PERSPECTIVE

For reprint orders, please contact: reprints@futuremedicine.com

Phages and immunomodulation

Andrzej Górski^{*1,2,3}, Krystyna Dąbrowska¹, Ryszard Międzybrodzki^{1,2,3},
Beata Weber-Dąbrowska^{1,2}, Marzanna Łusiak-Szelachowska¹, Ewa
Jończyk-Matysiak¹ & Jan Borysowski³

In the past years, the microbiome and its role in the pathophysiology of diseases have gained great interest. The progress of our knowledge in this field opens completely novel prospects for treating disorders, including those which are most challenging to medicine today. Of special interest are studies on the interactions of the microbiome with the immune system. Only recently has the presence of bacteriophages in the microbiome been highlighted, and their potential role in maintaining normal immunity has gained increasing attention. We summarize the available data pointing to the potential impact of phages in maintaining immunological homeostasis.

First draft submitted: 16 March 2017; Accepted for publication: 10 April 2017; Published online: 24 April 2017

Phages & the immune system

The current surge of interest in the microbiome and the recent application of advanced sequencing technology have allowed us to determine the microbiome's role in disease pathophysiology and its potential modulation for therapeutic purposes [1]. The interactions among the intestinal immune system, the epithelial barrier and the bacteria that reside on, are fundamental to maintaining a healthy gut homeostasis [2,3]. A schema of intestinal phage translocation and its protective role is shown in **Figure 1**. The microbiota has an important role in shaping the human immune system, for example, interindividual variation in responses to vaccines, chemotherapy and immune-modulating agents is influenced by differences in the gut microbiota [4]. This fascination with the gut microbiota shows no signs of slowing down, which is confirmed by the launch of the US National Microbiome Initiative in 2016 and similar activities worldwide [5]. The explosion of microbiome research is also illustrated by the fivefold increase in the number of relevant citations in the past 5 years [6].

From their discovery 100 years ago, until the end of the 20th century, the vast majority of studies concentrated on the well-known antibacterial effects of phages. Although phages have been used for immunization to evaluate humoral immunity in animals and patients with immunodeficiency syndromes, no data are available on phage interactions with the immune system and the potential implications of such phenomena for shaping the immune system. This contrasts with the knowledge about the interactions of pathogenic viruses with the immune system, which is quite rich even though pathogenic viruses were described at approximately the same time as phages. Yet – as already mentioned – review articles addressing the relationship between viruses and the immune system

KEYWORDS

• cytokines • gut • immunity
• lymphocytes • microbiome
• phages • platelets

¹Bacteriophage Laboratory, Ludwik Hirsfeld Institute of Immunology & Experimental Therapy, Polish Academy of Sciences (HIET PAS), Wrocław, Poland

²Phage Therapy Unit, Ludwik Hirsfeld Institute of Immunology & Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

³Department of Clinical Immunology, Transplantation Institute, The Medical University of Warsaw, Warsaw, Poland

*Author for correspondence: agorski@ikp.pl

– including the most recent one published by Nature Reviews Immunology – usually do not mention phages [4,7,8]. As rightly pointed out by a Nobel laureate Joshua Lederberg “...little is known of the interaction of bacteriophages with the mammalian host even while they are universally present... in the lower gut” [9].

Bacterial viruses (phages) have only recently been shown to coexist in great abundance, and many of them are persistent, highly personalized members of the microbiome. Most of them appear to be lysogenic, dsDNA viruses belonging

to the Caudovirales order, and their concentration may be as high as 10^9 per gram of feces [10]. Interestingly, the same individual was found to harbor a very similar fecal phage community over at least a 1-year period, which varied by less than 5% [11].

We hypothesized that intestinal phages may have protective potential, not only contributing to the elimination of bacteria but also inhibiting the local immune and inflammatory reactions, thus contributing to maintenance of immune homeostasis [12]. Furthermore, the phenomenon

Figure 1. The potential role of intestinal bacteriophages in maintaining immune homeostasis.

of translocation can enable phages to migrate to distant tissues and interact with cells of the immune system locally and at other sites [13]. We postulated that at least some phages (e.g., T4) could interact with cells of the immune system using the Lys-Gly-Asp (KGD) sequence present in the capsid protein gp24 and the respective cellular receptor β -3 integrin also used by some pathogenic viruses [14]. Interestingly, this sequence is also present within the CD40 ligand, known to activate endothelium and platelets, and stimulate inflammation, and is crucial for T- and B-lymphocyte activation – the interruption of CD40–CD40L interactions has strong immunosuppressive properties. Further evidence for our concept of endogenous phage-dependent support to our immune system has been provided by the data of Barr *et al.*, who described the symbiotic relationship between the phage and the metazoan host, providing antimicrobial defenses actively protecting intestinal mucosal surfaces from bacterial invasion [15]. The authors observed increased phage-to-bacteria ratios on all mucosal surfaces assayed, from cnidarians to humans. This enrichment occurs via binding interactions between mucin glycoproteins and immunoglobulin-like protein domains exposed on phage capsids and provides nonhost-derived mucosal immunity [15].

