Identification of adhesive structural proteins of phages T4, A3/R, 676/Z and their effect on mammalian cells

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Summary

Bacteriophages naturally occurre in human and animal bodies as an important component of the microbiome. They can also be used as the potential therapeutics in infections caused by drug-resistant bacterial strains. The detailed structure of most phages and their interactions with mammalian organisms are not well studied. These issues are important for understanding the role of phages naturally occurring in our bodies and for the safety and good effectiveness of phage use in medicine. The ability of phages to maintain in mammalian organisms suggests that these viruses in the course of evolution could evolve some mechanisms responsible for the interactions with higher organisms. Furthermore, the knowledge about the nature of these interactions, and especially which elements of phage virions are involved in them, is very limited. The aim of this work was to identify structural proteins of staphylococcal bacteriophages: A3/R and 676/Z, which give them the ability to interact with human cells.

Using the phage model (fluorescently-labeled T4), whose morphology is similar to staphylococcal phages, the ability to accumulate in the organs playing a major role in the effector functions of the immune system was assessed; particularly in capturing foreign elements, including phages. For the visualization of bacterial viruses, a gene coding green fluorescent protein (GFP), in a fusion with the gene encoding the decorative phage protein (hoc protein), was cloned and expressed in the E. coli bacterial system. Consequently, the in vivo phage display was used to introduce the GFP-hoc fusion into the bacteriophage capsid to obtain a fluorescent phage. The accumulation of bacteriophages in tissues was observed. Virions mainly accumulated in organs involved in the production of the immune system responses, including the induction of a specific response: in the spleen and lymph nodes.

In this study, the ability of staphylococcal therapeutic phages A3/R and 676/Z, and then their structural proteins, was also examined to induce specific immune response. It was found that in the mouse model, both bacteriophages effectively result in the production of specific antibodies: IgM and IgG, and their distribution is typical, as observed with many other types of protein antigens.

In the case of phages A3/R and 676/Z, the structure of the capsids has not been known so far. Therefore, the identification of the main structural proteins, that build the virions, was carried out. The bioinformatics methods have identified the genes encoding potential structural proteins, which were cloned into expression vectors, expressed and purified. The obtained proteins were used for immunoassays, immunolocalization of proteins in virions and for testing the adhesion of phages to eukaryotic cells.

The function of four genes present in the genome of the bacteriophage A3/R: AFN38122.1 (A2), AFN38181.1 (A4), AFN38113.1 (A6), AFN38152.1 (A8) and four homologous genes in the phage genome 676/Z: AFN38316.1 (A2), AFN38375.1 (A4), AFN38307.1 (A6), AFN38346.1 (A8) was determined. Using the immunoelectron microscopy, it was discovered that the proteins encoded by the above genes perform structural functions by building the phage capsid. A2 protein is a structural protein, which build the head of A3/R and 676/Z phage. Proteins A4 and A6 are placed on the tail in phages A3/R and 676/Z. A8 protein is located on the A3/R and 676/Z base plate. Moreover, only in the case of A8 protein, the complete inhibition of the phage's antimicrobial activity by the specific serum was observed. No neutralization of phages was detected for antibodies specific to other proteins (A2, A3, A4, A6). It suggests A8 protein may act as a ligand for the bacterial receptors used by these phages.

What is more, the frequency of phage neutralizing antibodies in case of phages: A3/R and 676/Z in the human population was examined. There were no significant differences in the presence of specific antibodies to phages A3/R and 676/Z; it was reported at 30% for the A3/R phage and 35% for the 676/Z phage. The occurrence of natural antibodies against the selected structural proteins (A2, A6, A8) of staphylococcal phages in healthy subjects was also analyzed. There were no substantial differences between the occurrence of antibodies specific for the tested proteins in the human population.

Apart from the expected interactions of the phages with the immune system, other types of relations between phages and the mammalian organisms are possible. The potential existence of such proteins is important, i.e. for the accumulation of phages placed in the sites of infections caused by Staphylococcus aureus. The ability of phage adhesion in these tissues, where infections occur, would give the phage accelerated contact with the bacteria as a host. It results in the effectiveness of treatment with adhering phages. In this study, the adhesive properties of A2, A4, A6, A8 proteins were tested against mammalian cells in vitro. The adhesive effects of A6 protein were observed in a human lung microvascular endothelial cell (HLMEC) cells and human skin cells (Hs294T tumor line was used). The adhesive interactions of other proteins

were not observed. The obtained results suggest the tested phages interact adhesively with certain mammalian cells via A6 protein, located on the tail. Bacteriophages, commonly found in mammalian organisms and administered for therapeutic purposes, may interact with cells of higher organisms. Interactions, both adhesive and those between phages and the immune system, were previously observed in the case of model phage (T4). In this work, these interactions have been studied and described for therapeutic phages. This knowledge can serve to the further develop of the phage practical importance, both as components of natural ecosystems, including human microbiome, and as the potential tools used in biotechnology and medicine.