Clin Exp Med (2009) 9:93–100
DOI 10.1007/s10238-008-0027-8

REVIEW ARTICLE

Bacteriophage interactions with phagocytes and their potential significance in experimental therapy

Aneta Kurzępa · Krystyna Dąbrowska · Grzegorz Skaradziński · Andrzej Górski

Received: 29 August 2008 / Accepted: 2 December 2008 / Published online: 30 January 2009

Abstract Bacteriophages are among the most numerous creatures on earth and they are omnipresent. They are thus in constant natural contact with humans and animals. However, the clinical and technological use of bacteriophages has also become more frequent, which is why all aspects of phage–mammal interactions need to be explored. Bacteriophages are able to interact with mammalian phagocytes. They may inhibit the phagocytosis of bacteria, but they may also undergo phagocytosis themselves. The ability of bacteriophages to reduce reactive oxygen species production by polymorphonuclear leukocytes in the presence of bacteria or their endotoxins was also confirmed. Studies show that the high immunogenicity of bacteriophages may also be employed in anti-tumor treatment. The present knowledge of phage interactions with cellular components of the mammalian immune system is sparse and insufficient, especially considering the increasing interest in the application of these viruses in human life. We believe that continuation of such research is indispensable.

Keywords Bacteriophages · Phagocytes · Human–mammalian interactions · Phagocytosis

A. Kurzępa (✉) · K. Dąbrowska · G. Skaradziński · A. Górski
L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Weigla 12, 53-114 Wrocław, Poland
e-mail: kurzepa@iitd.pan.wroc.pl

A. Górski
Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

Introduction

Bacteriophages (phages, BFs) are viruses that infect bacteria. Although their biology is mainly related to bacterial cells, they are constantly present in human life. This is not only because they occupy every environment where their host bacteria are present (e.g., water and soil), but also because they are more often used intentionally by medicine and industry. The branch of biotechnology connected with food production pins its hopes on involving bacteriophages in the fight against the bacterial infection of food products. For instance, in 2006 the FDA approved the use of a *Lysteria monocytogenes*-specific preparation on ready-to-eat meat and poultry products (http://www.cfsan.fda.gov/~dms/opabacqa.html). As bacteriophages are very efficient and, what is important, very specific antibacterial agents, their potential use in medicine seems invaluable. Nowadays, as contemporary medicine is facing the tremendous problem of antibiotic-resistant bacterial infections, bacteriophage therapy seems to present an interesting alternative to this popular form of medication. Studies have shown that interactions between bacteriophages and the mammalian immune system may be of great importance. Bacteriophages may play a significant role in clinical transplantation as they reduce cellular infiltration of allogenic skin allografts, which was observed in mice [1]. They are believed to have immunomodulatory properties [2]; they inhibit the adhesion of platelets and, to some extent, T cells to fibrinogen, a protein which plays an important role in transplant rejection, angiogenesis and metastasis [3]. They inhibit the activation of NF-κB, which has a positive influence during lung injuries accompanying acute allograft rejections, as observed in rats [1]. Bacteriophages may influence the polyclonal humoral response in mammals in different ways depending on phage type; *E. coli*

and *Pseudomonas* phages inhibit the antibody production of lymphocytes, while *Staphylococcus aureus* phages seem to cause its stimulation [3]. They may inhibit the production of (IL)-2, TNF and, to some extent, interferon γ by human leukocytes stimulated by PHA [4]. However, Kleinschmidt et al. [5] observed an increased level of interferon in mouse blood after injection of T4 phage preparations. Further studies excluded an involvement of the phage protein coat in the observed effect and indicated that viral DNA may be responsible for interferon production. Interestingly, isolated DNA has no such abilities and the phage coat is probably a container, which ensures safe delivery of the molecule in a configuration required to cause such an effect. The fact that bacteriophages may be used as such containers is applied in DNA vaccine technology. The vaccine gene is cloned into the bacteriophage genome and viral particles are injected into the eukaryotic host. Such a vaccine system is more effective than standard techniques, as was observed in mice and rabbits [6–8]. The very latest studies show that bacteriophages are also able to inhibit the production of reactive oxygen species (ROS) by granulocytes in the presence of bacteria, which undoubtedly may be beneficial in combating many diseases [9, 10]. They may even be used in anticancer therapies by involving the immune system in destroying tumor tissue cells [11].

Our observations indicating that phages have immunomodulatory properties raise the question of whether different phage strains mediate different effects on the immune system. This is confirmed by our findings showing that the HAP1 phage (a substrain of T4 phage defective in the *hoc* gene) causes a weaker inhibition of human T-cell activation in vitro than its parental strain T4 [1]. Furthermore, while both strains cause inhibition of platelet adhesion to fibrinogen, the effect of T4 is significantly stronger; similarly, the adhesive interactions of T4 with human T cells are more intense than those of HAP1 [3]. These observations are paralleled by in vivo data indicating that there are differences in the antimetastatic activities and clearance of T4 and HAP1 from the murine organism [12–14]. Thus, different phage strains could mediate different immunologic affects, which supports our theory of phage-mediated natural immunosuppression [15].