• Phage interactions with lymphocytes

Human T cells exhibit adhesive interactions with immobilized T4 and HAP1 phages; such interactions can be mediated by at least some phage capsid proteins. For example, it has been demonstrated that T cells adhere to gp24 (but not Hoc) protein. This interaction seems to be mediated by integrins of the β -1 and β -3 families as they can be blocked by respective monoclonal antibodies and the β -3 function inhibitor, eptifibatide [16]. T cells from spleen of naive mice exposed to Felix-01 *Salmonella* phages give weak proliferative responses *in vitro*. However, those responses are higher when mice had been immunized by prior phage administration. Those data suggest that some low level of T-cell sensitization to phage may exist in normal individuals and this reactivity can be upregulated by phage administration [17]. Purified T4 phage strongly inhibits CD3 receptor induced T-cell proliferation *in vitro*; however, it is unclear to what extent this effect is also dependent on lipopolysaccharide (LPS), which may induce similar effects even in very low concentrations. The same phage

preparations have been shown to inhibit skin allograft rejection in normal and sensitized mice, reducing allograft infiltration [18] and inhibiting alloantigen-induced immunoglobulin production *in vitro* as well as specific antibody responses in mice [16]. On the other hand, phage lysate of *Staphylococcus aureus* (but not purified phage preparation) is a potent polyclonal B-cell activator. B-cell activation is mediated by staphylococcal protein A present in the lysate [19]. Immune monitoring of patients on phage therapy suggests that it may cause transient fluctuations of T-, B- and NK-cell reactivities as well as their numbers in peripheral blood. But the treatment does not appear to significantly alter the immune status of patients when compared with their pretreatment immune parameters [16].

DĄbrowska *et al.* have shown that phage proteins differ in their ability to stimulate humoral responses in mice; in this regard, gp23 and Hoc induced significantly higher production of IgM and IgG, while production of gp24-specific IgM and IgG was weak, and the weakest antibody response was induced by Soc protein [20]. Moreover, oral phage application induced only very weak responses even with prolonged administration [21]. An extensive analysis of antiphage antibody responses in patients on phage therapy confirmed a virtual lack of immunogenic effect of orally applied phages. This confirms the data of Bruttin and Brüssow [22]. Interestingly, the level of antibody responses was not correlated with the outcome of therapy. What is more, high antibody levels were detected in 43% of patients treated with phage cocktails versus only in 17% of patients on monotherapy [23,24]. The *S. aureus* phage cocktails were composed of three phages: *S. aureus* A5/80, *S. aureus* P4/6409 and *S. aureus* 676/Z. Monotherapy was used with various monovalent *S. aureus* phages.

Phages vary in their immunogenicity, and the humoral response also depends on the route of administration, dose and the patient's immune status [23,24]. Recently, we presented a vision to develop and establish a bank of phages specific to the most threatening pathogens and armed with homing peptides enabling their selective localization in infected tissues and stable eradication of infection [25]. As shown by the Ruoslahti group, the endothelia of vessels of different organs are molecularly different (vascular zip codes). The studies have allowed for identification of such organ-specific peptides that may be expressed on the phage surface as tissue-homing peptides [25].

This methodology should reduce the exposure of therapeutic phages with cells of the immune system, thus contributing to downregulation of antiphage immune responses.

However, it cannot be excluded that in fact high antibody responses, measured in patients' sera, have a positive prognostic value reflecting phage therapy dependent recovery of the immune system that can cope more efficiently with infection [26]. The rate of phage inactivation (K) less than 5 was estimated as weak phage neutralization, between 5 and 18 as a medium level. K above 18 ($\geq 50\%$ neutralization at serum dilution $\geq 1:800$) was associated with high phage neutralization [23]. Recently, Jain *et al.* have demonstrated that *Brucella abortus* phage lysate absorbed on aluminum gel elicits strong humoral and cell-mediated immune responses in guinea pigs and protection comparable to a standard vaccine. This suggests that this methodology can be explored to produce a new class of immunotherapeutic agents [27]. The details of immune monitoring of patients on phage therapy have been described in detail [16].

• Phages & phagocytes

Intracellular killing of bacteria (IKB) is one of the fundamental defensive functions of granulocytes and monocytes against invading pathogens. Phages may induce only minimal oxygen species production by phagocytes and may lower their release induced by bacteria and LPS [28,29], whereas they appear to exert only minor effects on phagocytosis. In fact, the treatment of infected and uninfected mice with phages did not significantly alter the rate of phagocytosis by blood granulocytes and monocytes [30]. Therefore, it was important to determine phage effects on IKB. *In vitro* studies have shown that phage lysates and purified phage preparations did not influence IKB by human granulocytes and monocytes. This lack of effect was evident using different titers of phages homologous and heterologous to bacteria. Likewise, the majority of purified phage preparations and phage lysates did not affect human granulocyte and monocyte migration [16]. As already noted, A3R phage was also found not to induce degranulation of those phagocytes.

The effect of phage therapy on IKB has been studied by Jończyk-Matysiak *et al.* [31]. As could be expected, IKB of patients prior to therapy was generally reduced while the therapy did not cause further reduction in IKB of a standard

Escherichia coli B strain. Similar results were obtained when IKB of specific pathogens isolated from patients was analyzed. Interestingly, a significant increase of monocyte IKB was noted in patients with chronic urinary tract infections subjected to phage therapy. In addition, monocyte-dependent IKB of *E. coli* B was a good prognostic sign of therapy effectiveness.

