Correlation between the susceptibility of E. coli to phagocytosis and their ability to invade HeLa cells in vitro[1]

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Summary. The susceptibility of several strains of E. coli to phagocytic killing by polymorphonuclear leucocytes and the ability of the same strains to invade HeLa cells were studied. It was found that only the strains resistant to killing by leucocytes were able to penetrate and multiply within HeLa cells.

It has been shown that the ability of some species of enterobacteria to enter HeLa cells correlates with their invasiveness of the intestinal mucosa. Labrec et al. have demonstrated that a virulent strain of Shigella flexneri, which was able to penetrate the bowel epithelium and enter the lamina propria, was also able to infect HeLa cells and multiply within them, whereas an avirulent variant possessed neither of these capacities[2]. These findings have been confirmed by Calabi[3] and by Giammanco et al.[4]. The latter group of authors failed to show this property in strains of Shigella sonnei. Giannella et al. have shown that strains of S. typhimurium that invaded rabbit ileal mucosa also invaded HeLa cells, and strains failing to penetrate HeLa cells lacked the capacity to invade rabbit mucosa[5]. The data in the literature concerning the ability of E. coli to penetrate HeLa cells are conflicting. Formal et al. have shown that E. coli K-12 Hfr, a non-pathogenic strain, do not enter HeLa cells, whereas an E. coli-Shigella flexneri hybrid strain does, but cannot multiply in the cells[6]. In contrast, Calabi has reported that non-pathogenic E. coli are engulfed by HeLa cells and rapidly killed[3]. No data, however, are available on potentially invasive strains of E. coli. We have previously shown that several E. coli strains are not susceptible to phagocytosis in vitro by polymorphonuclear leucocytes (PMN) or macrophages[7–9]. This property may be an important factor in determining the invasiveness of E. coli. Indeed strains of E. coli which are resistant to phagocytosis are also virulent for mice[10]. Furthermore, in a study on Gram-negative rod bacteremia, it has been reported that 11 of 30 bacteremic isolates, all E. coli, showed absolute or relative resistance to phagocytosis[11]. In this report, we compare the property of E. coli to resist phagocytosis with their ability to invade HeLa cells in vitro.

Materials and methods. The following strains of E. coli were used: a) 2 standard strains for genetic studies, K-12 λ- and K-12 HfrR; b) 4 'enteropathogenic' strains, 026:B6, 055:B5, 0111:B4 and 0126:B16; c) 2 strains isolated from the stools of healthy children, E88 (06:H1) and ECR (018ac,23). The bactericidal activity was assayed according to the method of McRipley and Sbarra[12] with minor modifications as described in a previous paper[13]. To test the invasion of HeLa cells by E. coli, the method

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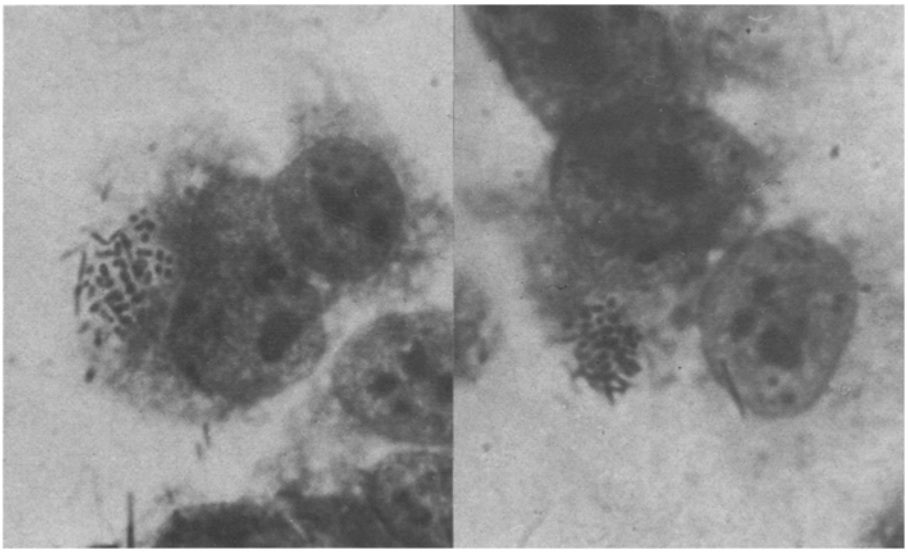

HeLa cell monolayer 3 h after inoculation with E. coli 026:B6. 2 isolated cells containing numerous bacteria are shown. Magnification ×1250.

