**Immunogenicity of PB1-like bacteriophages in humans and in mouse model**

*Pseudomonas* bacteriacause infections that are difficult to overcome in both humans and animals. A large part of these are antibiotic-resistant bacterial strains. The study of bacterial viruses attacking *Pseudomonas*, such as PB1-like ones, are important for the development of phage therapy. Phages are an alternative to antibiotics, which often are ineffective in the treatment of bacterial infections. However, PB1 bacteriophages are very poorly characterized, there is little literature data describing the structure of these phages. There is no literature data linking molecular characterization of PB1 bacteriophages with their immune reactivity.

The aim of this PhD thesis is to compare the immunogenicity of the F8 LMA2 and P1 phages and to link the differences in their immunogenicity with differences in their molecular structure. The research involved determining the effect of antibodies specific for the bacteriophage on antimicrobial activity of these viruses and the location of selected phage proteins.

The starting point for the research was to compare the in vivo and in vitro immunogenicity of bacteriophages from the BP1 group. Phages: F8 – used in the treatment of patients in the Wrocław Phage Therapy Unit (Institute of Immunology and Experimental Therapy, IIET); LMA2 – obtained from prof. Rob Lavigne, (Katholieke Universiteit Leuven, Belgium); and P1 – obtained through collaboration with prof. Joana Azeredo (University of Minho, Portugal).

The ability of the bacteriophage to induce the production of specific IgM and IgG antibodies in a murine model were compared. Mice were immunized with highly purified phages, and animal serum of IgM and IgG antibody levels specific for the phage were monitored for 160 days. Initially, the highest level of antibodies was observed in the case of the LMA2 phage. However, during the experiment the level of antibodies to LMA2 fell, and thus the P1 phage demonstrated the highest level of IgG antibodies.

Sera from a 55-person group of healthy people, who had never been subject to phage therapy,were tested *in vitro*. It was found that among the studied population the most frequent antibodies were those against the P1 phage – 40% of the samples. For LMA2 and F8 it was respectively 11% and 15% of the samples in the tested group.

Studies to determine the connection between the molecular structure of the phage and their ability to induce an immune response were initiated by selecting structural protein genes from these phages (ORF18, ORF22, ORF29). The selected genes were cloned and protein expression in *Escherichia coli* system was optimized. Protocols of the highly purified protein production were developed using affinity chromatography, proteolysis, size exclusion chromatography, LPS affinity chromatography (EndoTrap®HD) and dialysis.

The obtained proteins were used for immunization in the murine model and as antigens in the detection of antibodies specific for proteins following murine immunization with whole bacteriophages and in samples of human serum from healthy donors and the patients of the Phage Therapy Unit, IIET.

In the serum samples from animals immunized with the F8, LMA2 and P1 phages the levels of IgM and IgG antibodies were compared (specific for the expressed gp18, gp22, gp29 protein groups). The level of antibodies induced by the gpP18 from the P1 phage was significantly higher than that induced by gp18 from the F8 phage.

Most probably, strong immunogenic properties of the gpP18 result from the 'MMAFK' and 'KIAP' motifs present in this protein (data were obtained through a bioinformatic analysis made by Marek Harhala, IIET).

High immunogenicity of the P1 phage and the gpP18 protein has not been confirmed in studies of natural antibodies against selected structural proteins of the PB1 phages in human serum samples. The highest frequency of natural antibodies was observed for the LMA2 bacteriophage. These results do not coincide with the data on structural proteins obtained in studies on the murine model. However, they can be compared to the data obtained through animal immunization with whole phages, where the LMA2 bacteriophage initially showed the highest level of induced antibodies. These studies may indicate that brief contact with the LMA2 phage causes an increase in antibody levels, whereas during the experiment the number of antibodies to the LMA2 falls. Furthermore, the process of immune response is complex and may be dependent on various factors, ones that are not yet known.

With the use of immuno-electron microscopy techniques, the gpP18 protein was localized on the head of the P1 bacteriophage and the localization of the gpP22 and gpP29 proteins was confirmed. The gpP22 protein was found in the capsid, while gpP29 builds up the tail of the P1 phage.

The influence of specific antibodies on the *in vivo* phage activity was examined as an example of the therapeutic F8 phage by specifying phage concentration in the blood and other tissues in a murine model. Pre-immunized mice and ones that did not have contact with the phage were compared. Phage activity in the blood of mice after the initial immunization (high IgM) was significantly lower than in the control group after one hour from phage administration. In animals with high IgG levels, the phage was completely neutralized. A similar tendency was observed in the liver, spleen, kidney, lymph nodes and muscles. Laboratory results on the interaction of the F8 phage and the immune system were used to create a mathematical model (Dr. Jarosław Drapała, the Wroclaw University of Technology). This model has practical implications as it allows us to estimate the phage therapy process in given conditions, including the tripartite interaction of the bacteriophage, bacteria, and the immune system.

The results obtained in this thesis represent the first attempt to connect the features of the molecular construction of the PB1 group with their effect on the immune system. The complete knowledge of the phages’ interaction with the immune system may have a positive influence on optimization of the therapeutic use of bacteriophages. It can also be used in the process of selection of a phage for specific patients’ situations, including the immune response to achieve the most favorable solution.