Purified T4 phage and phage lysate of *E. coli* did not affect the expression of CD40, CD80 or CD86 on monocytes activated by LPS. This strongly suggests that phage therapy – at least in patients with infections caused by Gram-negative bacteria – does not overly stimulate monocytes [32]. In a parallel study, the same group assayed the effects of purified T4 and A3R phages and their lysates on the differentiation of human myeloid dendritic cells from monocytes [33]. This first study to evaluate the influence of phages on human dendritic cells revealed no detrimental effects of phages on their differentiation and the expression of molecules for some of those cells' main functions, especially their role in T-cell activation and phagocytosis [33]. Those results are in line with the results of Miernikiewicz *et al.* [34], who reported that T4 phage does not significantly affect the expression of CD40, CD80, CD86 or MHC class II molecules on murine dendritic cells. However – as already mentioned – the activation of mouse bone marrow derived dendritic cells with T4 phages and tumor antigens resulted in augmented expression of their differentiation markers associated with an enhanced ability to prime T cells for IFN- γ production and tumor growth inhibition [35]. In addition, An *et al.* reported that *Cronobacter sakazakii* phage can elicit maturation of murine dendritic cells [36]. In contrast, Bocian *et al.* [33] found that both T4 and A3R phages did not stimulate production of IL-12 by human dendritic cells, thus indicating that they do not activate immature human dendritic cells (IL-12 production is a feature of antigen-activated mature dendritic cells). Apparently, phage effects on dendritic cells may vary depending on phage type and host (effects in mice may be different from those manifested in humans). Evidently, more studies are necessary to shed light on phage effects on dendritic cells and their practical implications in immunotherapy.

• Phages & inflammation

Evidence has accumulated to indicate that phages and their proteins do not stimulate inflammatory mediators and reactive oxygen

species production *in vitro* and when administered in mice and in humans. What is more, phages may exert strong anti-inflammatory effects using mechanisms that appear to be unrelated to their well-known antibacterial action. For example, data derived from *in vivo* studies in noninfected animals and *in vitro* using bacteria-free systems clearly indicate that the effects observed are not dependent on mere reduction in the number of bacteria by phages but their direct interactions with immune cells. Those anti-inflammatory effects of phages have been recently summarized, so those phenomena will not be discussed here in detail [26].

Neutrophil degranulation can be induced by some animal and human viruses, and the phenomenon may be responsible for some pathologies related to viral infections. Our recent work has revealed that purified A3R staphylococcal phage and phage lysate of *S. aureus* – in contrast to *S. aureus* cells – do not induce exocytosis of primary and secondary neutrophil granules. Thus, unlike animal and human viruses, A3R phage and the products of A3R-mediated lysis of *S. aureus* should not result in the release of potentially toxic mediators from neutrophil granules [37].

Our recent publication on anti-inflammatory effects of purified recombinant phage tail adhesion protein (gp12) appears to be especially relevant. Gp12 has been shown to bind to LPS *in vitro* without affecting the viability and proliferation rate of mammalian cells (fibroblasts and endothelium). When administered in mice, the protein caused almost complete disappearance of IL-1 α circulation in the sera of animals challenged with LPS and a reduction in IL-6 serum levels by approximately 50%. Furthermore, the protein significantly reduced leukocytic infiltration of the lungs, spleen and liver, while not producing any inflammatory effect or injury in control animals [38]. These effects resemble similar phenomena caused by whole T4 phage administered to mice bearing skin allografts [18]. Moreover, the release of filamentous Pf *Pseudomonas* phage was also associated with reduced lung injury and lower cytokine levels [39]. Wang *et al.* demonstrated that phage therapy of uninfected mice does not alter the cytokine levels and significantly lowers those levels during therapy in infected mice, attenuating inflammation caused by *S. aureus* [40]. Similar findings were reported by Pabary *et al.*, whose data indicate that phage administration

reduced inflammation in a murine model of lung infection, lowering inflammatory cytokine and neutrophils levels in bronchoalveolar lavage fluid [41]. Also, when *E. coli* 0157:H7 phages in low and high doses were administered in mice, cytokine levels remained within normal ranges. Patterns of those cytokine differences were not dose related, indicating that phage treatment did not result in a strong immune response [42] (for normal ranges, see [43]). Tothova *et al.* confirmed that phage therapy of urinary tract infections in mice attenuates the expression of proinflammatory cytokines [44], and the same effect was reported by Hung *et al.* using a model of *Klebsiella pneumoniae* mediated liver abscess and bacteremia in mice [45]. In mice fed with murine norovirus, the inflammatory cytokine profiling showed increased levels of IL-1 α , IL-1 β , IL-2, IL-10, IL-12, IFN- γ and IFN- α . In contrast, none of those cytokines were increased in mice fed with phage T7 [46]. Phages can also inhibit tissue expression of inflammatory cytokines, as reported by Cao *et al.* They used a sublethal pneumonia model and discovered that phage-treated mice exhibited lower levels of inflammatory cytokines in their lungs [47]. It has been postulated that data suggesting cytokine-inducing ability of phage preparations *in vitro* and *in vivo* may be biased by endotoxin contamination of those preparations. This could result in activation of cytokine-producing cells by that agent rather than phages themselves [34,48]. **Figure 2** shows how phages can downregulate Th1- and Th2-lymphocyte derived cytokine production.