The effects that bacteriophages have on mammal tissues is one of the most significant areas of study. This review considers reports from observations regarding the interactions of bacteriophages with the mammalian immune system, in particular with phagocytes. We believe that this direction of research is extremely important not only for the development of bacteriophage treatment, but also for further applications of bacteriophages in human life.

Interactions between phagocytes and mammal-targeted viruses

Mammalian phagocytes, as part of the immune system, act as one of the most important tools during viral infection. Interactions between human/animal phagocytes and their pathogenic viruses are therefore well studied. Dendritic cells are crucial for the presentation of viral antigens to T lymphocytes. They are also a source of interferon α and induce NKT lymphocyte activity. However, because of their place of abode (skin, mucosa) and their function (transport of antigens to local lymph vessels), dendritic cells are also very efficient carriers for several viruses, such as HIV-1, HIV-2, CMV, Ebola virus and coronavirus. These viruses latch to the DC-SIGN receptor on dendritic cells and enter the lymphatic system. In contrast, measles virus, *Herpes simplex* virus and vaccinia virus infect dendritic cells [11, 16]. Macrophages are believed to be the first targets of HIV infection and they are the major reservoir of virions during all its stages. Although macrophages with HIV particles do not present cytopathic effects, they are a vector for spreading the disease and regulatory centers of infection [17].

Information on interactions between mammalian tissues and bacteriophages is scarce, unlike that related to mammal-targeted viruses. Moreover, it seems obvious that these interactions may be significantly different; for example, infection with animal-targeted viruses causes an increased production of ROS, while bacteriophage application inhibits this process in the presence of bacteria or their endotoxins (lipopolysaccharide, LPS). Even T4 phage without the presence of *E. coli* only slightly influences ROS production by granulocytes in comparison with the PBS group, while HSV virus stimulates it very strongly [9, 10]. Considering the constant presence and increased application of bacterial viruses in human life, it seems obvious that these interactions need to be investigated.

Interactions between phagocytes and bacteriophages

Phagocytosis of bacteriophages by phagocytes

Although there are not many studies referring to interactions between bacteriophages and phagocytes, there are some reports suggesting that such interactions are present and, moreover, they may be significant. Probably the earliest data were from the late 1950s and early 1960s and were presented by Kantoch [18, 19], who studied the topic of phage phagocytosis by leukocytes. He indicated that bacteriophages are able to bind leukocytes of the guinea pig. The longer the contact between the cells and

bacteriophage particles, the more were the leukocytes that bound to the virions. It is important to mention that the extent of binding was strongly dependent on the proportion of the concentration of the introduced bacteriophage particles to the concentration of leukocytes. It was also a specimen-, environment-, and temperature-dependant process. An increasing number of bound bacteriophages was accompanied by an increasing general number of leukocytes. Interestingly, the number of leukocytes involved in the binding process was relatively small. Furthermore, some portion of the bacteriophages remained unbound despite a long time of contact and an increasing concentration of leukocytes, of which most were unbound. The most important question regarding these observations was whether the bacteriophages only adhered to the leukocyte surface or were absorbed by them. The series of studies indicated that the bacteriophages were adsorbed as well as absorbed by leukocyte cells. These observations were confirmed in in vitro studies in which the percentage of phage particles bound by leukocytes was even higher [18, 19].

Studies (in vivo, confirmed subsequently in vitro) on bacteriophage phagocytosis by peritoneal macrophages conducted by Nelstrop et al. [20] indicated that this process occurs in two phases: a rapid first phase and a slower second phase. "Immune" macrophages (obtained by laparotomy after prior immunization of rabbit by the investigated phage T_1) inactivated bacteriophages faster than "non-immune" ones. It was proved that macrophages are capable of cellular immunity with no involvement of humoral factors, as the clearance of phages was observed with no simultaneous detection of produced antibodies.

The ability of stimulated polymorphonuclear leukocytes (PMNs) to inactivate lambda phages may be used to evaluate the stimulation level of PMNs exposed to some chemical and biological factors [21]. Since one phage virion results in the formation of one plaque, the residual plaques formed by phages after incubation with stimulated (by different agents) PMNs allow one to determine and compare the extent of the stimulatory ability of the applied reagents. It was also proved that bacteriophages themselves do not cause PMN stimulation. Although the mechanism of phage inactivation by PMNs is not clearly explained, there are some hypotheses indicating an involvement of hypochloric acid generated during PMN stimulation. Hypochloric acid is a highly reactive metabolite, so it is possible that it causes damage to phage nucleic acid and capsid proteins.

