MINIREVIEW

Bacteriophage translocation

Andrzej Górski1,2, Ewa Ważna2, Beata-Weber Dąbrowska1, Krystyna Dąbrowska1, Kinga Świtała-Jeleń1 & Ryszard Międzybrodzki1

1L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland and 2The Transplantation Institute, The Medical University of Warsaw, Warsaw, Poland

Correspondence: Andrzej Górski, L Hirszfeld Institute of Immunology and Experimental Therapy, Weigla 12, 53-114 Wrocław, Poland. Tel.: +48 71 3373491; fax: +48 71 3372171; e-mail: agorski@ikp.pl

Received 7 August 2005; revised 3 October 2005; accepted 4 October 2005. First published online 24 February 2006.

doi:10.1111/j.1574-695X.2006.00044.x

Editor: Willem van Leeuwen

Abstract

The occurrence of phages in the human body, especially in the gastrointestinal tract, raises the question of their potential role in the physiology and pathology of this system. Especially important is the issue of whether phages can pass the intestinal wall and migrate to lymph, peripheral blood, and internal organs and, if so, the effects such a phenomenon could have (such passage by bacteria, known as bacterial translocation, has been shown to cause various disturbances in humans, from immune defects to sepsis). Available data from the literature support the assumption that phage translocation can take place and may have some immunomodulatory effects. In addition, phages of the gut may play a protective role by inhibiting local immune reactions to antigens derived from gut flora.

Introduction

The passage of indigenous bacteria colonizing the intestine through the mucosa to local lymph nodes and internal organs is termed bacterial translocation and is a critical step in the pathophysiology of various disorders, from inflammatory bowel disease and sepsis to heart failure. Various drugs, chemicals, clinical procedures (e.g. bronchoscopy) and pathologic conditions (heart failure, burns) have been shown to influence this process, as discussed in detail in recent reviews (Guarner & Malagelada, 2003; Wiest & Garcia-Tsao, 2005).

There are three primary mechanisms in the promotion of bacterial translocation: bacterial overgrowth in the small intestine; increased permeability of the intestinal barrier; and immune system deficiencies (Guarner & Malagelada, 2003). Bacterial translocation may be of paramount importance for the normal development of gut-associated lymphoid tissue (GALT) and tolerance induction against indigenous flora (Gebbers & Laissue, 2004). Furthermore, translocation of endotoxins in small amounts may provide an important boost to the reticuloendothelial system (Schoeffel et al., 2000). What is more, bacterial translocation can also be detected in healthy people, with a frequency as high as 5% of the population assayed (Guarner & Malagelada, 2003).

The growing interest in bacteriophages (phages), their role in our environment, and their potential in treating antibiotic-resistant infections raises the important question as to whether phages also translocate and what the functional significance of such a phenomenon could be.

Viral translocation

While bacterial translocation is a well-described phenomenon that has been receiving greatly increased interest recently, little is known about the translocation of viruses. Experiments in mice have revealed that orally administered Coxsackie virus is concentrated in the lymphocytes of the mucosal layer of the small intestine and subsequently spread to internal organs (Harrath et al., 2004). Similarly, HIV-1 was shown to translocate through the epithelium using M cells, dendritic cells, and epithelial cells and to disseminate to systemic sites (Smith et al., 2003). Furthermore, some rotavirus strains are capable of escaping from the gut and disseminating to peripheral tissues via cells of the lymphoid system, although the exact mechanisms of translocation are obscure (Mossel & Ramig, 2003).

Viruses have evolved various strategies to translocate across the epithelial barrier and to act as pathogens. The binding and entry of viruses is a multistep process that involves the recognition of and attachment to the epithelial
cell surface using a variety of virus-attachment receptors. Those mechanisms have been described in detail (Bomsel & Alfsen, 2003).