Recently, another mechanism of phage-mediated downregulation of inflammation has been described. The Pf4 phage of *P. aeruginosa* has been demonstrated to inhibit metabolism and biofilm formation of *Aspergillus fumigatus* (Af). Phage inhibition of Af growth is caused by iron sequestration, and supplemental iron overcomes phage-mediated inhibition of Af metabolism [49]. It is well known that iron depletion may decrease humoral and cell-mediated immunity as well as cytokine production [50]. Moreover, low-dose iron chelation may markedly impair the activation of T lymphocytes and experimentally induced inflammatory liver damage [51]. Phage therapy does not aggravate anemia and may improve it [26,52]. The iron-chelating potential of other phages and the clinical significance of those findings are presently unclear and require further studies. However, this interesting phenomenon supports the notion of the ability of

Figure 2. Phage-mediated immunoregulation. Phages can downregulate both Th1- and Th2-derived cytokine production by eliminating bacteria (and bacteria-dependent upregulation of immunity and inflammation). They can also directly control the activity of lymphocytes present in gut-associated lymphoid tissue. Phages that translocate can also act at different tissues (Figure 1).

phages to control excessive immune reactions and inflammatory responses, also confirmed by our report showing that phage therapy downregulates laboratory markers of inflammation in the patients treated [53].

Toll-like receptors (TLRs) are the best-studied class of pattern recognition receptors which recognize conserved components of microbes called pathogen-associated molecular patterns. Recognition of pathogen-associated molecular patterns by pattern recognition receptors, including TLRs, is essential for the induction of innate immune responses to pathogenic viruses. However, while knowledge about the interactions between pathogenic viruses and TLRs is very extensive, data on the effects of phages on TLRs are extremely scanty. In fact, to the best of our knowledge, there are only two studies to suggest that phage virions can stimulate TLRs. In the first study, mice deficient in MyD88, a protein essential for signaling through all TLRs (except TLR3), unlike wild-type mice, did not respond to M13 phage immunization [54]. The second study was performed on a murine model of melanoma and showed that bacteriophage-induced tumor regression, production of pro-inflammatory cytokines and chemokines by

tumor-infiltrating macrophages, and neutrophil infiltration of tumors were almost completely abolished in MyD88-deficient mice compared with wild-type mice [55]. However, it needs to be stressed that no study has been conducted yet to evaluate direct interactions between phage particles and individual TLRs. Our studies have revealed that neither purified T4 phage nor *E. coli* phage lysate significantly affect the expression of TLR2 and TLR4 on human monocytes [32].

Although the vast majority of reports highlight the downregulation of immune reactions by phages, Lepage *et al.* suggested that bacterial viruses may contribute to the aberrant immune response in patients with Crohn's disease [56]. Higher phage concentrations were detectable in the gut intestinal mucosa of those patients than in healthy controls. Furthermore, decreased amounts of viral particles were present in ulcerated mucosa compared with nonulcerated mucosa [56]. These data, however, may also be interpreted to suggest the beneficial role of phages in protecting patients' mucosa, as suggested by Barr *et al.* [15]. Therefore, more studies are needed to elucidate the significance of those findings. Riley suggested that phages could play a role in the pathogenesis of autoimmune disease due to the fact that they

are immunogenic and – in theory – may act as antigens on epithelial surfaces, through molecular mimicry and as superantigens [57]. However, neither experimental nor clinical data available so far corroborate this hypothesis, and – as stated earlier – the data of Barr *et al.* [15] strongly suggest the protective role of phages present on epithelial surfaces. Moreover, it is well known that oral phage administration in mice and man allowing for subsequent phage–epithelial surface interactions in the gut induces only very weak antibody production [21,23,24].

Phages & platelets

Platelets are well-known modulators of the immune response, leading to inflammatory activation, allograft rejection and autoimmune diseases [58]. Some of those effects may be dependent on platelet-derived extracellular vesicles which can mediate inflammation and upregulation of immune reactions. Therefore, interest in the role of blood platelet derived microvesicles is constantly growing [59]. These properties in combination with the sheer amount of platelets circulating in the blood stream make them an important force in the immune response during health and disease [58,60].

Resting platelets can bind T phages, and this ability is markedly enhanced following collagen activation. The binding may be abolished by a monoclonal antibody to $\beta 3$ integrin as well as a peptide containing the KGD sequence. No binding took place using platelets from a patient with Glanzmann's thrombasthenia (whose platelets did not show expression of $\beta 3$ integrin). Platelets adhere to immobilized T4 and HAP1 phages, and their activity toward T4 is significantly stronger. Moreover, T4 phages significantly inhibit platelet adhesion to fibrinogen and only marginally inhibit T-cell adhesion; they also significantly inhibit thrombin-induced platelet aggregation *in vitro* [16,61]. Some eukaryotic viruses can invade and replicate in platelets causing thrombocytopenia which is common in patients with viral infections [62]. Our findings on phage interference with adhesion and replication of some viruses [63] combined with the observations made in patients on phage therapy in whom some minor fluctuations in platelet counts could be noted [52] as well as the data summarized above, suggest that further studies are needed to shed light on phage interactions with platelets, with potential practical implications for the development of novel therapies.

Anticancer effects of phages

In the past two decades, data have accumulated to suggest that phages may be effective in inhibiting tumor growth and metastases formation. This confirms observations made already in 1940 indicating that phages have antitumor activity in mice and rabbits [64].

