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Endotoxin-Induced Shedding of Viable Uroepithelial Cells Is an Antimicrobial Defense Mechanism

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ICR mice were infected intravesically with a virulent (7343) or a nonvirulent (U^+) *Escherichia coli* strain. The U^+ strain induced considerably more shedding of uroepithelial cells than did the 7343 strain. The stimulus for this shedding was shown to be associated with lipopolysaccharide and was abrogated by pretreatment with aprotinin. Desquamation commenced within 1 h postinjection, and the cells that were shed proved to be viable. Comparison of C3H/HeJ and C3H mice revealed that only the latter responded to shedding inducers. However, C3H/HeJ mice succumbed to a systemic infection on injection of 10^6 U^+ cells intravesically, whereas other mouse strains required a 100-fold dose of bacteria for this effect. Since the first stage of a bacterial infection entails adherence of the microbes to epithelial cells, inducible shedding is an antimicrobial defense mechanism.

We have recently developed a technique that enables follow-up of the incipience of inflammatory cells in the urine of ICR mice with experimental bladder infection (4). Since diuresing mice are employed in the experimental model (1), relatively large quantities of urine can be obtained by holding the animals manually and collecting their urine in test tubes. The urine is then cytocentrifuged and the cells are fixed, Giemsa stained, and counted. This method allowed us to monitor the type of exudate cells in the same animal throughout the infection stages. During the course of these experiments, we noticed that, occasionally, large quantities of uroepithelial cells appeared in the urine. Further investigation revealed that of the *Escherichia coli* strains that we used, the nonvirulent strain U^+ was much more effective in inducing epithelial shedding than was the virulent strain 7343.

Subsequent observation of the U^+ strain showed that this strain did not form pili under our culture conditions and that its ability to cause shedding remained unimpaired, even following its inactivation by gamma radiation. Likewise, boiling for 10 min did not impair the ability of the bacteria to cause shedding, and this raised the possibility that the activating element in the bacteria might be associated with lipopolysaccharide (LPS). We could indeed demonstrate that LPS prepared from strain U^+ by the method of Westphal and Jann (6) was a much more potent shedding inducer than was the LPS from strain 7343 (each mouse was injected with LPS extracted from 2×10^8 bacteria).

It became clear early in the study that a more quantitative approach was required because the amounts of urine obtained from the animals varied considerably, so all subsequent experiments were conducted by the procedure described below.

In this report we provide evidence that LPS may induce shedding of uroepithelial cells and discuss the specificity of this effect. In addition, an attempt was made to verify whether the ability of the *E. coli* strains to cause shedding is also negatively related to their virulence. Finally, the notion that the shedding response is part of an antimicrobial defense mechanism is discussed in light of the pertinent information that is available in the literature.

MATERIALS AND METHODS

Bacterial endotoxins. The *E. coli* endotoxins O55:B5 and O111:B4 were purchased from Difco Laboratories (Detroit, Mich.). Endotoxins O128:B12, O26:B6, and a chromatographically purified preparation of O55:B5 were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cultivation of bacteria. The bacteria (all of them isolates from humans with pyelonephritis) were grown on nutrient agar (Difco) supplemented with 0.1% yeast extract–0.1% glucose at 37°C for 20 h.

After the bacteria were harvested, they were washed three times in saline. The stocks for shedding induction were lyophilized and subjected to ^{60}Co radiation of 1.5 megarads.

The procedure for the determination of bacterial virulence in the urinary tract was based on that described previously (1), in which urine collected from the infected mice was plated.

Procedure for evaluating the extent of shedding. (i) Normal (i.e., nondiuresing) animals were inoculated intravesically with bacteria or endotoxins (volume injected, 0.05 ml). (ii) At the desired interval postinoculation the animals were anesthetized and their ureters were clamped (to avoid the loss of liquid). (iii) Then, 0.2 ml of saline was injected into the bladder of each animal, and immediatly after this the bladder fluid contents were aspirated with the same syringe. (iv) The aspirate containing the shed epithelial cells was subjected to the usual treatment, namely, cytocentrifugation, fixation, staining, and counting (Fig. 1).

