ADSORPTION OF BACTERIA ON MAMMALIAN CELLS CULTURED UNDER MIXED INFECTION CONDITIONS

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It is generally considered that the initial essential stage in certain infectious processes due to bacteria is their adsorption on epithelium and subsequent colonization [9, 11]. Adsorption of bacteria on epithelial cells is due to a specific reaction of binding of their surface ligands with receptors on the cell surface. Primary virus infection of cells, through the action of which on the surface new receptors may appear or changes may take place in existing receptors [1, 12], in all probability affects subsequent adsorption of bacteria. This is confirmed indirectly by epidemiological data [5, 6] and also by the results of experiments on animals showing that mixed bacterial–virus infection is realized only if the virus is introduced a few days before infection with bacteria, and that it differs from monoinfections in the severity of its course [8, 10].

However, quantitative measurements of the principles governing adsorption under mixed infection conditions, which have been undertaken traditionally and successfully in the study of adsorption during monoinfection, are difficult in experiments *in vivo*, where the action of viruses on adsorption of bacteria may be not only direct, but also indirect through the immune system [7], and for that reason there have been very few such investigations [12, 14], and those have been conducted only on bacteria of the genera *Streptococcus* and *Staphylococcus*.

The quantitative principles of adsorption of bacteria on the surface of host cells under mixed bacterial–virus infection conditions were studied in the investigation described below on a model of cultures of human and animal cells.

EXPERIMENTAL METHOD

Bacteria with the best studied adsorption apparatus were used, namely *Escherichia coli*, which has morphologically clearly defined adsorption structures, or fimbriae, the presence of which can be reliably verified; this is particularly important, because they are lost during laboratory subcultures [4]. As the virus component of the test system in the model, we used measles virus, which is known to cause substantial modification to the surface of mammalian cells, and oncoviruses, which as a rule persist in all cell lines and often are not considered as active components of the infectious process. To assess quantitative relations between the reacting components in the model a technique based on measurement of relative radioactivity was developed.

A cell monolayer of transplantable line L-41 (generously provided by Dr. N. E. Gulevich, N. F. Gamaleya Research Institute of Epidemiology and Microbiology), a transplantable culture of E5 rat fibroblasts (obtained from Dr. A. Rauko, Czechoslovakia), and a variant of it, infected and chronically producing endogenous xenotropic Balb v2 mouse virus were grown on coverslips of standard size in Leighton's tubes in medium 199 with the addition of 10% inactivated bovine serum. At different stages of growth the L-41 cells were infected with living measles vaccine virus and, on the appearance of a marked cytopathic action (consisting of symplast formation), confirmed by intravital observation, the experiment began.

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Escherichia coli cells of strain H_{10407} C_F A, grown at 37°C with aeration up to the late logarithmic phase in medium A [3] with 0.2% glucose and 1 μCi/ml of uridine-5 labeled with $^3H_1$ (30 Ci/mmole), were harvested by centrifugation, washed, and suspended in Hanks' solution to a concentration of $2 \cdot 10^9$ bacteria/ml. The efficiency of incorporation of the radioactive label under these conditions averaged $2 \cdot 10^{-3}$ cpm per bacterium. The presence of fimbriae on the bacteria and their preservation in the course of the experiments were confirmed electron-microscopically by negative staining.

The mean number of cells grown on coverslips was determined, after their removal with trypsin, by counting in a Goryaev's chamber. The bacterial suspension was applied to the washed cell monolayer and incubated under conditions corresponding to the experimental aims. The multiplicity of infection did not exceed 10^4 bacteria per cell.

To determine the level of physical adsorption of the bacteria [2], flasks previously cooled to 4°C, with cells and added bacterial suspension, were incubated at 4°C.

The level of adsorption and physical sorption on glass was determined during the experiments at 37°C and at 4°C.

