



THE INFLUENCE OF *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGE ON CYTOKINE SYNTHESIS IN MICE

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Introduction. Earlier we show (Filipova T. *et al.*, 2007), that *Clostridium perfringens* bacteriophage can amplify inflammatory reactions that cause carragenan and zymosan due to increasing a proinflammatory cytokines production. It was interesting to know, are other bacteriophages commercial preparations can possess the same effects.

Aim. We have shown previously that a commercial preparation of *Staphylococcus aureus* bacteriophage induced an inflammatory reaction on carragenan and zymosan after oral use. Taking into consideration a significant role of cytokines in inflammation, the aim of the current study was to evaluate the effect of *Staphylococcus aureus* bacteriophage *in vivo* and *in vitro* on TNF- α , IFN- γ and IL-10 production.

Materials and Methods. *Mice.* Male BALB/c mice (10 weeks old) were obtained from the vivarium of the Medical Institute (Odessa, Ukraine). After acclimatization (2 weeks) the animals were housed in solid-bottomed cages containing bedding of wood shavings and were allowed food and water *ad libitum*. The room temperature was maintained at 21–24 °C and a 12/12 h light/dark cycle was employed. At the end of the experiment, the mice were sacrificed by cervical dislocation and their lymphoid organs were weighed.

Bacteriophage (10^7 pfu/ml) were obtained from Perm, Russia.

In vivo experiments. *Staphylococcus aureus* bacteriophage was administered per os at dose 0,5 ml 1- and 3-fold. Each time point and treatment group was composed of five animals per experiment. Sera and supernatants from spleen homogenates were collected and frozen until used for cytokine determination by ELISA.

In vitro experiments. Isolated peritoneal and spleen macrophages were incubated in a 96-well plate at the concentration of 10^5 /well in culture medium RPMI 1640 supplemented with 10 % serum, 2 mM L-glutamine and antibiotics. Cells were incubated without or with *Staphylococcus aureus* bacteriophage at the concentration 10^5 and 10^6 pfu/ml. After 24 h incubation (37 °C, 0.5% CO₂) cell culture supernatants were collected and stored at -70°C until analyzed.

Mouse cytokine response. Concentrations of the cytokines tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ) and interleukin-10 (IL-10) were measured in triplicate by using the Mouse Cytokine Ready-SET-Go! ELISA Kit (eBioscience, USA). The assay was carried out as indicated by the manufacturers. The optical density was determined by a Microplate Reader Uniplan (Russia) at 450 nm.

Results. The fact, that *Staphylococcus aureus* bacteriophage can possess not only an anti-inflammatory effect, but in other hand, can suppress phagocytic activity and active oxygen form generation by peritoneal macrophages *in vivo* and *in vitro*, were shown earlier by our work group. A potent of these effects depend on the duration of uses the *Staphylococcus aureus* bacteriophage and its concentration in the incubation medium. Because these effects can be determined by the cytokines synthesis modulation with the *Staphylococcus aureus* bacteriophage, in our work we were study a contents of the proinflammatory - TNF- α , IFN- γ ; and anti-inflammatory – IL-10 cytokines in mice after one- and three times injection. Results of this investigation show that even one injection of the *Staphylococcus aureus* bacteriophage, possess a strong changes in the level of the examined cytokines.

IFN- γ content in serum and spleen of mice increase in 2.5 and 3.4 times, and content of the TNF- α increase for 24 % and 35 %. After three times administration of the *Staphylococcus aureus* bacteriophage, level of this cytokines increase were higher, then after one time injection, especial for IFN- γ . Its level in serum and spleen was in 7.8 and 10.2 times higher in contrast the control animals. Tumor necrosis factor content increased for 50 %. In contrast, content of the IL-10 were decrease in the dependence on dose of the staphage,

and after three time administration its level compiled a half over there starting level. This data suppose that *Staphylococcus aureus* bacteriophage can posses activate Th1 helper lymphocytes subpopulation and NK-cells that are major producents of the IFN- γ . In this way, we can suppose, that increasing of the IFN- γ and TNF- α levels after *Staphylococcus aureus* bacteriophage administration, make usage of this phage one of the perspective strategies to increase antiviral and antitumor resistance of the organism on the background of the phagotherapy. In other way, inhibitory effect of this bacteriophage on the IL-10 production, suppose that it can decrease activity of Th2 helpers and, accordingly, decrease humoral immune reactions.

In the in vitro experiments, were studded *Staphylococcus aureus* bacteriophag ability to cause action to the cells that produce cytokines, which levels were studded in *in vitro* experiments.

Our results show, that staphage can directly activate cytokines-produce cells. This type of action displayed in activation of the dose-depended IFN- γ production by spleen lymphocytes and TNF- α by macrophages. In the same time, *Staphylococcus aureus* bacteriophage did not posses any effects on the IL-10 production in vitro.

Conclusion. Thus, our investigation show that strengthening of the inflammatory answer after *Staphylococcus aureus* bacteriophag injection is the result of the activation of producing major proinflammatory cytokines - IFN- γ and TNF- α . Similar, increasing TNF- α level were shown after bacteriophage therapy at human. Moreover, at this people were inhibiting phagocytic activity of the neutrophils even up to three month period (Reynaud A. *et al.*, 1992; Weber-Dabrowska *et al.*, 2002). Absence the direct action of *Staphylococcus aureus* bacteriophage on the IL-10 production by splenocytes can evidence that decreasing its level in vivo may be connected with stimulation of the proinflammatory cytokines production instead of the action immediately to the cells that produce ones. But there is unknown, is this effects connect directly with phage or with other components, such as lysate of *Staphylococcus aureus* cells. So, this investigation show, that *Staphylococcus aureus* bacteriophage posses the same effects on the pro- and anti-inflammatory cytokines level, like a *Clostridium perfringens* bacteriophage.

References

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