RESULTS

Comparison of shedding induction by the virulent strain 7343 and the nonvirulent strain U^+. After we verified that the gamma-irradiated bacteria closely resembled the viable cells in their ability to induce shedding, we conducted all the experiments with lyophilized irradiated cells, to ensure the homogeneity of the stimulus and to avoid possible complications resulting from bacterial proliferation.

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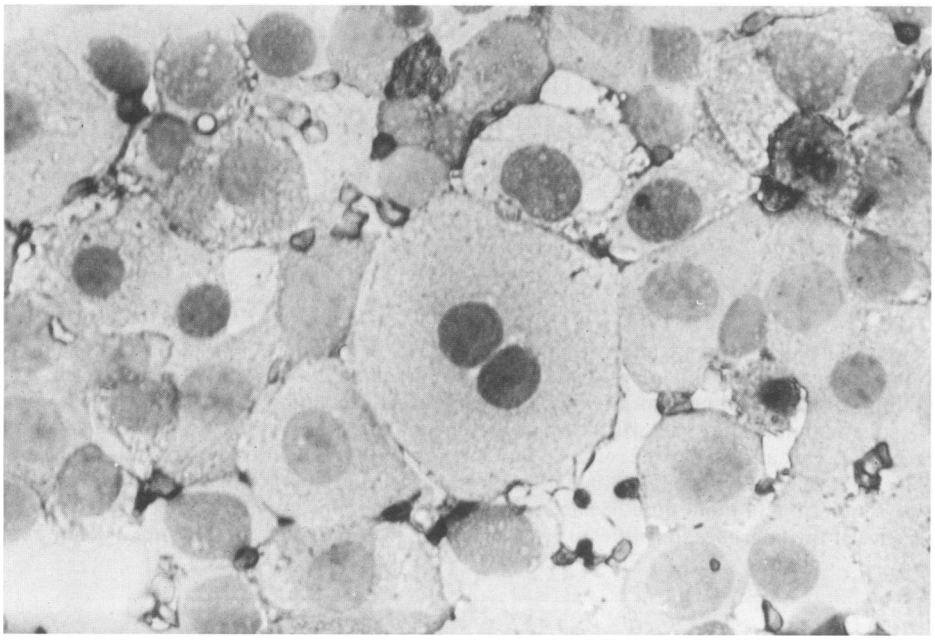

FIG. 1. Shedding of uroepithelial cells following injection of the U^+ strain of *E. coli*. The cells were aspirated, cytocentrifuged, fixed, and stained with Giemsa. Magnification, ×2,000.

The U^+ strain was about 100 to 200 times more potent as a shedding inducer than was the 7343 strain, and the saline injection per se caused only negligible shedding (Table 1). Other noteworthy findings not given in Table 1 were that desquamation may have commenced at 1 h after injection of the stimulus, that the sloughed cell population at that time was entirely epithelial (polymorphonuclear cells appeared several hours later), and that the aspirated cells appeared to be alive. This last finding was substantiated by treatment of the cells with trypan blue, which showed that over 90% of them were viable. It was established with the Papanicolaou stain that cells from various depths of the epithelium, including basal cells, occurred in the aspirate after bacterial inoculation.

Testing of some commercial endotoxins again showed specificity, in that O55:B5 was quite effective in inducing shedding at a concentration of 200 μg per mouse (Table 1), whereas O111B4, O128:B12, and O26:B6 were not. The chromatographically purified preparation of O55:B5 was again a potent inducer.

Shedding was effectively abolished by treatment with the well-known protease inhibitor aprotinin (80 to 90% inhibition following intraperitoneal injection of the mice with 140 μg of Trasylol [Bayer] 2 h before administration of endotoxin mixed with 35 μg of Trasylol). Since the U^+ preparations and the endotoxins were entirely devoid of proteolytic activity, it is plausible that shedding was effected via the release of proteolytic enzymes by the epithelial cells.

