) tissue colonization by the pathogen in the animal model would occur as it does naturally on human epithelial surfaces; (d) gonococcal ocular lesions could be elicited in a controlled manner with relative ease because the infection site is readily accessible; (e) symptoms of infection could be evaluated with a noninvasive grading scheme based upon direct visual observations; and perhaps most important to our objective, (f) the initiation of pathogenesis of ocular gonorrhea in the in vivo model would be mediated by functional human gonococcal attachment receptors incorporated into non-human epithelium.

To determine whether the human gonococcal attachment receptors remained functional following the electrofusion process, bacterial adherence assays, utilizing *Neisseria gonorrhoeae* strain Pgh 3-2 (colony type 2), were performed in situ using rabbit corneas which were histologically modified by the electrofusion of human or non-human cells. These experiments were performed previously and are described in detail by Grasso et al. [1]. Corneas that were exposed to DC pulses in the absence of cells or had human cells placed on their surface in the absence of DC pulses (no fusion), were unable to facilitate the attachment of Pgh 3-2 organisms as observed by scanning electron microscopy. In addition, no attachment of the gonococci were observed on corneas which had either rabbit skin or monkey vero kidney cells fused to their surface. However, when either human HL60 or U937 cells were fused to the rabbit corneal epithelium we observed clusters of the gonococcal organisms attached to the surface of the rabbit cornea (Fig. 1).

The procedures employed for the in vivo experiments were performed as described for the in situ experiments of Grasso et al. [1]. Briefly, human and nonhuman cells were washed in phosphate-buffered saline (PBS) and collected by centrifugation onto Millipore filters. After discarding supernatants, the filters containing about 10^7 cells were removed with forceps and placed cell side down on the PBS washed corneal surfaces of rabbits anesthetized with nitrous oxide. Mechanical pressure (700 g/cm^2) plus three 20 μs DC pulses at 1 Hz were applied simultaneously, with a custom designed 'eye-shaped' concave titanium electrode, to all corneas except the right cornea of rabbit No. 1, which was subjected only to mechanical pressure. All eyes were washed with PBS which removed unfused cells. After 30 min, the eyelids were held open and both eyes of rabbit No. 1 and the right eyes of rabbits 2, 3 and 4 received 125 μl of *N. gonorrhoeae* strain Pgh 3-2 (colony type 2) suspended in brain heart infusion broth, at a concentration of 10^9 organisms/ml, for 5 min. All eyes were washed

TABLE I

Purulent gonococcal keratoconjunctivitis mediated by human receptors in rabbits infected with N. gonorrhoeae Pgh 3-2
The data represent the mean for four determinations.

Treatment of corneas	Rabbit 1		Rabbit 2		Rabbit 3		Rabbit 4	
	left	right	left	right	left	right	left	right
Experimental design [a]								
Rabbit skin cells	−	−	+	+	−	−	−	−
Mouse WEHI-3 cells	−	−	−	−	+	+	−	−
Human HL60 cells	−	+	−	−	−	−	+	+
dc fusion pulses	+	−	+	+	+	+	+	+
N. gonorrhoeae pgh 3-2	+	+	−	+	−	+	−	+
Clinical evaluation [b] 3 h postinfection								
Injection	−	−	−	−	−	−	−	+
Chemosis	−	−	−	−	−	−	−	+ +
Exudate	−	−	−	−	−	−	−	+
Clinical evaluation [b] 6 h postinfection								
Injection	−	−	−	−	−	−	−	+ +
Chemosis	−	±	−	−	−	−	−	+ +
Exudate	−	±	−	−	−	−	−	+ +

[a] The pluses and minuses listed under Experimental design refer to performing (+) or not performing (−) the indicated treatments on the respective left and right corneas of the four rabbits.

[b] Ocular symptoms of injection (reddening of the sclera), chemosis (conjunctival edema) and exudate (extravascular cells and fluid) were clinically evaluated for their severity with the following grading scheme: − = no symptoms; +/− = trace; + = slight; + + = mild; + + + = moderate.

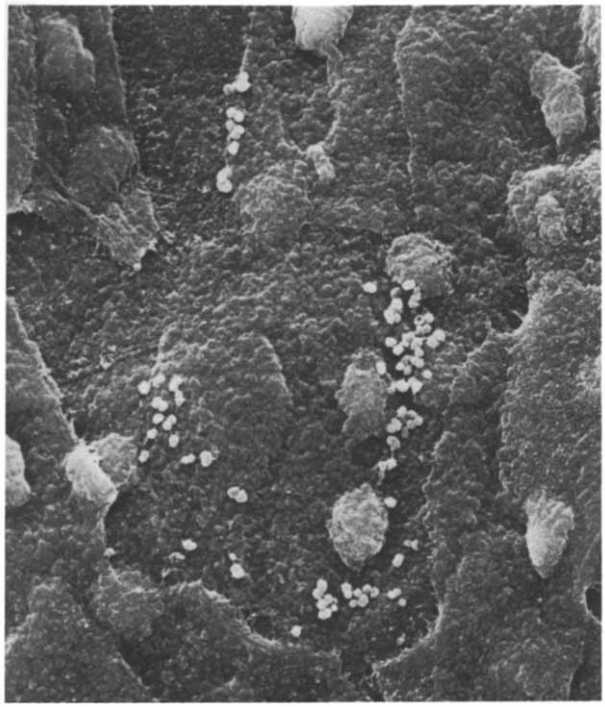

Fig. 1. Receptor-mediated attachment of *N. gonorrhoeae* Pgh 3-2 to somatic human-rabbit cell hybrids formed within rabbit corneal epithelium by electrofusion in situ. After electrofusing human HL60 cells to corneal epithelial tissue, the corneas were excised, employed in gonococcal adherence assays, and examined by scanning electron microscopy. (1260×).

with sterile PBS to remove unattached bacteria. The rabbits' eyes were clinically evaluated over a 24 h period based on the evaluation scheme described in Table I.