The immunoactivity of dendritic cells has been investigated for many years. Although in vivo experiments indicated their having phagocytic activity, verification of these observations in vitro was very difficult. In vitro phagocytosis of "latex" microspheres was probably the first convincing proof of the phagocytic abilities of dendritic cells observed outside a living organism. Barfoot et al. [22] also observed the phagocytosis of bacteriophage T4 by dendritic cells, which was stronger than for some artificial particles. Electron microscope images showed an agglomeration of viral particles around dendritic cells. Moreover, the phages seemed to be trapped in phagolysosomes and devoid of their outer coat during phagocytosis.

Studies on the phagocytosis of bacteriophages introduced into mammalian organisms showed that the most significant function is probably fulfilled by the liver. It is responsible for the phagocytosis of 99% of intravenously introduced phages. Phages accumulated in the liver at a 12 times higher titer than in the spleen. At the same time, the rate of phagocytosis of bacteriophages by Kupffer cells was four times faster than by splenic macrophages. This resulted in a more rapid decrease in viral titer detected in the liver than in the spleen [23]. Retention of phages in the spleen may be explained in two ways: the greater phagocytic activity of Kupffer cells than splenic macrophages and the fact that phages are objects of the nondestructive capture of antigens, a mechanism involving Schweigger-Seidel reticulum cells. This allows saving a high titer of phages in the spleen, which makes it a constant source of stimulation of antibody production [24].

In 2003, Gaubin et al. [25] described in detail the uptake and processing of the filamentous bacteriophage fd. Studies involving fluorescently labeled virions showed that they may be efficiently processed by the MHC class I and class II pathways. The ability to induce a strong cytotoxic T lymphocytic (CTL) response is an important feature, especially considering the development of phage display-based vaccines.

Phage influence on phagocytosis

Kantoch and Dubowska-Inglot studied the inhibition of phagocytosis in horse and guinea pig leukocytes by Coxsackie virus [26]. The number of bacteria destroyed was lower when the number of leukocytes with viruses was high. The complete inactivation of phagocytosis was related to the changes in leukocyte structure, i.e., shrinkage of its diameter and a more compact nucleus. The process was not influenced by the kind of leukocytes and bacteria used. These studies involved the enterovirus; however, other reports showed similar effects for different viruses, for example heat-inactivated influenza and mump viruses [27] and vaccina virus [28]. These observations might have suggested that the effect of phagocytosis inhibition might be common to most viruses, including bacteriophages.

Research into phagocytosis inhibition in the presence of virus was undertaken years later with reference to

bacteriophages. In 2006, Przerwa et al. [9] studied the influence of phages on phagocytosis by neutrophils and monocytes. Both homologous and heterologous phages inhibited phagocytosis after preincubation with phagocytes. The inhibition was even stronger in the case of the *Pseudomonas aeruginosa*-specific phage F8 and *E. coli* bacteria. Incubation of homologous phages with host bacteria resulted in stimulation of phagocytosis, which might be a result of bacteria opsonization by bacteriophages facilitating phagocytosis, in accordance with a hypothesis by Gorski et al. [2]. This effect was not observed for heterologous phages. Competitive preincubation of phages, phagocytes and bacteria resulted in inhibition of phagocytosis in the presence of high titers of host T4 phage. In the presence of the heterologous phage, a slight inhibition of phagocytosis was observed. The effect of *E. coli* phagocytosis by phagocytes was also studied in vivo; however, no significant influence on this process was observed.

Phage–bacteria–phagocyte interactions were also used in the design of some biological tests. These involved tests evaluating the number of bacteria absorbed and adsorbed on the surfaces of phagocytic cells. The phagocytic test described by Slopek et al. [29] used a *Staphylococcus aureus*-specific bacteriophage suspension to remove bacterial cells from the surfaces of leukocytes after inhibition of phagocytosis. Shaw et al. [30] used UV-irradiated bacteriophage T6. This inactivation prevented the replication of the phages in bacteria. No evidence that T6 affected the effectiveness of macrophages was found, and the phagocytosis of bacteriophages by these cells also did not seem to be sufficient to decrease bacterial destruction by these viruses.

Although data concerning phage–phagocyte interactions are not extensive, it seems obvious that such bidirectional effects occur. Considering the potential role of these interactions, further studies are indispensable.

The prospect of the involvement of phage–phagocyte interactions in anti-tumor therapies

Interactions between bacteriophages and mammalian immune systems has also been used in developing new microbiological strategies combating cancer development. Erikson et al. [11] described in 2007 the inhibition of tumor growth by tumor-specific phages, which induced the infiltration of PMNs and the secretion of IL-12 (p70) and interferon γ. Two types of tumor-specific phages were obtained: phages selected by screening phage display libraries and phages expressing an Fab fragment with a previously described specificity for tumor tissue. Tumor specificity resulted in the accumulation of phage particles in tumor tissue. Phages considered foreign agents for the mammalian body are able to induce humoral and cellular responses; therefore localizing phage virions to the site of pathology caused directing the immune response to phage–tumor complexes. Treatment of tumor-bearing mice resulted in regression of tumor growth, prolonged survival of the animals or even complete clearance of tumor cells. Massive infiltration of polymorphonuclear neutrophils was observed 24 h after the application of phages, and tumor tissue damage and a small number of viable tumor cells were observed already after 72 h. Induction of Th1 cytokines by the introduced phages was also observed. Although this effect might have been a consequence of the presence of endotoxin in the phage preparation, the studies showed that phages without endotoxins may induce IL-12 and IF-γ as well. Although the mechanism of the described interactions is still not precisely known, the destruction of tumor tissue may be caused by induced cytokines, which might activate neutrophils to release ROS and other cytotoxic agents, resulting in tissue damage [31]. Although phages can stimulate the production of antibodies specific to them, the described phenomenon seems to have no connection with these kinds of interactions, as previous phage-immunization does not enhance the therapy's effect.