Interestingly, similar to those described above, molecular mechanisms may be at least partly responsible for the antimetastatic effects of T4 and its substrain HAP1 phages, against melanoma cells in murine experimental cancer models [65]. Furthermore, using various murine models, we noted that oral phage application was more effective than intraperitoneal phage administration: 3% of metastases inhibition was noted using intraperitoneally purified T phage administration versus 29% inhibition using oral route; these values were 19 and 80%, respectively, for purified HAP1 phage [66]. Another anticancer effect of phages may depend on their ability to enhance antitumor responses initiated by dendritic cell based vaccines [35]. We demonstrated that migration of human and mouse melanoma cells on fibronectin was inhibited by purified phage T4 and HAP1 preparations, an effect independent of the residual LPS [67]. Subsequently, staphylococcal phage lysates have also been shown to inhibit melanoma cell migration *in vitro* [68]. Moreover, phage lysates of Gram-positive and Gram-negative bacteria did not significantly affect migration of leukemia cells [69]. Eriksson *et al.* proposed another mechanism of TLR-dependent phage-mediated anticancer effects related to phage ability to activate tumor-associated macrophages, subsequent neutrophil recruitment and neutrophil-mediated tumor destruction [55]. Using a breast cancer model, phages armed with anticancer peptides were shown to mediate antitumor effects while maintaining their antibacterial activities [70]. Those data derived from *in vitro* and *in vivo* animal experiments suggest that phages may mediate anticancer effects, both by interfering with metastasis formation and by local destruction of tumor tissue. What is more, T4 phage preparations were able to potentiate the antimetastatic effect of cyclophosphamide [71]. The clinical significance of these findings remains unclear at the present time; however, the results of four trials seem to be encouraging. A trial performed in 131 cancer patients with postoperative wound infections caused by different Gram-positive and Gram-negative bacteria has revealed phage

therapy effectiveness in >80% of patients, and three other trials yielded similar effects; interestingly no significant side effects were reported even during prolonged (several months) phage administration [72].

Phages versus eukaryotic viruses

Phages may interact directly and indirectly with viruses. Borecky's group has described some clinical data on the effectiveness of native bacteriophage RNA in the treatment of viral infections, a phenomenon that may be partly related to interferon production [73]. The other mechanism may be the direct competition of phages with viruses for cellular receptors for viral cell entry [74]. Recently, we have provided data to support the latter option: T4 phage was found to significantly downregulate both the adsorption and replication of human adenovirus in a dose-dependent manner [63]. These data indicate that T4 can inhibit infection by the eukaryotic virus, however, further studies are needed to determine if the phage may be considered a novel antiviral agent. Obviously, potential antiviral effects of other phages should also be investigated.

Future perspective

The data presented in this article clearly indicate that evidence on phage engagement in regulating immunity is accumulating. This knowledge sheds new light on the potential immunoregulatory activities of phages which constitute an abundant part of the microbiome. As recently confirmed, intestinal phages provide a nonhost-derived antibacterial immune

defense. Furthermore, there is now ample evidence suggesting that those phages may downregulate activation of immune responses, contributing to maintenance of immune homeostasis in the gut, and – through the process of phage translocation – mediate similar effects on other tissues and organs. Further confirmation of this hypothesis may lead to the development of novel therapies based on phage-mediated immunoregulation.

Financial & competing interests disclosure

A Górski, R Międzybrodzki, B Weber-Dąbrowska, K Dąbrowska, J Borysowski are co-inventors of patents owned by the Institute and covering phage preparations. This work was supported by grant DEC-2013/11/B/NZ1/02107 from National Science Center (NCN) and statutory funds from the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, PAS (grant no. 16) and Warsaw Medical University (1/MG/2015-2016). This publication was also supported by Wrocław Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014–2018. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Open access

This work is licensed under the Attribution-NonCommercial-NoDerivatives 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

EXECUTIVE SUMMARY

Phages & gut-associated lymphoid tissue

- Phages are present in high concentrations in the intestinal tract, where they may interact not only with bacteria but also with cells of the gut-associated lymphoid tissue.

Phage receptors mediating interactions with immune cells

- Phages may interact with cells of the immune system through their proteins and cellular receptors. Some of these receptors appear to belong to the β -integrin family.

Phage-dependent immunoregulation

- Phage interactions with immune cells seem to have immunomodulatory action, downregulating the heightened responses both *in vitro* and *in vivo* without causing immune deficits.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

- 1 Relman D. The human microbiome and the

future practice of medicine. *JAMA* 314(11), 1127–1128 (2015).

- A comprehensive review of current knowledge about the relationship between the human

microbiome and potential for using this knowledge for treating and preventing disease.