Table 1. Susceptibility of several strains of E. coli to phagocytic killing by guinea-pig polymorphonuclear leucocytes (PMN)

E. coli strains	Number of viable bacteria					
	Bacteria alone			Bacteria with PMN		
	0 time	30 min	60 min	0 time	30 min	60 min
K-12 λ-	1.0×10^7	1.2×10^7	9.0×10^6	1.1×10^7	1.3×10^5	1.3×10^5
K-12 HfrR	2.6×10^7	2.8×10^7	2.7×10^7	2.6×10^7	2.2×10^6	3.5×10^5
026:B6	3.1×10^7	3.3×10^7	3.5×10^7	3.0×10^7	1.9×10^7	1.9×10^7
055:B5	1.5×10^7	1.5×10^7	1.8×10^7	1.6×10^7	1.4×10^7	1.2×10^7
0111:B4	2.2×10^7	2.0×10^7	2.0×10^7	2.0×10^7	2.1×10^7	2.0×10^7
0126:B16	2.4×10^7	3.0×10^7	3.3×10^7	2.1×10^7	2.3×10^7	2.4×10^7
E88 (06:H1)	2.6×10^7	2.5×10^7	2.2×10^7	3.1×10^7	3.8×10^7	3.4×10^7
ECR (018ac,023)	2.5×10^7	3.0×10^7	3.2×10^7	2.8×10^7	3.0×10^7	3.3×10^7

E. coli were incubated with or without PMN (ratio cells to bacteria 1:5) for 30 or 60 min. After addition of saponin to the mixture to lyse the PMN, the number of colony forming units was determined. The mean values of 2 different experiments are given.

described by Calabi[3] was employed. Each Leighton tube, that contained a coverslip in its flattened side, received 1.5 ml of HeLa cells suspension in Eagle MEM supplemented with antibiotics (2×10^5 cells/ml). The tubes were incubated horizontally at 37 °C to let the cell settle on the coverslip. After 24 h, the medium was changed, and after additional 24 h, it was replaced with the same medium without antibiotics. At 72 h, 1.5 ml of E. coli suspension in Eagle MEM without antibiotics (10^8 bacteria/ml) was added to each tube and the tubes were incubated horizontally at 37 °C. At various intervals, the coverslips were removed from the tubes, washed 3 times gently with Krebs-Ringer-phosphate at 37 °C, fixed with methanol and stained with May-Grünwald and Giemsa solutions. The presence of bacteria-containing cells was determined under a light microscope.

Results. Table 1 shows the susceptibility of several strains of E. coli to the phagocytic killing by polymorphonuclear leucocytes. The 2 K-12 derived strains were readily killed by the phagocytes, whereas the other strains were either partially susceptible (026:B6 and 055:B5) or totally insensitive to phagocytic killing. Table 2 shows the results of E. coli invasiveness test in vitro using HeLa cells. No bacteria were present within the cells when the monolayers were infected with the 2 K-12 derived strains. The monolayers infected with the other 6 strains of E. coli showed many bacteria-containing cells 3 h after infection, the number of infected cells varying with the different strains. With E. coli 0111:B4 and E88 (06:H1) an appreciable number of HeLa cells contained bacteria even 1 h after infection. Bacteria were usually present as small clumps (figure) within the invaded cells, which is suggestive of bacterial growth after penetration[2,6].

Table 2. Correlation between the invasion of HeLa cells by several strains of E. coli and the susceptibility of the same strains to phagocytic killing by PMN

E. coli strains	Bacteria-containing cells		Percent of bacteria killed by PMN after 60 min incubation
	60 min after infection	180 min after infection	
K-12 (λ-)	0	0	98.8
K-12 (Hfr)	0	0	98.7
026:B6	0	+	36.7
055:B5	0	+	25.0
0111:B4	+	+	0
0126:B16	0	+	0
E88 (06:H1)	+	+	0
ECR (018 ac, 023)	0	+	0

The data reported in the last column are taken from table 1. The presence of bacteria containing cells was determined by light microscopic observation on stained monolayers of HeLa cells. For experimental details see 'Materials and methods'.

Discussion. At least 2 different mechanisms underly the enteropathogenicity of E. coli: a) production of enterotoxins which effects the secretion of water and electrolytes into the gut lumen; b) invasion of the gut wall which may result in inflammatory reaction and ulcer formation[13,14]. The ability to invade epithelial cells seems to play an important pathogenetic role even in those strains of E. coli that produce the enterotoxins. It has been shown, infact, that a toxinogenic strain, which is poorly invasive, causes diarrhea in a lower percentage of cases than a strain which is both invasive and toxinogenic[15,16]. The explanation offered by the authors is that the toxinogenic, but poorly invasive strain, is rapidly swept out of the gut by villi movement and intestinal peristalsis. An additional requirement for E. coli pathogenicity might be the resistance to phagocytosis, which would favour the invasion of the lamina propria of the intestinal mucosa, spreading into the blood stream and possible localization in extraintestinal organs. It is shown here that a direct correlation exists between the ability of E. coli to invade HeLa cells (and, by extrapolation, the epithelial cells) and their resistance to the phagocytic killing. 2 of the 6 non-phagocytosable and invasive strains tested in our study do not belong to the group of the so-called 'enteropathogenic' E. coli, as defined by serotyping criteria[17]. This suggests that the strains which are provided with those properties should be considered, regardless their serotype, as potential pathogens whose role in disease has to be more precisely defined. The mechanism of E. coli penetration into HeLa cells and the factors that modulate the phenomenon have not been investigated. We have previously shown that whether or not E. coli are phagocytosed by PMN and by macrophages, depends on the nature of the surface polysaccharide[7]. Studies are in progress to investigate the role of the polysaccharide on the interaction between E. coli and HeLa cells.

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