The significance of Toll-like receptor (TLR-9) activation has also been debated. Phages can activate this receptor on such cells as antigen-presenting cells (APCs), neutrophils, macrophages and dendritic cells, which may lead to inflammation. Tissue damage may also be caused by neutrophils and NK cells, which, as elements of the innate immune system, are highly effective destroyers of invading pathogens, not only by phagocytosis, but also by the involvement of other immunological cells. It is possible that local dendritic cells may also be involved in the presentation of tumor antigens to T cells. The interactions may then be very complicated. Regardless of the mechanisms and the involvement of phages in immune responses, the role of these viruses seems significant. Phages' specificity to tumor allows one to steer the immunological response in the direction of the tumor tissue [11]. This is extremely important considering the devastating effects on non-tumor tissues in patients undergoing chemotherapy, which is currently in general use.

A new anti-tumor immunotherapeutic strategy has recently been developed by Pajtasz-Piasecka et al. [32]. Activation of bone marrow-derived dendritic cells (BM-DCs) by T4 bacteriophage and their further loading with tumor antigens (TAgs) resulted in the induction of an anti-tumor response in mice bearing MC38 colon carcinoma tumor. Because of the previously mentioned observations related to the inhibition of phagocytosis of molecules in the presence of phages [1], the ability of TAg uptake by DCs

after preincubation with phages was also studied. The observations showed that prior contact of phages with dendritic cells had no effect on subsequent antigen uptake. Preincubation of DCs with T4 bacteriophage with or without further loading with TAg resulted in augmentation of maturation marker expression. The contact with phages also caused increased production of interferon γ by splenocytes. Although the effect was strongest for BM-DC/T4 + TAg groups, it was also observed for cells incubated with bacteriophages only. The anti-tumor effect of phage-activated dendritic cells was studied in vivo. Mice inoculated with tumor cells were injected with BM-DC/T4 + TAg cells. The results were compared with those of control groups in which the cells were activated only with bacteriophage T4 (BM-DC/T4), TAg (BM-DC/TAg) or lipopolysaccharide (BM-DCs/"solvent"). Treatment resulted in prolonged ΔTRV (time at which the tumor volume is 1 cm^3), which was 19 days in BM-DC/T4+TAg animals, but about 3.5–7 days in the other experimental groups. Tumor growth inhibition (TGI) was also highest in BM-DC/T4 + TAg groups, reaching 76%. Although the mechanisms responsible for the anti-tumor actions are not precisely known, activated DCs are responsible for priming $DC8^+$ T lymphocyte activity connected with T-cell cytotoxicity and the production of interferon γ. A role of macrophage activity is also postulated.

The anti-tumor activity of bacteriophages may also be based on interactions other than immunological. Although bacteriophages are believed to have no natural tropism to mammalian cells, such interactions were observed years ago. In 1940, Bloch et al. [33] proved the ability of bacteriophages to accumulate in tumor tissue. Moreover, they seemed to inhibit tumor growth. The antitumor activity of phages was later confirmed by Dabrowska et al. [13, 14]. A mechanism of those interactions was proposed by Gorski et al. [15]. It involves the potential ability of β3 integrins on the surfaces of some (including cancer) cells to bind the KGD (Lys–Gly–Asp) motif on some phages' capsids. The models of the studies were the bacteriophages T4 and HAP1 containing the KGD motif in gp24, a pentameric protein occurring on the phages' heads. The studies confirmed that blocking β3 integrins by ligand analogs inhibits the binding of phages to cancer cells, which seems to confirm the hypothesis. Phages significantly inhibited lung metastasis of B16 melanoma cells (T4 by 47% and HAP1 by 80%) [12]. The increased antitumor activity of HAP1 may be related to the fact that this phage has a damaged Hoc protein, a protein which symmetrically protrudes from the capsid. Removal of this seric barrier and the free exposition of KGD ligand may be the reason for the stronger inhibition of metastasis. Regardless of the mechanisms of action, the involvement of phages in oncology seems very promising.

