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Short Communication

Adsorption of Coliphage T4D to the Mammalian Cells and Interaction between the Phage and Smegmatocin 14468 on the Mammalian Cells

(Coliphage T4D/mycobacteriocin/receptors)

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Coliphage T4D particles can attach not only to the cell surfaces of *Escherichia coli* B cells but also to the cell surfaces of mammalian cells. The adsorption of T4D phage particles was specific because their adsorptions was strongly blocked by N-acetyl-D-glucosamine and plant lectins such as concanavalin A and wheat germ agglutinin. Cytotoxic effects of bacteriocin (smegmatocin) derived from *Mycobacterium smegmatis* ATCC 14468 on simian virus 40-transformed BALB/c mouse tumor cells was inhibited by T4D phage particles. These results suggest the possibility that the receptors for coliphage T4D are shared by those for mycobacteriocin molecules on the cell membrane of the transformed cells.

Bacteriocins produced by various bacteria are proteinaceous, bactericidal substances, and usually active against bacteria of the same or closely related species (1—3). Recently, we reported that bacteriocin (smegmatocin) 14468 obtained from *Mycobacterium smegmatis* ATCC 14468 cells attacked not only susceptible mycobacteria but also mammalian tumor cells (4, 5). T4D phage is a bacterial virus which can propagate within *Escherichia coli* B cells as a host. Although the mode of actions of bacteriocins and bacteriophages differ, they have a common point which is defined by the initial adsorption to the binding sites on the cell surfaces of host. A receptor area for T4D phage is a lipopolysaccharide moiety in the cell wall from *E. coli* B (6) and its determinant group is the glucosyl glucose or the N-acetyl-glucosaminyl glucose residue (7). However, whether or not T4D phage can adsorb to the mammalian cell surfaces and show cytotoxicity as in cases of several bacteriocins remains unknown (4, 5, 8). We studied the adsorption of T4D phage and the interaction between T4D phage particles and smegmatocin 14468 molecules on the mammalian cell surfaces.

Tumor virus-transformed cells (XC, 155-4 T2, TSV-5 and mKS-A TU-7 cells) (5) and normal cells (NRK, BHK 21 and BALB/3T3 cells) (5) in the

same species were suspended in 5 ml of Eagle's minimal essential medium (MEM, Wako Pure Chemical, Osaka, Japan) supplemented with 10% fetal bovine serum (MBA Inc., Walkersville, Maryland, U.S.A.) at a density of 10^5/ml and cultured in plastic tissue-dishes (50 mm, Wako Pure Chemical) at 37°C for 24 hr in a humidified atmosphere containing 5% CO_2 and 95% air. Adherent cells grown on the dishes were rinsed with MEM lacking serum and then incubated with 0.2 ml of T4D phage suspension at a density of 5×10^8 plaque-forming units (PFU) per ml at 37°C for 120 min. After incubation, the phage suspension was withdrawn, diluted 10-fold with Tris-hydrochloride buffer (pH 7.5) containing 0.1 M NaCl, and the residual PFU was assayed by using *E. coli* B cells as an indicator, as described previously (7). As can be seen in Fig. 1, the number of attached phage particles (PFU)

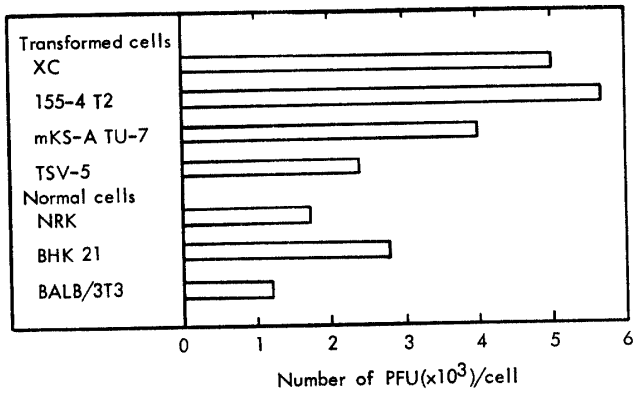

Fig. 1. Adsorption of T4D phage to various mammalian cells. Adherent cells (5×10^5) grown on the plastic tissue-dishes (50 mm) were incubated with 0.2 ml of T4D phage suspension (5×10^8 PFU/ml*) at 37°C for 120 min. After incubation, the residual PFU were assayed, and the number of adsorbed phage particles per cell was calculated as follows.

$$\text{The number of adsorbed phages/cell} = \frac{\text{PFU in the control*} - \text{Residual PFU in the test}}{\text{The number of adherent cells}}$$

to XC, 155-4 T2 and mKS-A TU-7 cells was about 5,000, 5,800 and 4,000 per cell, respectively, while PFU per cell of normal cells ranged from 1,000 to 3,000. Simian virus (SV) 40-transformed hamster cells (TSV-5) which were relatively resistant to smegmatocin 14468 (5) showed less ability of adsorption of T4D phage (about 2,500/cell). Thus, except for TSV-5 cells, the phage-adsorbing abilities of tumor cells which were sensitive to smegmatocin 14468 were much higher than those of normal cells which were relatively resistant to the bacteriocin. T4D phage showed no toxic effect on the mammalian cells (data not shown).