The experimental design and results from a representative experiment of the four replicate experiments, each utilizing four rabbits, that were performed is presented in Table I. Rabbit 1 served as the electrofusion and human cell control. The left eye of this animal was subjected to DC fusion pulses in the absence of cells and the right eye subjected to human cells in the absence of the pulses. These treatments failed to produce ocular inflammation over the 24 h test period after being exposed to the pathogen for 5 min. Both eyes of rabbits 2, 3, and 4 were electrofused with rabbit, murine and human cells, respectively, as indicated in Table I. The left eye of each animal served as the inflammation control and was not exposed to gonococci. When the right eyes of rabbits 2 and 3 bearing electrofused non-human cells were exposed to the bacteria, neither inflammatory reaction nor ocular lesions developed. In striking contrast, a purulent keratoconjunctivitis developed only in the right eye of rabbit 4 bearing electrofused human cells as indicated in Table I. Clinical symptoms of these ocular lesions appeared approx. 2 h after infection and peaked between 8 and 12 h (Fig. 2).

Based upon the observations of these experiments, it appears to be possible to incorporate gonococcal attachment receptors into the tissue of anesthetized animals by the process of cell-tissue electrofusion. Gonococcal attachment (in situ) and inflammation reaction (in vivo) were only observed following the formation of human-rabbit somatic cell hybrids (fusion process) and subsequently exposing the histologically modified tissue, which now contained the necessary human cell membrane components, to the pathogen. These results, although qualitative, are our initial evidence that cell-tissue electrofusion may be useful in developing animal models for the study of species specific diseases.

Among the four in vivo experiments, at the peak of inflammation, the severity of the clinical signs observed in the right eye of rabbit 4 varied, i.e., from mild (+ +) to moderately severe (+ + +). Hence, undesirable variables need to be identified and controlled in order to standardize the severity of the lesions for quantitative

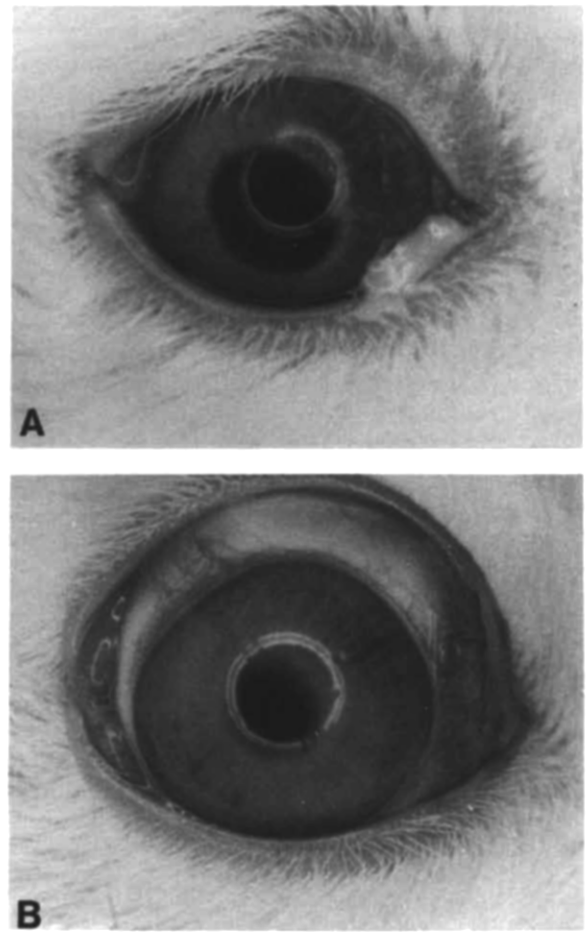

Fig. 2. (A) Photograph of a mild gonococcal lesion that occurred in the right eye of rabbit 4 (Table I) only 4 h after initiating the bacterial infection. Human-rabbit somatic cell hybrids were formed, 30 min prior to the addition of the bacteria, by electrofusing human HL60 cells to the rabbit corneal epithelium. (B) Photograph of the right eye of rabbit 3 (Table I), that had been similarly infected 4 h earlier. Murine-rabbit somatic cell hybrids were formed, 30 min prior to the addition of the bacteria, by electrofusing murine WEHI-3 cells to the rabbit corneal epithelium.

purposes in order to develop a suitable animal model to study gonococcal pathogenesis. However, these results taken together with our previous report [1] provide evidence that: (a) individual cells can be electrofused to anesthetized animals without causing lethality or ocular inflammation; (b) human gonococcal membrane receptors remain functional after their incorporation into human-rabbit somatic cell hybrids; and (c) an obligate human pathogen can produce a purulent inflammatory response mediated by transferred human microbial attachment receptors in a common laboratory animal.

Future research will examine a variety of gonococcal strains as well as other human cell types which may possess higher receptor densities. Additional experiments will include the isolation and quantitation of the gonococci following inflammation and optimizing the electrofusion process to increase the number of human cells incorporated into animal tissue. This new knowledge may lead to the future development of an animal model which could be utilized for examining the pathogenic mechanisms of gonococcal infections and extend our current concepts of pathogenesis and immunobiology.

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