Bacteriophages and reactive oxygen species production by PMNs

A very important direction of study deals with the influence of phages on the production of ROS by neutrophils. This was recently investigated by Przerwa et al. (2006) and Miedzybrodzki et al. (2007). Reactive oxygen species are powerful weapons of neutrophils and monocytes. However, intensified production of those molecules may result in serious tissue damage and involvement in many disorders. ROS are believed to be responsible for the initiation as well as progression of cancer [34]. They are also considered to be connected with cardiovascular and neurodegenerative diseases, including Parkinson's, Alzheimer's, and ALS (amyotrophic lateral sclerosis) [35–38]. The pathogenesis of sepsis involves the dysfunction of some immune cells as well as endo- and epithelial cells and is connected with the activity of ROS (also reactive nitrogen species) [39]. The studies showed that bacteriophages can reduce the production of ROS by phagocytes in the presence of bacteria. Phages alone do not cause this. While Przerwa et al. [9] indicated that only homologous phages may inhibit ROS production, Miedzybrodzki et al. [10] showed such ability also for heterologous phages (T4 and the bacterial strain *E. coli* R4 resistant to T4 infection); however, in this case preincubation with the stimulating bacteria did not enhance the inhibitory effect. Miedzybrodzki et al. observed the inhibitory effect on ROS production in PMNs stimulated by live bacteria as well as by their endotoxins (LPS). The interactions seem to be complex and involve not only phage–phagocyte interaction, but also phage–LPS interaction and bacterial lysis.

Discussion

The issue of bacteriophage interactions with the mammalian immune system and its components is still not precisely defined. The significance of such interactions may be crucial for the development of bacteriophage therapy, which is undisputed. The recruitment of patients who may undergo such therapy might become less restricted when all aspects of human–phage interactions are strictly known. However, the clinical applications of phages are not limited to phage therapy. Bacteriophages may also be beneficial in transplantation. They may inhibit the activation of allograft-induced T cells and the nuclear transcription factor NF-κB, which is believed to be strongly related to transplant tolerance [40]. Diminution of NF-κB activity by bacteriophages is an effect opposite to that caused by HSV-1 virus, which was proved to promote activation of this factor [1]. The effect of phage presence on graft infiltration (mononuclear cells and neutrophils)

was also studied. Skin transplants removed and examined on days 1–3 post-transplant did not reveal any differences between the phage and control groups. However, on subsequent days the intensity of graft infiltration differed in both the analyzed groups. On days 7–8 post-transplant, the phages appeared to diminish the infiltration of mostly mononuclear cells, but also partially that of neutrophils. Reducing inflammatory infiltration may be extremely beneficial in preventing graft injury or its loss and may also result in allograft-induced T-cell activation [1].

The constant development of molecular biological techniques may lead to more significant application of bacteriophages than now. Bacteriophages, as objects of intense study, may have a great influence on human life in the future. The phage-display technique, which is based on genetic modifications of phages, allows the display of foreign proteins on their capsids. This method has been successfully used for the development of vaccines with phage capsids as platforms for antigens. Significantly, the creation of a vaccine is not limited by the protein's size. It is possible to obtain phage particles displaying several kinds of antigens, as was shown by, among others, Sathaliyawala et al. [41] in 2006 for HIV p24, Nef and g41 proteins. These proteins were displayed simultaneously on T4 phages' capsids deprived of Hoc protein. In vivo studies showed that such vaccines may elicit a strong humoral and cellular immune response [41–44]. Because the treatment of animals with these vaccines brought good results and considering the abundant advantages of this technique, it seems an interesting direction for further study. Another application of phage vaccines is anti-cancer immunotherapy. Identified tumour antigens are subsequently displayed on the phage capsid to induce an immunological response. Such a strategy was used, for example, with antigens of 4T1 breast adenocarcinoma. Peptides were displayed on T7 virions and orally applied to mice, inducing a specific immune response. As a result of this response, tumor growth and metastasis were inhibited [45]. The development of anticancer phage-based vaccines seems to be one of the most meaningful applications of phages. The good results of studies conducted so far suggest the necessity of their continuation.

Bacteriophages are also one of the best known genetic vectors. Increased interest in genetic methods may also result in augmented bacteriophage application in biotechnological branches connected with genetic modifications.

The developing techniques of molecular engineering may also soon bring the possibility of designing phages with particular properties and their usage in a directed way. In 2001, Di Giovine et al. [46] introduced a gene encoding a protein responsible for the adhesion and internalization of adenovirus into the bacteriophage M13 genome. Modified phages could bind integrin receptors on mammalian cells and penetrate and transduce them; however, they could neither propagate nor induce cell lysis. It is possible that the inevitable future development of molecular biology will allow free manipulation of interactions between phages and organisms other than bacterial ones.

The studies involving phage–mammal interactions also include the influence of individual proteins on the biological activity of normal and cancer cells. Especially interesting from the immunological point of view seems to be the recently described Hoc protein of bacteriophage T4, which possesses an immunoglobulin-like domain. Since the immunoglobulin superfamily (IgSF) includes members of fundamental importance in the mammalian immune system, the similarity of its domains and Hoc protein domains may be significant [47]. Moreover, analyses show that many phage peptides more frequently show closer sequence similarity with eukaryotic than with prokaryotic homologs [48], which may be of great importance when considering phage–mammal interactions.