We examined whether or not the attachment of T4D phage particles to these cell surfaces is specific. Adherent mKS-A TU-7 cells (5×10^5, SV 40-transformed BALB/c mouse cells) were incubated with or without 0.2 ml of 0.5 M N-acetyl-D-glucosamine (GlcNAc) and 0.5 M L-rhamnose at 37°C for 15 min, and further incubated with 0.2 ml of T4D phage suspension (5×10^8 PFU/ml) at 37°C for up to 180 min. At various time intervals, the

mixture was withdrawn and the number of unadsorbed phages was assayed. As shown in Fig. 2, when the adherent cells were incubated with the phage

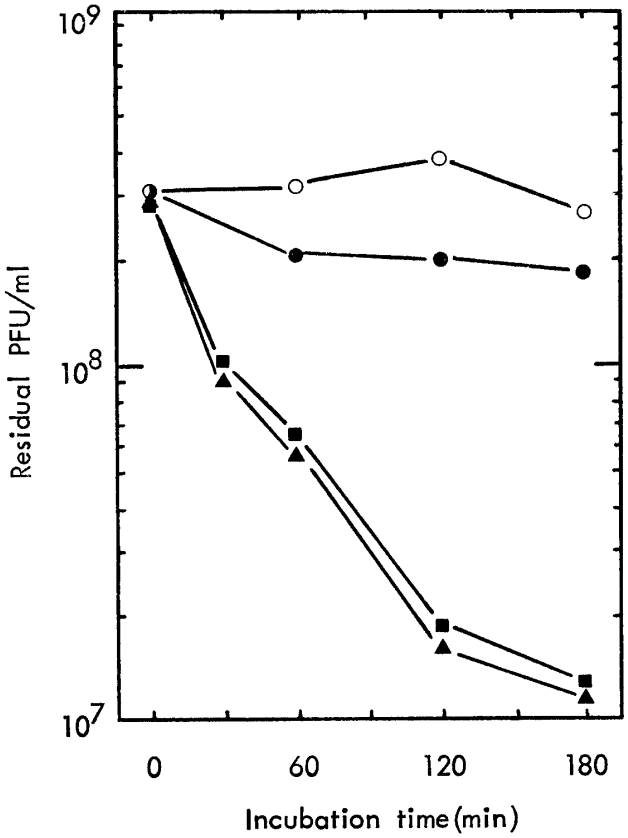

Fig. 2. Inhibition of adsorption of T4D phage to mKS-A TU-7 cells by sugars. Adherent cells (5×10^5) were incubated with or without 0.2 ml of N-acetyl-D-glucosamine (GlcNAc, 0.5 M) and L-rhamnose (0.5 M) at 37°C for 15 min, and further incubated with 0.2 ml of T4D phage suspension (5×10^8 PFU/ml) at 37°C for up to 180 min. At indicated intervals, the residual PFU were assayed. Phage only (—○—), Phage +cells (—▲—), Cells+GlcNAc + phage (—●—), Cells + L-rhamnose+ phage (—■—).

suspension in the presence of 0.5 M L-rhamnose, an evident decrease in PFU was observed. On the contrary, when the adherent cells were incubated with the phage suspension in the presence of 0.5 M GlcNAc, only a slight decrease in PFU was seen. These results suggest that the adsorption of T4D phage particles to mKS-A TU-7 cells is blocked by GlcNAc. For clarification, concanavalin A (Con A) and wheat germ agglutinin (WGA) which specifically bind to the glucose (and/or mannose) residue (9) and the N-acetylglucosaminyl residue (10), respectively, were examined for blocking activity against the adsorption of T4D phage particles to cell surfaces. Adherent mKS-A TU-7 cells were incubated with or without 10 mM Con A and WGA at 37°C for 30 min and further incubated with 0.2 ml of T4D phage suspension (5×10^8 PFU/ml) at 37°C for 120 min. The incubation mixture was withdrawn and the residual PFU was assayed. As shown in Fig. 3, the number of PFU per cell was markedly reduced by preincubation with Con

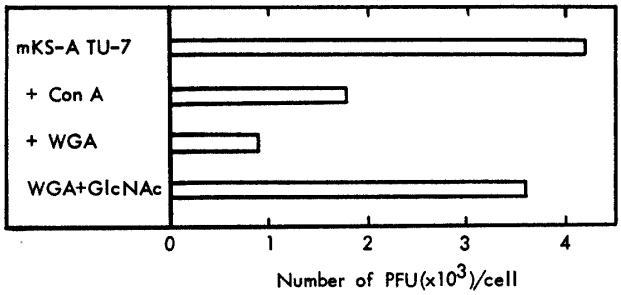

Fig. 3. Inhibition of adsorption of T4D phage to mKS-A TU-7 cells by plant lectins. Adherent cells (5×10^5) were incubated with or without 10 mM concanavalin A (Con A) and wheat germ agglutinin (WGA) at 37°C for 30 min, and further incubated with 0.2 ml of T4D phage suspension (5×10^8 PFU/ml) at 37°C for 120 min. Cells treated with WGA was incubated with 0.5 M GlcNAc at 37°C for 30 min, rinsed with MEM and further incubated with T4D phage suspension. In all cases, the residual PFU were assayed and the number of PFU per cell was calculated as mentioned in the legend of figure 1.

