Title: Studies of T4 bacteriophage capsid proteins modulatory effect on inflammatory reaction

Summary

Viruses cause a strong inflammatory response in animals and humans. Capsid proteins usually confer immunostimulatory character to viral particles. By activating signaling pathways, they contribute to the induction of inflammatory mediators. The potential effect of bacterial viruses on the mediators of the inflammatory process in higher organisms should therefore be considered due to the perspective of their medical or veterinary applications.

The aim of this study was to assess the ability of T4 bacteriophage capsid proteins to modulate the inflammatory response. Out of a large and ubiquitous group of tailed T4-like phages, T4 was the first phage to be completely sequenced and thoroughly characterized. It is also a virus representing a large group of similar phages, very common in the environment and in the body of humans and animals.

In the first part of this work, proteins that build the mature form of the bacteriophage T4 head were examined: gp23 *, gp24 *, Hoc and Soc. The genes encoding the abovementioned proteins were cloned into expression vectors, and their expression and purification were then optimized. Highly purified protein preparations were obtained and used for further studies.

The effect of obtained phage protein preparations on the production of factors involved in the inflammatory reaction was assessed. Screening tests for production of several dozen immunological factors (RayBio® Cytokine Array cytokine matrices) in the mouse model and *ex vivo* in human blood showed that gp23 *, gp24 *, Hoc and Soc do not affect the activity of inflammatory cytokines, e.g. IL-1α, IL -1β, IL-2, IL-6, IL-12 p40 / p70, IFN-γ, TNF-α; chemokines e.g. IL-8, MCP-1, MIG, RANTES; growth factors, e.g. MCSF, GCSF, GM-CSF, VEGF; mediators of inflammation associated with allergies, e.g. IL-4, IL-5, IL-13; cytokines and anti-inflammatory agents, e.g. IL-10, sTNF RI, sTNF RII; or adhesion molecules e.g. , P-selectin, VCAM-1. Additionally, the tested phage proteins did not affect the release of ROS by human PBMCs and PMNs.

The structure of T4 phage is complex and there are other elements that could potentially influence the occurrence and course of the inflammatory reaction. For this reason, the whole particle of T4 phage was subjected to the same tests to assess the possible effect of the entire capsid. It was shown that T4 phage does not induce production of neither inflammatory regulators in mice nor ROS in human

blood cells. Therefore, it can be concluded that both the T4 phage and its gp23 *, gp24 *, Hoc and Soc head proteins do not induce inflammation in mammals.

Staphylococcal phages A3R and 676Z were also included in the study, in order to compare the properties of the model phage T4 with other phages. The results of screening tests for the production of several dozen immunological factors in the mouse model using RayBio® Cytokine Array indicated that A3R and 676Z phages also do not affect the activation of the inflammatory reaction. These phages and T4 phage are taxonomically distant and do not share homology, which suggests that the inability to induce an inflammatory response is a characteristic of a wider group of bacterial viruses.

Tests were carried out to determine the effect of *Escherichia coli* T4 and HAP1 phages and *Staphylococcus aureus* A3R and 676Z on complement activity. All three complement activation pathways were examined: the classical pathway, the alternative pathway and the lectin pathway. It was shown that phages T4, HAP1, A3R and 676Z do not affect the activity of the alternative pathway. HAP1, a natural T4 mutant lacking Hoc protein, reduced the activity of the classical pathway, while staphylococcal phages A3R and 676Z suppressed the activity of the lectin pathway.

The next part of this work regarded the protein forming short tail fibers of bacteriophage T4 baseplate, which is capable of binding LPS. First, cloning and production of gp12 were optimized. A final purified preparation of gp12 that was obtained retained its native structure and thus its biological properties, i.e. LPS binding capacity, as confirmed by the hydrodynamic diameter of the gp12 and LPS mixture.

The purified gp12 preparation was used for *in vivo* studies. It was shown that recombinant gp12 was able to counteract the inflammatory response to LPS in a murine model *in vivo*. When administered simultaneously with LPS, gp12 preparation reduced the level of inflammatory mediators in mice and histopathological analysis of selected animal tissues revealed that gp12 may reduce leucocytes infiltration to spleen and liver. It was also shown that gp12 is not toxic to animals and eukaryotic cells *in vitro*.

Lack of immunostimulatory effect of both capsid proteins and whole phage particles confirms the safety of phage application in medicine. These observations are important for any medical or veterinary use of bacteriophages. Acute, chronic infections leading to sepsis still present a serious challenge and a problem for clinicians around the world. Gp12 seems to have properties that could offer a useful medical solution in cases of acute infections or sepsis associated with LPS. This work presents pioneering results, which should be further developed

and expanded in the future, but are in line with the trend of the latest research interests regarding immunobiology of phages and safety of their application.