SHORT COMMUNICATION

Specificity of Attachment of Certain Enterobacteriaceae to Mammalian Cells

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The specificity of adherence of various Enterobacteriaceae to different mammalian cells was studied *in vitro*. ³H-Labelled organisms of the same species isolated from various clinical sources differed significantly in their abilities to adhere to the same mammalian cells. Bacteria frequently adhered better to cells derived from sites other than those analogous to their original source. Bacteria did not display consistently 'high' or 'low' attachment to a variety of human and tissue-cultured cells and little selective adherence was demonstrable.

INTRODUCTION

During the past two decades, members of the Enterobacteriaceae have become increasingly important causes of morbidity and mortality in patients in hospital (Johanson *et al.*, 1972; Pierce & Sanford, 1974). An understanding of the mechanisms by which organisms of this group adhere to and colonize mammalian cells might lead to ways of preventing or altering infections with such agents. The results of several previous studies have suggested a high degree of bacterial-tissue specificity (Ellen & Gibbons, 1974; Gibbons & van Houte, 1971). Common pathogens of bovine mastitis adhere better to bovine udders than do bacteria which do not frequently cause mastitis (Frost, 1975). Group A streptococci associated with rheumatic fever were reported to attach in greater numbers to buccal cells of patients with rheumatic fever than to normal subjects (Selinger *et al.*, 1978). It has also been reported that various Enterobacteriaceae adhere better to uroepithelial cells derived from women with recurrent urinary tract infections than to similar cells derived from normal subjects (Fowler & Stamey, 1977; Kallenius & Winberg, 1978), and that *Escherichia coli* isolated from patients with urinary tract infections adheres better to normal uroepithelial cells than does *E. coli* isolated from patients with asymptomatic bacteruria (Svanborg-Eden *et al.*, 1976). The greater attachment to heart valves of bacteria which frequently cause endocarditis compared with the unusual causative organisms (Gould *et al.*, 1975), and the greater adherence of *Pseudomonas aeruginosa* to damaged heart valves compared with normal heart valves (Ramirez-Ronda, 1978), also suggest that the adherence process is specific.

Despite the suggestive nature of the evidence, no systematic examination of the specificity of the adherence process has been conducted. The current study was designed, therefore, to determine the extent of specificity, using clinical Enterobacteriaceae isolates of the same and of different species incubated with (i) mammalian cells of similar type to those at the site of origin, (ii) cells from other sites and (iii) tissue-cultured cells. The use of a radio-adherence assay facilitated more precise quantification of the adherence process.

METHODS

Bacteria. Strains of Enterobacteriaceae isolated from patients with urinary tract infections ($\geq 10^5$ per ml) and from sputa and wounds ($\geq 50\%$ of the total growth on blood agar plates) were obtained from the University of Iowa and Veterans Administration Medical Center Clinical Microbiology Laboratories and stored at 4 °C on heart infusion agar slants for no longer than 1 month. The organisms used in all of the experiments had not undergone further subculture. Sputum, urine and wounds were simultaneously cultured from each patient; each isolate used for study was present in only one patient site.

Mammalian cells. Human buccal cells and cutaneous epithelial cells were collected by scraping the oral mucosal membranes and the volar aspects of the forearms of one of the investigators; uroepithelial cells were obtained from the first morning urine samples from the same investigator. Mouse adrenal (Y1), Chinese hamster ovary and rat hepatoma tissue-cultured cells were propagated in Eagle's minimal essential medium (MEM) supplemented with 10% (v/v) foetal calf serum at 37 °C under a humidified atmosphere of 95% air/5% CO₂.

Radioadherence assay. This assay was described in detail by Sugarman & Donta (1979). Briefly, the bacteria were incubated in MEM at 37 °C for 16 h with 25 μ Ci [*methyl*-³H]thymidine (New England Nuclear; specific activity 50 mCi μ mol⁻¹). After centrifuging and washing three times with 50 mM-phosphate buffered saline pH 7.4 (PBS), the bacteria were resuspended at a final concentration of 10⁸ organisms ml⁻¹, as determined by the amount of radioactivity and verified by quantitative pour-plate colony counts. The mammalian cells were also washed three times in PBS and resuspended at concentrations of 10⁵ cells ml⁻¹ (or 10⁶ ml⁻¹ for the smaller tissue-cultured cells), as determined by haemocytometry.