A (10 mM) or WGA (10 mM) as compared with the control (about 4,000 particles/cell). The blocking activity of WGA was also effectively reversed by addition of 0.5 M GlcNAc. These results indicate that the attachment site of T4D phage particles to mKS-A TU-7 cells may be the glucosyl or the N-acetyl-glucosaminyl residue on the cell surface.

Smegmatocin 14468 molecules strongly adsorb to mKS-A TU-7 cell surfaces and show cytotoxic effects (5). On the other hand, the adsorption of smegmatocin 14468 to indicator bacteria (*M. diernhoferi* ATCC 19340) was blocked by glucose derivatives (11). If the binding sites for T4D phage particles are the same as those for smegmatocin 14468 molecules on the cell membranes of mKS-A TU-7 cells, the cytotoxic activity of the bacteriocin would be blocked by the phage particles. Thus, adherent mKS-A TU-7 cells (5×10^5), preincubated with or without 0.2 ml of the phage suspension (5×10^8 PFU/ml) at 37°C for 120 min, were exposed to 0.2 ml of the bacteriocin solution (256 arbitrary units/ml) at 37°C for 180 min. The cells were rinsed with MEM and further incubated at 37°C for up to 96 hr. At various time intervals, the adherent cells were detached with 0.2% EDTA, suspended in 0.85% NaCl and counted with a hemocytometer as described previously (12). As shown in Fig. 4, the toxic effect of smegmatocin 14468 against mKS-A TU-7 cells was strongly inhibited by T4D phage particles. Therefore, it is quite possible that the binding sites for T4D phage particles are the same as those for smegmatocin 14468 molecules on the cell membranes of mKS-A TU-7 cells, and further, that the glucosyl and/or the N-acetyl-glucosaminyl residues are the determinant constituents.

On the basis of these experiments, bacteriophages are expected to provide useful experimental tools for studies on the functions and constituents of mammalian cell membranes.

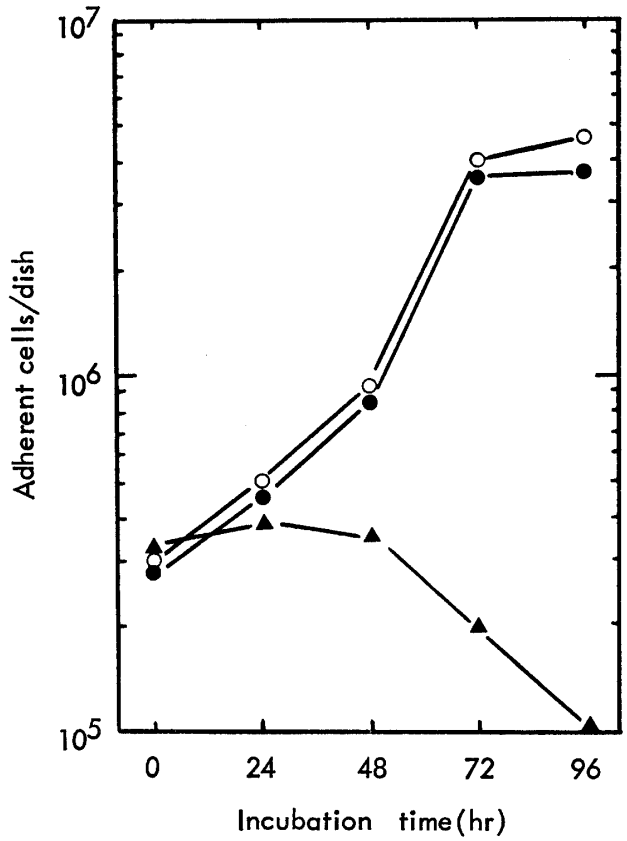

Fig. 4. Inhibition of growth-inhibitory activity of bacteriocin 14468 by T4D phage particles. Adherent mKS-A TU-7 cells (5×10^5), preincubated with or without 0.2 ml of T4D phage suspension (5×10^8 PFU/ml) at 37°C for 120 min, were exposed to 0.2 ml of bacteriocin solution (256 arbitrary units/ml) at 37°C for 180 min. At indicated intervals, the adherent cells were counted. Cells only (—○—), Cells+bacteriocin (—▲—), Cells+bacteriocin+phage (—●—).

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