Further evidence of the role of endotoxin in shedding induction was obtained by comparing the endotoxin-resistant C3H/HeJ mice with the parent strain. Gamma-irradiated U^+ and LPS O55:B5 proved to be as effective in C3H as in ICR mice, whereas C3H/HeJ mice were not responsive (Table 1). However, on injection of live U^+ cells into the bladder of ICR, C3H, and C3H/HeJ mice, it was found that the latter succumbed to as few as 10^6 cells within 24 h due to a systemic infection; bacteria were isolated from the lungs, liver, and spleen. A 100-fold increase in bacterial dosage was required to obtain the same result in ICR and C3H mice.

We also tested additional isolates of *E. coli* from humans with pyelonephritis. Of these, five isolates were not pathogenic in the mouse urinary tract, nor did they cause epithelial shedding. Although in this latter respect they behaved like the virulent 7343 strain, they presumably lacked other com-

TABLE 1. Effect of various stimuli on the shedding of uroepithelial cells

Mouse strain[a]	Material injected	Amt	Duration (h) of stimulus	No. of epithelial cells shed
ICR	Saline (control)	0.05 ml	2	100–400
ICR	7343 (gamma-irradiated)	2.5×10^8	2–6	600–1,000
ICR	U^+ (gamma-irradiated)	2.5×10^7	2	10,000–20,000
ICR	LPS (O55:B5)	200 μg	2	10,000–20,000
C3H	Saline	0.05 ml	2	100–500
C3H	U^+ (gamma-irradiated)	2.5×10^7	2	10,000–20,000
C3H	LPS (O55:B5)	200 μg	2	10,000–20,000
C3H/HeJ	U^+ (gamma-irradiated)	2.5×10^7	2	100–500
C3H/HeJ	LPS (O55:B5)	200 μg	2	100–500

[a] Results for ICR mice were obtained from 30 to 50 individual animals, and those for C3H and C3H/HeJ mice were obtained from 15 animals of each strain.

TABLE 2. Comparisons among various clinical isolates of *E. coli* in terms of infectivity, adherence, and capacity to induce epithelial shedding[a]

Strain	Infectivity following injection of bacteria		Induction of shedding following injection of heat-killed bacteria		Adherence to uroepithelium in vivo
	10^6	10^7	10^7	10^8	
D3	None	None	Very low	Very low	—[b]
D16	None	None	None	None	—
D23	None	None	None	Very low	—
D27	None	Very low	None	Very low	—
D28	None	None	High	High	None
D29	None	None	None	Very low	—
D41	Very high	Very high	Low	Low	High
7343	Very high	Very high	Low	Low	High
U^+	None	None	High	Very high	None

[a] Results are for female ICR mice; at least two independent experiments were done in which 10 mice were used in each experiment.
[b] —, Not tested.

ponents of virulence. On the other hand, one strain (D28) resembled U^+ (it also lacked pili), and another strain (D41) resembled strain 7343 both in pathogenicity and in the capacity to induce shedding (Table 2).

DISCUSSION

A survey of the literature revealed that the same phenomenon has been described by Orikasa and Hinman (5) (the morphological details of the phenomenon were later elaborated by Fukushi et al. [3]). However, Orikasa and Hinman (5) provided evidence that the desquamation is preceded by cellular disintegration; this finding is possibly attributable to the fact that they employed live bacteria, and these bacteria probably damaged the cells to which they adhered. These findings were later corroborated by Asscher et al. (2). We fully concur, however, with the suggestion by these authors that desquamation may serve as a defense mechanism. Considering the generally accepted tenet that infection is begun by the adherence of the pathogens to the epithelial mucosae, removal of the involved epithelial cells from the body should affect the infecting pathogen as well. This was dramatically demonstrated with C3H/HeJ mice; having failed to clear the bladder of U^+ bacteria, they died from a systemic infection within 24 h.

As for the possible clinical implications of these results, we note that urologists are unanimous in their belief that the appearance of a large number of epithelial cells in the urine, such as that which is also known to occur spontaneously, has an uncertain etiology and significance. In such cases, however, we suggest that an overt or covert bladder infection with a bacterial strain capable of inducing shedding may have taken place. Finally, we are currently investigating, in an animal model, the possible application of our shedding induction technique to the early detection of tumors of the bladder in animals to which appropriate carcinogens are administered.

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