Bacteria and mammalian cells (1 ml of each) were incubated together at 37 °C with mild agitation for 60 min, with bacteria incubated in PBS alone as the controls. After incubation, the suspensions were filtered on membrane filters (12 μ m, Nucleopore), which were then washed extensively with PBS. The residual radioactivity on the filters was determined and converted to numbers of bacteria; the results were then expressed as the number of adherent bacteria per cell. Control counts were all $\leq 2 \times 10^3$ c.p.m. (equivalent to < 1 bacterium per cell). All experiments were done in duplicate and repeated on at least three occasions. The results obtained were analysed using the standard *t* test. Significant differences were considered to exist when *P* values were < 0.05.

RESULTS

Bacterial adherence properties

Different strains of the same bacterial species isolated from the same sites of different individuals varied markedly in their ability to adhere to mammalian cells derived from a similar site (Table 1). For example, *Klebsiella pneumoniae* strains 1 to 5 were all isolated from sputa, each representing at least 50% of the total growth on blood agar plates; yet, following incubation of each strain with identical buccal cells, strains 1 and 2 adhered significantly better than strains 3, 4 and 5, and strain 5 adhered less well than any of the other four strains. Similar differences were observed for each bacterial species, regardless of the clinical source of the isolate. In addition, no differences between bacterial species in their ability to adhere to analogous cells could be demonstrated.

Mammalian cell effect

Approximately 50% of the isolates adhered best to homologous cells. Overall, however, no significant differences in the abilities of isolates to adhere to cells similar to the site of origin could be demonstrated. Frequently, individual isolates attached in significantly greater numbers to tissue-cultured cells, which were smaller than other mammalian cells. Sputum isolates *K. pneumoniae* 5, *E. coli* 2, *Proteus mirabilis* 2 and wound isolate *E. coli* 2 each attached in higher numbers to the tissue-cultured cells than to any of the three types of human cells.

Certain bacteria (e.g. *E. coli* urine isolate 4) attached poorly to all cell types tested, while others (e.g. *K. pneumoniae* sputum isolate 2) attached in high numbers to all of the mammalian cells. However, most bacterial isolates and mammalian cell types did not display consistently 'high' or 'low' adherence properties.

