Phage-specific diverse effects of bacterial viruses on the immune system

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> **"**prophages are the major contributor to bacterial inter-strain immune heterogeneity manifested as variation of adaptive T- and B-cell immune responses of human lymphocytes, *in vitro*, to *S. aureus* and *Streptococcus pyogenes***"**

With the increasing threat of antibiotic resistance, the interest in phage therapy (PT) as a potential solution to this crisis has rapidly grown. Recently, several reports have been published describing successful treatment of patients with life-threatening antibiotic-resistant bacterial infections, including recipients of lung allografts and treatment using genetically modified phage. Moreover, the first PT center has been opened in the USA, following the establishment of a similar unit in Belgium. These developments give credence to our decision to establish, in 2005, the first such unit operating in accordance with EMA and national regulations, which helped pave the way for future progress in PT as an option to combat the antimicrobial resistance crisis. Ample evidence derived from observational studies indicates the safety of PT. Furthermore, several clinical trials have been completed (including one performed according to all required standards of good medical practice and evidence-based medicine) and are ongoing. However, these trials are yet to provide definitive proof of the effectiveness of PT [1–4]. As the struggle to register phage as a medicinal product and to introduce it onto the market continues, parallel data have been accumulating to indicate that phage may interact not only with bacteria but also with eukaryotic cells (including cells of the immune system). Therefore, it cannot be excluded that in the future, after phage discovery, research may shift towards phage–immune system interactions, whereas, to date, work on phage interactions with their natural target (bacteria) has prevailed. Hopefully, simultaneous advancements in both research fields can bring beneficial results for human health, both in terms of combating antibiotic-resistant bacterial infections and developing novel anti-inflammatory and immunomodulatory agents with minimal toxicity and satisfactory efficacy [4,5].

We have formulated a hypothesis suggesting that phage present in the gut may migrate to blood, lymph and organs, mediating anti-inflammatory effects, and contributing to immunological tolerance and immune homeostasis – both *in situ* and at other sites of the body [6]. Study results have subsequently confirmed this and what is more, over 30 billion phages undergo transcytosis of the gut epithelium every day and disseminate to the blood, lymph and organs [7]. Furthermore, other cell types, including immune cells, may also take up phage through the endocytic pathway [8].

The new emerging concept of phage, as not only bacterial predators but as potential anti-inflammatory and immunomodulatory agents, needs detailed further study. One critical issue requiring clarification is phage specificity in mediating particular immune reactions. Phages are known for their high specificity toward bacteria which has

been established for decades and is applied in phage typing for classification of different bacterial strains. Are immunotropic activities also phage specific or do phages induce similar responses, regardless of phage type?

It is believed that phage capsid proteins may primarily be responsible for phage biological properties unrelated to interactions with bacteria. These proteins vary in their immunogenicity and may elicit different antibody responses to phage, which also depends on route of administration. Further, different strains of a homologous phage recognizing a given bacterium, may express different proteins [9,10] and confer various functions to phage (e.g., persistence in circulation and antimetastatic effects). For example, a T4 phage mutant, HAP1, with a nonfunctional Hoc protein is more susceptible to the liver's Kupffer cells and is cleared more rapidly than its parental strain. Also, there are differences between HAP1 and T4 phage in their interactions with T cells and fibrinogen [11,12].

Initial studies on the effects of phage on other immune functions suggest that the effects may also differ depending on phage type. For example, purified T4 coliphage inhibits human T-cell proliferation induced via CD3-TCR complex, whereas purified staphylococcal phage exerts a co-stimulatory effect [12]. A detailed study on *Staphylococcus* and *Pseudomonas* phage revealed that although these phages induced similar responses in human peripheral blood mononuclear cells, through upregulation of gene expression of anti-inflammatory cytokines IL-1 receptor antagonist and of suppressor of cytokine signaling 3, their influence on other immune functions were restricted to the specific phage. A protolerogenic and anti-inflammatory cytokine IL-10 was induced by all tested *Pseudomonas* phage but not by any staphylococcal phage. On the other hand, the latter phage-induced TNFα, whereas only two out of four assayed *Pseudomonas* phage had similar effects. Furthermore, the *TLR4* gene was downregulated solely by a *Pseudomonas* PMN phage, thus suggesting its anti-inflammatory action (TLR4 activation induces secretion of pro-inflammatory cytokines) [13]. Diversity of phage action on the immune system was also confirmed by recent data showing that a filamentous *Pseudomonas* Pf phage inhibits TNF production and phagocytosis while *Escherichia coli* filamentous Fol phage does not have such effects [8]. Moreover, our data indicate that both T4 coliphage and A5/80 *Staphylococcus aureus* phage significantly reduce the expression of human adenovirus genes, but synthesis of viral DNA is inhibited only by T4 coliphage [14]. Additionally, there have been suggestions that temperate and lytic phage may differ in their actions on the immune system [8]. In fact, prophages are the major contributor to bacterial inter-strain immune heterogeneity manifested as variation of adaptive T- and B-cell immune responses of human lymphocytes, *in vitro*, to *S. aureus* and *Streptococcus pyogenes* [15].