At the end of the period of adsorption the coverslips were removed from the flasks, washed 8 times in Hanks' solution, dried, fixed with 96° ethanol, and placed on the bottom of scintillation flasks with the cells uppermost. Radioactivity was counted in a "Beckman 250" liquid scintillation counter (USA) with counting efficiency of 25%, using standard toluene scintillation fluid.

The efficiency of adsorption of bacteria on cells of the monolayer (Ef, the number of bacteria per cell) was calculated by the equation:

$$Ef = \frac{A - (a - B_2 + B_1)}{y \cdot C},$$

where A is the count with adsorption of bacteria on the cell monolayer at 37°C (in cpm), a the count for physicochemical sorption (4°C) of bacteria on the cell monolayer (in cpm), B_1 the count for adsorption of bacteria on empty coverslips at 37°C (in cpm), B_2 the value of physicochemical sorption of bacteria (4°C) on glass (in cpm), y the efficiency of incorporation of label into bacteria (in cpm/bacterium), and C is the number of cells on the coverslip.

EXPERIMENTAL RESULTS

Choosing optimal conditions of adsorption of bacteria on eukaryote cells *in vitro* showed that the level of adsorption rises with an increase in concentration of bacteria, but complete saturation cannot be achieved because of peeling of the cells from the glass on account of the cytopathic action of the bacteria.

The optimal multiplicity of infection was 10^4 bacteria per cell. Binding of bacteria with cells of the monolayer reached a maximum under these conditions after combined incubation for 30 min (Fig. 1), but not after 1-2 min [13]. Electron-microscopic examination of specimens of bacteria taken at different stages of adsorption revealed the presence of fimbriae on more than 90% of microorganisms throughout the duration of the experiment.

The process of adsorption of bacteria on cells of the monolayer was found to depend on temperature and to reach a maximum at 37°C; this indicates that it is actively biological in character (Table 1), in contradistinction to some data in the literature [13].

TABLE 1. Comparison of Levels of Physical Sorption and Specific Adsorption of Bacteria on Different Lines of Mammalian Cells

Cell line	Physical sorption (4°C), cpm per cell	Specific adsorption (37°C), cpm per cell
E5	52 086±148	109 196±224
E5+Balb v2	52 515±117	112 190±261
L 41	86 624±158	181 457±259
L 41+ living measles vaccine virus	118 180±261	211 654±311

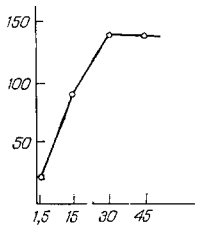

Fig. 1. Level of radioactivity bound with cells of monolayer depending on duration of contact with bacteria. Abscissa, duration of contact of monolayer of L-41 cells with ^{3}H-labeled bacteria (in min); ordinate, radioactivity bound with standard number of cells of monolayer (in cpm × 10^3). Each point on curve represents mean value of three independent measurements.

TABLE 2. Dependence of Level of Adsorption of Bacteria on Cells of Monolayer on Presence and Nature of Virus

Cell line	Level of specific adsorption on cells, number of bacteria per cell
E5	159±11
E5+Balb v2	169±9
L-41	564±16
L-41+ living measles vaccine virus	853±21

It will be clear from Table 1 that adsorption of bacteria is possible also on cell lines not infected beforehand with virus, contrary to existing data [12]. However, this does not rule out the role of virus infection in the level of binding of bacteria with the host cells. The data in Tables 1 and 2 are evidence that, depending on the nature of the virus, its reproduction in mammalian cells can exert a significant influence on the level of binding of the cells with the infecting bacteria. For instance, the development of measles virus in cell line L-41 (Table 2) is 1.5 times greater than the level of adsorption of the bacteria. Meanwhile oncovirus does not change adsorption of bacteria by fibroblasts of cell line E5. The results given above, and, in particular, the increase in the level of physical sorption of bacteria on cells of line L-41 when the latter are infected with measles virus, are indirect evidence in support of the hypothesis [14] that virus infection causes generalized changes in the cell surface and not just of individual receptors. It seems less likely that new receptors can appear [12, 14].

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