- 2 Marino E. The gut microbiota and immunoregulation: the fate of health and

- disease. *Clin. Transl. Immunol.* 5(11), e107 (2016).
- 3 Burcelin R. Gut microbiota and immune crosstalk in metabolic disease. *Mol. Metab.* 5(9), 771–781 (2016).
 - 4 Brodin P, Davis MM. Human immune system variation. *Nat. Rev. Immunol.* 17(1), 21–29 (2017).
 - **Summary of current knowledge on how the bacterial microbiota affects the immune system.**
 - 5 Garrett WS. Gut microbiota in 2016: a banner year for gut microbiota research. *Nat. Rev. Gastroenterol. Hepatol.* 14(2), 78–80 (2017).
 - 6 Hutch Magazine. www.fhcrc.org
 - 7 Braciale TJ, Hahn YS. Immunity to viruses. *Immunol. Rev.* 255(1), 5–12 (2013).
 - 8 Alcamí A, Ghazal P, Yewdell JW. Viruses in control of the immune system. Workshop on molecular mechanisms of immune modulation: lessons from viruses. *EMBO Rep.* 3(10), 927–932 (2002).
 - 9 Lederberg J. Smaller fleas...ad infinitum: therapeutic bacteriophage redux. *Proc. Natl Acad. Sci. USA* 93(8), 3167–3168 (1996).
 - 10 Abeles SR, Pride DT. Molecular bases and role of viruses in the human microbiome. *J. Mol. Biol.* 426(23), 3892–3906 (2014).
 - **An article presenting what is currently known about the presence of viruses in the human microbiome.**
 - 11 Mills S, Shanahan F, Stanton C, Hill C, Coffey A, Ross RP. Movers and shakers: influence of bacteriophages in shaping the mammalian gut microbiota. *Gut Microbes* 4(1), 4–16 (2013).
 - 12 Górski A, Weber-Dąbrowska B. The potential role of endogenous bacteriophages in controlling invading pathogens. *Cell. Mol. Life Sci.* 62(5), 511–519 (2005).
 - **Presentation of our hypothesis on the protective role of endogenous phages.**
 - 13 Górski A, Ważna E, Weber-Dąbrowska B, Dąbrowska K, Światała-Jeleń K, Międzybrodzki R. Bacteriophage translocation. *FEMS Immunol. Med. Microbiol.* 46(3), 313–319 (2006).
 - **Presentation of our hypothesis on phage translocation and its significance for controlling immunity.**
 - 14 Górski A, Dąbrowska K, Światała-Jeleń K *et al.* New insights into the possible role of bacteriophages in host defense and disease. *Med. Immunol.* 2(1), 2 (2003).
 - 15 Barr JJ, Auro R, Furlan M. Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proc. Natl Acad. Sci. USA* 110(26), 10771–10776 (2013).
 - 16 Górski A, Międzybrodzki R, Borysowski J *et al.* Phage as a modulator of immune responses: practical implications for phage therapy. *Adv. Virus Res.* 83, 41–71 (2012).
 - 17 Kim KP, Cha JD, Jang EH *et al.* PEGylation of bacteriophages increases blood circulation time and reduces T-helper type 1 immune response. *Microb. Biotechnol.* 1(3), 247–257 (2008).
 - 18 Górski A, Kniotek M, Perkowska-Prasińska A *et al.* Bacteriophages and transplantation tolerance. *Transplant. Proc.* 38(1), 331–333 (2006).
 - 19 Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. *Nat. Rev. Microbiol.* 13(9), 529–543 (2015).
 - 20 Dąbrowska K, Miernikiewicz P, Piotrowicz A *et al.* Immunogenicity studies of proteins forming the T4 phage head surface. *J. Virol.* 88(21), 12551–12557 (2014).
 - 21 Majewska J, Beta W, Lecion D *et al.* Oral application of T4 phage induces weak antibody production in the gut and in the blood. *Viruses* 7(8), 4783–4799 (2015).
 - 22 Bruttin A, Brüßow H. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.* 49(7), 2874–2878 (2005).
 - 23 Łusiak-Szelachowska M, Żaczek M, Weber-Dąbrowska B *et al.* Antiphage activity of sera during phage therapy in relations to its outcome. *Future Microbiol.* 12(2), 109–117 (2017).
 - 24 Żaczek M, Łusiak-Szelachowska M, Jończyk-Matysiak E *et al.* Antibody production in response to staphylococcal MS-1 phage cocktail in patients undergoing phage therapy. *Front. Microbiol.* 7, 1681 (2016).
 - 25 Górski A, Dąbrowska K, Hodyra-Stefaniak K, Borysowski J, Międzybrodzki R, Weber-Dąbrowska B. Phages targeting infected tissues: novel approach to phage therapy. *Future Microbiol.* 10(2), 199–204 (2015).
 - 26 Górski A, Międzybrodzki R, Weber-Dąbrowska B. Phage therapy: combating infections with potential for evolving from merely a treatment for complications to targeting diseases. *Front. Microbiol.* 7, 1515 (2016).
 - **A review summarizing the current status and potential of phage therapy.**
 - 27 Jain L, Rawat M, Ramakrishnan S, Kumar B. Active immunization with *Brucella abortus* S19 phage lysate elicits serum IgG that protects guinea pigs against virulent *B. abortus* and protects mice by passive immunization. *Biologicals* 45, 27–32 (2017).
 - 28 Borysowski J, Wierzbicki P, Kłosowska D, Korczak-Kowalska G, Weber-Dąbrowska B, Górski A. The effects of T4 and A3/R phage preparations on whole-blood monocyte and neutrophil respiratory burst. *Viral Immunol.* 23(5), 541–544 (2010).
 - 29 Międzybrodzki R, Światała-Jeleń K, Fortuna W *et al.* Bacteriophage preparation inhibition of reactive oxygen species generation by endotoxin-stimulated polymorphonuclear leukocytes. *Virus Res.* 131(2), 233–242 (2008).
 - 30 Przerwa A, Zimecki M, Światała-Jeleń K *et al.* Effects of bacteriophages on free radical production and phagocytic functions. *Med. Microbiol. Immunol.* 195(3), 143–150 (2006).
 - 31 Jończyk-Matysiak E, Łusiak-Szelachowska M, Kłak M *et al.* The effect of bacteriophage preparations on intracellular killing of bacteria by phagocytes. *J. Immunol. Res.* 2015, 482863 (2015).
 - 32 Bocian K, Borysowski J, Zarzycki M *et al.* LPS-activated monocytes are unresponsive to T4 phage and T4-generated *Escherichia coli* lysate. *Front. Microbiol.* 7, 1356 (2016).
 - 33 Bocian K, Borysowski J, Zarzycki M *et al.* The effects of T4 and A3/R bacteriophages on differentiation of human myeloid dendritic cells. *Front. Microbiol.* 7, 1267 (2016).
 - 34 Miernikiewicz P, Dąbrowska K, Piotrowicz A *et al.* T4 phage and its head surface proteins do not stimulate inflammatory mediator production. *PLoS ONE* 8(8), e71036 (2013).
 - 35 Pajtasz-Piasecka E, Rossowska J, Duś D, Weber-Dąbrowska B, Zabłocka A, Górski A. Bacteriophages support anti-tumor response initiated by DC-based vaccine against murine transplantable colon carcinoma. *Immunol. Lett.* 116(1), 24–32 (2008).
 - 36 An TW, Kim SJ, Lee YD, Park JH, Chang HI. The immune-enhancing effect of the *Cronobacter sakazakii* ES2 phage results in the activation of nuclear factor κB and dendritic cell maturation via the activation of IL-12p40 in the mouse bone marrow. *Immunol. Lett.* 157(1–2), 1–8 (2014).
 - 37 Borysowski J, Międzybrodzki R, Wierzbicki P *et al.* A3R phage and *Staphylococcus aureus* lysate do not induce neutrophil degranulation. *Viruses* 9(2), 36 (2017).
 - 38 Miernikiewicz P, Kłopot A, Soluch R *et al.* T4 phage tail adhesin gp12 counteracts