Although phage influence on the migration of mammalian cells seems to be extremely important, there are no reliable and abundant data related to this problem. The inhibition of melanoma cell migration and the resulting inhibition of tumor spread by phages discussed earlier are evidence that this phenomenon may be of great importance. The study of the influence of phages on the metastatic migration of melanoma cells has been continued in our laboratory. Recently, we have also undertaken studies on the influence of phages on the migration of human immune cells, i.e., granulocytes, mononuclear cells and also human leukemia tissue. The studies include the T-family phage preparations investigated previously and also preparations used in the treatment of staphylococcal and pseudomonal infections of patients of our therapy center. We have also studied the biological properties of T-family phage proteins, which in our opinion is extremely important and to our knowledge a pioneering direction of studies. It is important to mention that, because of virion propagation, phage preparations contain some amounts of bacterial endotoxins occurring after bacterial lysis. Complete endotoxin removal from a protein solution was previously described [49]. However, such procedures lead to marked phage loss and may therefore be of limited practical value. LPS is highly immunogenic and its effects on the immune system have to be considered along with the actions of phages. We have observed that bacteriophage T4 preincubated with granulocytes (1 h) caused a slight stimulation of cell migration compared with the PBS group. This effect was, however, stronger than that of LPS and the phages seemed to suppress the inhibiting effect of lipopolysaccharide. Bacteriophage HAP1 caused inhibition of granulocyte migration only slightly more weakly than LPS (data not shown). Considering the great importance of

this issue and the sparse (if any) data related to the described interactions, we believe that this direction of study is indispensable.

Bacteriophages introduced into the mammalian organism may penetrate it quite freely. They are able to enter the bloodstream almost irrespective of the way of administration [50–55]. Recent studies showed that bacteriophages may pass the intestinal wall by exploiting gut immune cells (enterocytes, M cells and, particularly, dendritic cells) [56]. Interactions with dendritic cells and downregulation of their actions may be significant in preventing inflammation leading to gut injuries. The easy and direct contact with human/animal tissues implies the necessity of a narrow circumscription of all the possible interactions between introduced virions and mammalian cells. One of the most important areas of interest seems to be the immunological system, which is the first target of phage contact. The small amount of data on the interactions of bacteriophages with cells of the mammalian immune system, especially that of humans, in connection with increasing the significance of these viruses in contemporary medicine and biotechnology seems to be a problem. We believe that explaining all the aspects of such interactions and the influence of bacteriophages on the animal/human immune system is indispensable and that this direction of research is pivotal.

Acknowledgments This review was supported by KBN (Polish State Committee for Scientific Research) grants: NN402 2855 35 and NN401 1305 33.

Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

1. Gorski A, Kniotek M, Perkowska-Ptasinska A, Mroz A, Przerwa A, Gorczyca W (2006) Bacteriophages and transplantation tolerance. Transplant Proc 38:331–333
2. Gorski A, Dabrowska K, Switala-Jeleń K, Nowaczyk M, Weber-Dabrowska B, Boratynski J, Wietrzyk J, Opolski A (2003) New insights into the possible role of bacteriophages in host defense and disease. Med Immunol 2:2
3. Kniotek M, Ahmed AMA, Dabrowska K, Switała-Jelen K, Opolski A, Gorski A (2004) Bacteriophage interactions with T cells and platelets. In cytokine network, Regulatory, cells signalling, and apoptosis (immunology). Monduzzi Editors, Bologna, pp 189–192
4. Przerwa A, Kniotek M, Nowaczyk M, Weber-Dabrowska B, Switala-Jelen K, Dabrowska K, Gorski A (2005) Bacteriophages inhibit IL-2 production by human T lymphocytes. Abstract of the 12th Congress of European Society of Organ Transplantation, Geneva, October 2005
5. Kleinschmidt WJ, Douthardt RJ, Murphy EB (1970) Interferon production by T4 coliphage. Nature 228:27–30
6. Clark J, March J (2004) Bacteriophage-mediated nucleic acid immunization. FEMS Immunol Med Microbiol 40:21–26
7. March J, Clark J, Jepson C (2004) Genetic immunization against hepatitis B using whole bacteriophage lambda particles. Vaccine 22:2413–2419
8. Clark J, March J (2006) Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. Trends Biotechnol 24:212–218
9. Przerwa A, Zimecki M, Switała-Jeleń K, Dabrowska K, Krawczyk E, Łuczak M, Weber-Dabrowska B, Syper D, Miedzybrodzki R, Górski A (2006) Effects of bacteriophages on free radical production and phagocytic functions. Med Microbiol Immunol 195:143–150
10. Miedzybrodzki R, Switala-Jelen K, Fortuna W, Weber-Dabrowska B, Przerwa A, Lusiak-Szelachowska M, Dabrowska K, Kurzepa A, Boratynski J, Syper D, Pozniak G, Lugowski C, Gorski A (2008) Bacteriophage preparation inhibition of reactive oxygen species generation by endotoxin-stimulated polymorphonuclear leukocytes. Virus Res 131:233–242
11. Eriksson F, Culp WD, Massey R, Egevad L, Garland D, Persson MA, Pisa P (2007) Tumor-specific phage particles promote tumor regression in a mouse melanoma model. Cancer Immunol Immunother 56:677–687
12. Dabrowska K, Zembala M, Boratynski J, Switala-Jelen K, Wietrzyk J, Opolski A, Szczaurska K, Kujawa M, Godlewska J, Gorski A (2007) Hoc protein regulates the biological effects of T4 phage in mammals. Arch Microbiol 187:489–498
13. Dabrowska K, Opolski A, Wietrzyk J, Switala-Jelen K, Boratynski J, Nasulewicz A, Lipinska L, Chybicka A, Kujawa M, Zabel M, Dolinska-Krajewska B, Piasecki E, Weber-Dabrowska B, Rybka J, Salwa J, Wojdat E, Nowaczyk M, Gorski A (2004) Antitumor activity of bacteriophages in murine experimental cancer models caused possibly by inhibition of beta3 integrin signaling pathway. Acta Virol 48:241–248
14. Dabrowska K, Opolski A, Wietrzyk J, Switala-Jelen K, Godlewska J, Boratynski J, Syper D, Weber-Dabrowska B, Gorski A (2004) Anticancer activity of bacteriophage T4 and its mutant HAP1 in mouse experimental tumour models. Anticancer Res 24:3991–3995
15. Gorski A, Weber-Dabrowska B (1998) The potential role of endogenous bacteriophages in controlling invading pathogens. Cell Mol Life Sci 62:511–519
16. Kruse M, Rosorius O, Krätzer F, Stelz G, Kuhnt C, Schuler G, Hauber J, Steinkasserer A (2000) Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. J Virol 74:7127–7136
17. Meltzer MS, Gendelman HE (1992) Mononuclear phagocytes as targets, tissue reservoirs, and immunoregulatory cells in human immunodeficiency virus disease. Curr Top Microbiol Immunol 181:239–263
18. Kantoch M (1958) Studies on phagocytosis of bacterial viruses I. Arch Immunol Ther Exp 6:63
19. Kantoch M (1958) Studies on phagocytosis of bacterial viruses II. Arch Immunol Ther Exp 6:417
20. Nelstrop AE, Taylor G, Collard P (1968) Studies on phagocytosis. II: in vitro phagocytosis by macrophages. Immunology 14:339–346
21. Ferrini U, Mileo AM, Nista A, Mattei E, Orofino A (1989) Polymorphonuclear leukocyte stimulation measured by phage inactivation. Int Arch Allergy Appl Immunol 90:207–212
22. Barfoot R, Denham S, Gyure LA, Hall JG, Hobbs SM, Jackson LE, Robertson D (1989) Some properties of dendritic macrophages from peripheral lymph. Immunology 68:233–239
23. Inchley CJ (1969) The activity of mouse kupffer cells following intravenous injection of T4 bacteriophage. Clin Exp Immunol 5:173–187
24. Geier MR, Trigg ME, Merril CR (1973) Fate of bacteriophage lambda in non-immune germ-free mice. Nature 246:221–223