Immunomodulatory and anti-inflammatory effects of phage may also be cell- and tissue-specific. The intranasal administration of 536_P1 (but not LM33-P1) coliphage in mice with experimental pneumonia caused an increase in lung antiviral cytokines and chemokines. Neither phage evoked changes in blood cytokine/chemokine levels, which also suggests that phage effects on the immune system may have various manifestations in different compartments of the body [16]. Phage ability to mediate tissue-specific activity is confirmed by Pincus *et al.* [17], where staphylococcal phage did not induce proinflammatory cytokines in human peripheral blood mononuclear cells but may have induced IFN-γ in human keratinocytes. Furthermore, we have shown that A5/80 staphylococcal phage increases the expression of Il-2 in the A549 cell line [18]; an activity that has yet to be reported for phage action on other cell types in *in vitro* studies. An increase of serum Il-2 levels in response to phage administration was also recently reported in mice treated with *Acinetobacter baumannii* phage but its cellular source is unknown [19].

As mentioned, recent data indicates that phage may be internalized by mammalian cells and large numbers transcytose across gut epithelial cells while immune cells also internalize phages, especially dendritic cells (DCs), monocytes and B cells [7,8]. Recently, we described a marked phage-dependent stimulation of *Hsp72* gene [18]. This induction of a known cellular chaperone may be a mechanism for protecting cells undergoing transcytosis from potential injury by intracellular phage. What is more, *Hsp72* is known to decrease T-cell proliferation and cytokine secretion regardless of stimuli used and inhibits DC ability to stimulate allogeneic T cells. This may suggest that *Hsp72* could be used as an immunomodulator [20]. It has also been shown to suppress experimental arthritis in rats [21]. We have reported that phage can inhibit the development of collagen-induced arthritis in mice, an experimental model of rheumatoid arthritis [22]. Interestingly, *Hsp72* was also shown to suppress arthritis in this model [23]. It may well be that phage-dependent induction of *Hsp72* is at least partly responsible for inhibition of aberrant immune reactions (including autoimmunity and hyperinflammation) caused by phage [24].

Phage interactions with immune cells may be dependent on specific phage receptors enabling those interactions. Data are scarce at present on the nature of such receptors. Pruzzo *et al.* [25] suggested that coliphages T3 and T7 could adhere to epithelial cells using their receptors for *Klebsiella pneumoniae*. Our hypothesis pointed to a Lys-Gly-Asp (KGD) sequence present in one of the capsid proteins of T4 phage as a potential ligand for cell integrin receptors [24]. Lehti *et al.* showed that *E. coli* phage may recognize and bind neuroblastoma cells displaying polysialic

acid on their surface [26]. If indeed polysialic acid is a ligand for receptors of some phages it could enable those phages to bind to immune cells as the presence of polysialic acid has also been demonstrated on human DCs, NK cells and a subpopulation of T cells [27,28]. Thus, it is likely that various phages may use different cellular ligands to attach to and transcytose target cells including those of the immune system. Notably, even a single amino acid substitution in a phage capsid protein may cause >1000-fold enhancement of phage survival in mouse circulation, which probably reflects modified interactions between phage and phagocytes (and perhaps other cells endocytosing phage) [29].

Phage not only target specific bacteria but – at least to some extent – can also cause phage-specific immune responses. These findings open a new exciting field for further research on the significance of such responses in health and disease. Furthermore, these data suggest that a specific phage could be optimally selected for use in PT from different phage strains recognizing a given bacterium, considering both its anti-bacterial activity and the type of immune response it may evoke. This is important in patients with immunodeficiencies, autoimmunity, allograft recipients, etc. who – pending the nature of their disease – may require immunostimulation or immunosuppression. Evidently, further research in this field may pave the way for the use of specific phage in immunomodulation.

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