- LPS-induced inflammation *in vivo*. *Front. Microbiol.* 7, 1112 (2016).
- An article presenting experimental data strongly suggesting anti-inflammatory action of phages.
- 39 Secor PR, Michaels LA, Smigiel KS *et al.* Filamentous bacteriophage produced by *Pseudomonas aeruginosa* alters the inflammatory response and promotes noninvasive infection *in vivo*. *Infect. Immun.* 85(1), pii:e00648-16 (2016) (Epub ahead of print).
 - 40 Wang Z, Zheng P, Ji W *et al.* SLPW: a virulent bacteriophage targeting methicillin-resistant *Staphylococcus aureus* *in vitro* and *in vivo*. *Front. Microbiol.* 7, 934 (2016).
 - 41 Pabary R, Singh C, Morales S *et al.* Antipseudomonal bacteriophage reduces infective burden and inflammatory response in murine lung. *Antimicrob. Agents Chemother.* 60(2), 744–751 (2015).
 - 42 Hong Y, Thimmapuram J, Zhang J *et al.* The impact of orally administered phages on host immune response and surrounding microbial communities. *Bacteriophage* 6(3), e1211066 (2016).
 - 43 Stenina MA, Krivov LI, Voevodin DA, Savchuk VI, Kovalchuk LV, Yarygin VN. Cytokine profile of the blood in mice with normal and abnormal heart rhythm. *Bull. Exp. Biol. Med.* 152(6), 692–695 (2012).
 - 44 Tothova L, Celec P, Babickova J *et al.* Phage therapy of *Cronobacter*-induced urinary tract infection in mice. *Med. Sci. Monit.* 17(7), BR173–BR178 (2011).
 - 45 Hung CH, Kuo CF, Wang CH, Wu CM, Tsao N. Experimental phage therapy in treating *Klebsiella pneumoniae* mediated liver abscesses and bacteremia in mice. *Antimicrob. Agents Chemother.* 55(4), 1358–1365 (2011).
 - 46 Park K, Cha KE, Myung H. Observation of inflammatory responses in mice orally fed with bacteriophage T7. *J. Appl. Microbiol.* 117(3), 627–633 (2014).
 - 47 Cao F, Wang X, Wang L *et al.* Evaluation of the efficacy of a bacteriophage in the treatment of pneumonia induced by multidrug resistant *Klebsiella pneumoniae* in mice. *Biomed Res. Int.* 2015, 752930 (2015).
 - 48 Dufour N, Henry M, Ricard JD, Debarbieux L. Commentary: morphologically distinct *Escherichia coli* bacteriophages differ in their efficacy and ability to stimulate cytokine release *in vitro*. *Front. Microbiol.* 7, 1029 (2016).
 - 49 Penner JC, Ferreira JA, Secor PR *et al.* Pf4 bacteriophage produced by *Pseudomonas aeruginosa* inhibits *Aspergillus fumigatus* metabolism via iron sequestration. *Microbiology* 162(9), 1583–1594 (2016).
 - 50 Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. *Hematol. J.* 5(7), 579–583 (2005).
 - 51 Bonaccorsi-Riani E, Danger R, Lozano JJ *et al.* Iron deficiency impairs intra-hepatic lymphocyte mediated immune response. *PLoS ONE* 10(8), e0136106 (2015).
 - 52 Międzybrodzki R, Borysowski J, Weber-Dąbrowska B *et al.* Clinical aspects of phage therapy. *Adv. Virus Res.* 83, 73–121 (2012).
 - 53 Międzybrodzki R, Fortuna W, Weber-Dąbrowska B, Górski A. A retrospective analysis of changes in inflammatory markers in patients treated with bacterial viruses. *Clin. Exp. Med.* 9(4), 303–312 (2009).
 - 54 Hashiguchi S, Yamaguchi Y, Takeuchi O, Akira S, Sugimura K. Immunological basis of M13 phage vaccine: regulation under MyD88 and TLR9 signaling. *Biochem. Biophys. Res. Commun.* 402(1), 19–22 (2010).
 - 55 Eriksson F, Tsagozis P, Lundberg K *et al.* Tumor-specific bacteriophages induce tumor destruction through activation of tumor-associated macrophages. *J. Immunol.* 182(5), 3105–3111 (2009).