25. Gaubin M, Fanutti C, Mishal Z, Durrbach A, De Berardinis P, Sartorius R, Del Pozzo G, Guardiola J, Perham RN, Piatier-Tonneau D (2003) Processing of filamentous bacteriophage virions in antigen-presenting cells targets both HLA class I and class II peptide loading compartments. DNA Cell Biol 22:11–18
26. Kantoch M, Dubowska-Inglot A (1960) Inhibition of the phagocytic activity of leukocytes by Coxsackie viruses. I: the influence of viral concentration and temperature on the inhibition of phagocytosis. Pathol Microbiol (Basel) 23:83–94
27. Merchant DJ, Morgan HR (1950) Inhibition of the phagocytic action of leucocytes by mumps and influenza viruses. Proc Soc Exp Biol Med 74:651–653
28. Nishmi M, Bernkopf H (1958) The toxic effect of vaccinia virus on leucocytes in vitro. J Immunol 81:460–466
29. Slopek S, Steuden W, Kaczmarek M, Krukowska A, Durlak I (1983) Application of bacteriophage in the phagocytic test. Arch Immunol Ther Exp 31:75–78
30. Shaw DR, Maurelli AT, Goguen JD, Straley SC, Curtiss R 3rd (1983) Use of UV-irradiated bacteriophage T6 to kill extracellular bacteria in tissue culture infectivity assays. J Immunol Methods 56:75–83
31. Fossati G, Bucknall RC, Edwards SW (2002) Insoluble and soluble immune complexes activate neutrophils by distinct activation mechanisms: changes in functional responses induced by priming with cytokines. Ann Rheum Dis 61:13–19
32. Pajtasz-Piasecka E, Rossowska J, Duś D, Weber-Dabrowska B, Zabłocka A, Górski A (2007) Bacteriophages support anti-tumor response initiated by DC-based vaccine against murine transplantable colon carcinoma. Immunol Lett 116:24–32
33. Bloch H (1940) Experimental investigation on the relationships between bacteriophages and malignant tumors. Arch Virol 1:481–496
34. Møller P, Wallin H (1998) Adduct formation, mutagenesis and nucleotide excision repair of DNA damage produced by reactive oxygen species and lipid peroxidation product. Mutat Res 410:271–290
35. Bergamini CM, Gambetti S, Dondi A, Cervellati C (2004) Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des 10:1611–1626
36. Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Rev Neurosci 5:S18–S25
37. Shah AM, Channon KM (2004) Free radicals and redox signalling in cardiovascular disease. Heart 90:486–487
38. Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14
39. Biswal S, Remick DG (2007) Sepsis: redox mechanisms and therapeutic opportunities. Antioxid Redox Signal 9:1959–1961
40. Zhou P, Balin SJ, Mashayekhi M, Hwang KW, Palucki DA, Alegre ML (2005) Transplantation tolerance in NF-κB-impaired mice is not due to regulation but is prevented by transgenic expression of Bcl-xL. J Immunol 174:3447–3453
41. Sathaliyawala T, Rao M, Maclean DM, Birx DL, Alving CR, Rao VB (2006) Assembly of human immunodeficiency virus (HIV) antigens on bacteriophage T4: a novel in vitro approach to construct multicomponent HIV vaccines. J Virol 80:7688–7698
42. Shivachandra SB, Li Q, Peachman KK, Matyas GR, Leppla SH, Alving CR, Rao M, Rao VB (2006) Multicomponent anthrax toxin display and delivery using bacteriophage T4. Vaccine 25:1225–1235
43. Cao YC, Shi QC, Ma JY, Xie QM, Bi YZ (2005) Vaccination against very virulent infectious bursal disease virus using recombinant T4 bacteriophage displaying viral protein VP2. Acta Biochim Biophys Sin (Shanghai) 37:657–664
44. Jijang J, Abu-Shilbayeh L, Rao VB (1997) Display of PorA peptide from *Neisseria meningitidis* on the bacteriophage T4 capsid surface. Infect Immun 65:4770–4777
45. Shadidi M, Sørensen D, Dybwad A, Furset G, Sioud M (2008) Mucosal vaccination with phage-displayed tumour antigens identified through proteomics-based strategy inhibits the growth and metastatis of 4T1 breast adenocarcinoma. Int J Oncol 32:241–247
46. Di Giovine M, Salone B, Martina Y, Amati V, Zambruno G, Cundari E, Failla CM, Saggio I (2001) Binding properties, cell delivery, and gene transfer of adenoviral penton base displaying bacteriophage. Virology 282:102–112
47. Bateman A, Eddy SR, Mesyanzhinov VV (1997) A member of the immunoglobulin superfamily in bacteriophage T4. Virus Genes 14:163–165
48. Bernstein H, Bernstein C (1989) Bacteriophage T4 genetic homologies with bacteria and eucaryotes. J Bacteriol 171:2265–2270
49. Aida Y, Pabst M (1990) Removal of endotoxin from protein solutions by phase separation using Triton-X114. J Immunol Methods 132:191–195
50. Carrera MR, Kaufmann GF, Mee JM, Meijler MM, Koob GF, Janda KD (2004) Treating cocaine addiction with viruses. Proc Natl Acad Sci USA 101:10416–10421
51. Frenkel D, Solomon B (2002) Filamentous phage as vector-mediated antibody delivery to the brain. Proc Natl Acad Sci USA 99:5675–5679
52. Merril CR, Biswas B, Carlton R, Jensen NC, Creed GJ, Zullo S, Adhya S (1996) Long-circulating bacteriophage as antibacterial agents. Proc Natl Acad Sci USA 93:3188–3192
53. Bogozova GG, Voroshilova NN, Bondarenko VM (1991) The efficacy of *Klebsiella pneumoniae* bacteriophage in the therapy of experimental *Klebsiella* infection. Zh Mikrobiol Epidemiol Immunobiol 4:5–8
54. Georgakopoulos PA (1968) Permeability of the female genital system to virus. Arch Gynakol 205:211–218
55. Hoffmann M (1965) Animal experiments on the mucosal passage and absorption viremia of T3 phages after oral, tracheal and rectal administration. Zentralbl Bakteriol 198:371–390
56. Gorski A, Wazna E, Dabrowska BW, Dabrowska K, Switała-Jelen K, Miedzybrodzki R (2006) Bacteriophage translocation. FEMS Immunol Med Microbiol 46:313–319