**Presence of phages in peripheral blood/serum (‘phagemia’)**

If bacteria and viruses can translocate through the intestinal barrier, it could be expected that bacteriophages (phages) can also pass the gut wall. If phage translocation indeed takes place, this should lead to phage circulation in the peripheral blood (‘phagemia’), a phenomenon which could have some functional consequences, especially in view of our novel data suggesting that phages may exert immunobiological activities (Górski & Weber-Dąbrowska, 2005). There are several reports describing the presence of phages in sera used for in vitro tissue culturing (Chu et al., 1972; Merrill et al., 1972; Vieu et al., 1974; Fong et al., 1975; Orr et al., 1975) (summarized in Table 1). Importantly, there was no correlation between the presence of phages, their titers and the growth-promoting characteristics of the sera (in fact, several lots of sera containing phages had good growth-supporting ability), which suggests that phages had no harmful effects on cultured cells (Chu et al., 1972). It should be emphasized that the level of phages detected in those studies must be considered minimums, since only particular strains of their bacterial targets were used for phage detection. It is possible that the use of more bacterial targets would reveal more significant phagemia. The situation may thus be similar to the low sensitivity of detecting bacterial translocation using standard blood culture technique. Enteric bacterial and Candida albicans DNA were detected using PCR in patients with a predisposition to this pathology (bowel obstruction, ulcerative colitis, supramesenteric artery occlusion, and those receiving chemotherapy for colon cancer); all these patients were negative for bacterial translocation using the blood culture technique (Ono et al., 2005).

Both endogenous and exogenous sources of phagemia have been suggested (physiological viremia vs. contamination) (Merril et al., 1972; Vieu et al., 1974), and the problem therefore merits further study. Isolation of viruses from fetal calf serum without concurrent bacteria suggests that viremia and phagemia can indeed occur in normal animals (Molander et al., 1972).

Can phages also circulate in the human bloodstream? Literature addressing this fundamental question is very scarce. The novelty of the problem of phage translocation and distribution in the body can be illustrated by the number of records retrieved from PubMed in response to a combination of terms: ‘bacteria + urine’ yielded almost 14000 records but ‘bacteriophage + urine’ only 57, of which only one deals with the issue of phage detection in human urine (!), and the material involves only one patient. Our search of the available literature revealed that there is only one paper on the issue of phage presence in human urine that addresses this problem in a more systematic way, published more than 70 years ago (Caldwell, 1928). We have found two reports confirming such ‘natural’ phage occurrence in human sera (i.e. unrelated to their exogenous administration). Mankiewicz & Liivak (1967) demonstrated the presence of mycobacteriophages in the sera of up to 75% of patients with sarcoidosis, whereas no phages could be isolated from serum samples of healthy persons or patients with tuberculosis. Parent & Wilson (1973) found mycobacteriophages in three of 19 patients with Crohn’s disease; interestingly, four of 18 age-matched normal subjects were also positive (Parent & Wilson, 1973). The latter paper is probably the only report in the literature describing the natural occurrence of phages in the blood of healthy individuals.

**Table 1.** Phage presence in animal sera used for tissue culture

<table>
<thead>
<tr>
<th>No. of positive lots</th>
<th>Phage type (bacterial target)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/37 ( &gt; 60%)</td>
<td><em>Escherichia coli</em> C-3000 strain</td>
<td>Chu et al. (1972)</td>
</tr>
<tr>
<td>Not specified; in addition to fetal bovine serum, calf serum, fetal calf serum, lamb, horse and chicken serum also positive</td>
<td><em>Escherichia coli</em> C-3000 strain</td>
<td>Merrill et al. (1972)</td>
</tr>
<tr>
<td>13/39 (33%)</td>
<td><em>Escherichia coli</em> B, <em>Escherichia coli</em> K12</td>
<td>Vieu et al. (1974)</td>
</tr>
<tr>
<td>23/24 ( &gt; 90%)</td>
<td><em>Escherichia coli</em> 36, <em>Escherichia coli</em> C-3000 strain</td>
<td>Orr et al. (1975)</td>
</tr>
<tr>
<td>17/25 (68%)</td>
<td>Not specified (electron microscopy)</td>
<td>Fong et al. (1975)</td>
</tr>
</tbody>
</table>