Table 1. Attachment of *Enterobacteriaceae* to various mammalian cells

Bacteria		No. of adherent bacteria per mammalian cell $\pm$ s.d.					
		Human cells			Tissue-cultured cells		
		Buccal	Urine	Cutaneous	Mouse adrenal	Chinese hamster ovary	Rat hepatoma
Sputum isolates							
<i>K. pneumoniae</i>	1	94 $\pm$ 7	21 $\pm$ 5	9 $\pm$ 5	17 $\pm$ 3	2 $\pm$ 3	28 $\pm$ 4
	2	88 $\pm$ 6	53 $\pm$ 7	61 $\pm$ 8	71 $\pm$ 10	38 $\pm$ 2	49 $\pm$ 2
	3	62 $\pm$ 3	12 $\pm$ 2	24 $\pm$ 3	7 $\pm$ 2	4 $\pm$ 4	6 $\pm$ 3
	4	39 $\pm$ 5	58 $\pm$ 4	7 $\pm$ 1	45 $\pm$ 6	15 $\pm$ 3	33 $\pm$ 2
	5	9 $\pm$ 2	22 $\pm$ 4	17 $\pm$ 1	33 $\pm$ 4	39 $\pm$ 4	17 $\pm$ 2
<i>E. coli</i>	1	75 $\pm$ 4	6 $\pm$ 5	51 $\pm$ 2	6 $\pm$ 3	8 $\pm$ 5	34 $\pm$ 7
	2	27 $\pm$ 2	30 $\pm$ 3	18 $\pm$ 3	43 $\pm$ 3	9 $\pm$ 4	7 $\pm$ 5
	3	6 $\pm$ 1	18 $\pm$ 2	11 $\pm$ 1	3 $\pm$ 3	6 $\pm$ 2	5 $\pm$ 1
<i>P. mirabilis</i>	1	80 $\pm$ 4	19 $\pm$ 2	6 $\pm$ 1	7 $\pm$ 1	9 $\pm$ 2	5 $\pm$ 1
	2	6 $\pm$ 1	6 $\pm$ 2	15 $\pm$ 3	8 $\pm$ 4	11 $\pm$ 3	29 $\pm$ 3
Urine isolates							
<i>K. pneumoniae</i>	1	28 $\pm$ 1	63 $\pm$ 3	44 $\pm$ 3	31 $\pm$ 2	7 $\pm$ 3	5 $\pm$ 2
	2	53 $\pm$ 3	50 $\pm$ 4	38 $\pm$ 2	19 $\pm$ 2	8 $\pm$ 1	26 $\pm$ 5
	3	19 $\pm$ 1	8 $\pm$ 4	7 $\pm$ 3	2 $\pm$ 2	6 $\pm$ 2	14 $\pm$ 3
<i>E. coli</i>	1	49 $\pm$ 4	58 $\pm$ 4	44 $\pm$ 3	16 $\pm$ 3	12 $\pm$ 3	11 $\pm$ 2
	2	38 $\pm$ 4	41 $\pm$ 2	24 $\pm$ 2	11 $\pm$ 1	4 $\pm$ 2	16 $\pm$ 1
	3	11 $\pm$ 1	6 $\pm$ 1	32 $\pm$ 2	16 $\pm$ 4	11 $\pm$ 2	20 $\pm$ 2
	4	8 $\pm$ 4	6 $\pm$ 2	5 $\pm$ 4	2 $\pm$ 1	3 $\pm$ 2	2 $\pm$ 2
<i>P. mirabilis</i>	1	16 $\pm$ 2	57 $\pm$ 4	21 $\pm$ 1	14 $\pm$ 2	10 $\pm$ 1	4 $\pm$ 2
	2	18 $\pm$ 3	39 $\pm$ 4	31 $\pm$ 3	12 $\pm$ 4	7 $\pm$ 1	28 $\pm$ 3
Wound isolates							
<i>K. pneumoniae</i>	1	36 $\pm$ 2	16 $\pm$ 3	47 $\pm$ 3	16 $\pm$ 3	13 $\pm$ 2	21 $\pm$ 6
	2	19 $\pm$ 3	49 $\pm$ 4	22 $\pm$ 5	37 $\pm$ 5	9 $\pm$ 1	17 $\pm$ 4
<i>E. coli</i>	1	6 $\pm$ 3	4 $\pm$ 1	3 $\pm$ 3	2 $\pm$ 1	4 $\pm$ 3	6 $\pm$ 4
	2	16 $\pm$ 3	23 $\pm$ 4	18 $\pm$ 4	39 $\pm$ 3	17 $\pm$ 4	9 $\pm$ 2

DISCUSSION

The results of the experiments reported here do not provide strong support for the concept of bacterial-mammalian cell adherence specificity. The results indicate that the adherence process may be quite complex with both bacterial and mammalian cell properties of paramount importance in determining selective adherence. That isolates of the same species of bacteria from the same clinical sites adhere in such a divergent manner to the same mammalian cells attests to the importance of subtle differences amongst bacteria which underlie this process. While different isolates attached better to only certain cell lines, adherence to almost every cell line was readily demonstrated. Similar results obtained at 4 °C negate significant phagocytosis as an explanation for such differences.

The use of different mammalian cells was also instrumental in delineating the complexity of the process of adherence of bacteria to mammalian cells. Some of the results *in vitro*, especially those using non-human mammalian tissue-cultured cells, may have little relevance to situations *in vivo*. Alternatively, bacterial adherence to such cells may be the result of specific interactions that are of importance *in vivo*, and the use of such tissue-culture models could facilitate our understanding of the mechanisms underlying the specificity of adherence. It is also conceivable that bacteria could be 'typed' according to which cell line(s) they best adhered, and such typing might be of potential epidemiological value.

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