 - 56 Lepage P, Colombet J, Marteau P, Sime-Ngando T, Dore J, Leclerc M. Dysbiosis in inflammatory bowel disease: a role for bacteriophages? *Gut* 57(3), 424–425 (2008).
 - 57 Riley PA. Bacteriophages in autoimmune disease and other inflammatory conditions. *Med. Hypotheses* 62(4), 493–498 (2004).
 - 58 Iannaccone M. Platelet-mediated modulation of adaptive immunity. *Semin. Immunol.* 28(6), 555–560 (2016).
 - 59 Melki I, Tessandier N, Zufferey A, Boilard E. Platelet microvesicles in health and disease. *Platelets* doi:10.1080/09537104.2016.1265924 (2017) (Epub ahead of print).
 - 60 Koenen RR. The prowess of platelets in immunity and inflammation. *Thromb. Haemost.* 116(4), 605–612 (2016).
 - 61 Świtała-Jeleń K. Studies on bacteriophage interactions with blood platelets and leukocytes. Doctoral thesis, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław (2005).
 - 62 Rondina MT, Weyrich AS. Dengue virus pirates human platelets. *Blood* 126(3), 286–287 (2015).
 - 63 Przybylski M, Borysowski J, Jakubowska-Zahorska R, Weber-Dąbrowska B, Górski A. T4 bacteriophage-mediated inhibition of adsorption and replication of human adenovirus *in vitro*. *Future Microbiol.* 10(4), 453–460 (2015).
 - An article presenting experimental data on phage-dependent interference with adenovirus attachment and replication within the target cells.
 - 64 Bloch H. Experimentelle untersuchungen über beziehungen zwischen bakteriophagen und malignen tumoren. *Archiv für die Gesamte Virusforschung.* 1(4), 481–496 (1940).
 - 65 Dąbrowska K, Opolski A, Wietrzyk J *et al.* Antitumor activity of bacteriophages in murine experimental cancer models caused possibly by inhibition of beta3 integrin signaling pathway. *Acta Virol.* 48(4), 241–248 (2004).
 - Experimental data suggesting phage interference with the formation of melanoma metastases in an experimental model in mice.
 - 66 Dąbrowska K, Opolski A, Wietrzyk J *et al.* Activity of bacteriophages in murine tumor models depends on the route of phage administration. *Oncol. Res.* 15(4), 183–187 (2005).
 - 67 Dąbrowska K, Skardziński G, Jończyk E *et al.* The effect of bacteriophages T4 and HAP1 on *in vitro* melanoma migration. *BMC Microbiol.* 9, 13 (2009).
 - 68 Dąbrowska K, Skardziński G, Kurzępa A *et al.* The effects of staphylococcal bacteriophage lysates on cancer cells *in vitro*. *Clin. Exp. Med.* 10(1), 81–85 (2010).
 - 69 Kurzępa-Skardzińska A, Skardziński G, Weber-Dąbrowska B *et al.* Influence of bacteriophage preparations on migration of HL-60 leukemia cells *in vitro*. *Anticancer Res.* 33(4), 1569–1574 (2013).
 - 70 Dąbrowska K, Kaźmierczak Z, Majewska J *et al.* Bacteriophages displaying anticancer peptides in combined antibacterial and anticancer treatment. *Future Microbiol.* 9(7), 861–869 (2014).
 - 71 Szczauńska-Nowak K, Dąbrowska K, Celka M *et al.* Antitumor effect of combined treatment of mice with cytostatic agents and bacteriophage T4. *Anticancer Res.* 29(6), 2361–2370 (2009).
 - 72 Borysowski J, Górski A. Is phage therapy acceptable in the immunocompromised host? *Int. J. Infect. Dis.* 12(5), 466–471 (2008).
 - 73 Borecky L. Use of bacteriophage nucleic acid inducers of interferon in man. *Tex. Rep. Biol. Med.* 35, 535–540 (1977).
 - 74 Międzybrodzki R, Fortuna W, Weber-Dąbrowska B, Górski A. Bacterial viruses against viruses pathogenic for man? *Virus Res.* 110(1–2), 1–8 (2005).