**Can phages pass the gut barrier and reach peripheral lymph and blood?**

**Phage migration in animals**

While the physiological and immunobiological importance of phages circulating in the blood of otherwise healthy animals and humans presents an intriguing phenomenon which clearly requires further study and confirmation, the question of whether orally administered phages (e.g. for therapeutic purposes) can penetrate the intestinal wall is equally important, especially from a clinical perspective. The group of Keller & Engley (1958) have shown that Bacillus megathericum phages introduced into the gastrointestinal tracts of mice could be recovered as early as 5 min after...
gastric lavage or oral inoculation, although the quantity of viable phages recovered from blood was random and irregular (Keller & Engly, 1958). Hildebrand & Wolochow have shown that T1 coliphages instilled into the duodenum of rats can pass directly into lymph and then reach the peripheral blood. Further work of this group revealed that properties other than size alone appear to influence the capability of microorganisms (including phages) to translocate. The ratio of instilled to recovered phages became larger, with the numbers instilled subsequently reaching a plateau (Hildebrand & Wolochow, 1962; Wolochow et al., 1966). On the other hand, there was no correlation between recovery and the volume of instilled suspension. Hoffman has shown that 15 min after oral administration in mice, T3 coliphages can be demonstrated in the majority of animals, with high fluctuations among individual mice. Rectal application seems to be a very efficient route of phage administration, causing large numbers of phages to enter the bloodstream in all animals. Phagemia is as high as after intramuscular injection, but increases more rapidly and peaks after 5 min (15 min after intramuscular administration) (Hoffmann, 1965). Reynaud et al. have shown that following gastric inoculation of rabbits, CF 0103 coliphages can be recovered from blood on the 4th day after administration in quite high titers (3 × 10^7 PFU mL^-1 of plasma), but phage recovery required lysis of erythrocytes. The authors thus suggest that coliphages may adhere to erythrocytes and, possibly, leukocytes (Reynaud et al., 1992). [Phage adhesion to erythrocytes was described by Bystricky et al. (1964).] In contrast, Keller & Engley (1958) did not observe erythrocyte binding of T1 coliphages circulating in the blood. Schubbert et al. (1994) provided evidence that not only intact M13 phages but phage DNA fed to mice can be retrieved from their bloodstream (Schubbert et al., 1994).

As stated earlier, the ability of microorganisms to translocate is determined not only by their size, but also by a variety of other factors (e.g. receptors for gut epithelium and M-cell ligands). Duerr et al. used in vivo phage display to identify the sequences which may facilitate phage translocation through the intestine wall. The authors showed that M13 phage is unable to pass the intestinal mucosal barrier; however, enterocytes are able to recognize specific peptides displayed by engineered phages and transport them across the mucosal barrier, thus preserving their biological activities. In addition, a novel model of macromolecular transport has been suggested for phages bearing a specific peptide (Duerr et al., 2004). These data indicate that at least some phages may translocate; however, their passage may also be regulated by possible phage ligands which can be recognized by intestinal cells responsible for their transport (enterocytes, M cells and dendritic cells) (Nagler-Anderson, 2001; Elson & Cong, 2002; Kelsall & Leon, 2005). Very subtle changes in the protein structure of the phage capsid may evoke major changes in phage transport and bioavailability: a single specific substitution of glutamic acid to a lysine upgrades a phage's capacity to remain in the mouse circulatory system up to 16 000-fold (Vitiello et al., 2005). This rule could also apply to phages crossing the gut barrier on their way to lymphatic and blood vessels.

**Phage migration in humans**

While phage translocation in animal gut has been studied in some detail, there are very few data available for humans. Weber-Dąbrowska et al. (1987) demonstrated the presence of circulating phages (against *Staphylococcus, Escherichia*, *Pseudomonas* and *Proteus*) in the majority of blood samples taken on day 10 of oral treatment of patients with various bacterial infections resistant to antibiotics (no phages could be detected in these patients prior to the therapy) (Weber-Dąbrowska et al., 1987). This is the only study we could find in Medline-covered literature reporting phage circulation in patients’ blood following oral administration. Recently, Bruttin & Bruessow measured the bioavailability of oral T4 phage in humans and showed that fecal phage was detected in all volunteers receiving a higher phage dose (10^7 PFU mL^-1). No phage was observed in the serum of the subjects after the end of the study. On the other hand, the authors cite a Russian paper describing phage presence in the blood of patients who were receiving phages orally for therapeutic purposes (Bruttin & Bruessow, 2005). It should be noted that the group in our Institute used different phages in much higher (1000-fold and more) concentrations and phage administration was preceded by neutralization of gastric juice; the titers of phages in the sera examined on the 5th day during their administration varied from 2 × 10^5 PFU mL^-1 (Ps68 *Pseudomonas* phage), 1.2 × 10^7 PFU mL^-1 (676/Forys *Staphylococcus* phage), to 1.7 × 10^7 PFU mL^-1 (*Staphylococcus* phi 131 phage). Furthermore, phage translocation in patients may be much higher than in normal donors (similar to bacterial translocation), as the gut barrier in disease is often much more permeable to microorganisms. Evidently, the issue of phage penetration after oral administration requires more study. The phenomenon is also important in view of new data suggesting that phage penetration can be used for clinical purposes by expressing therapeutic agents on translocating phages. Janda’s group has shown the therapeutic potential of a phage-displayed cocaine-binding antibody and cocaine esterase transported by phages penetrating the central nervous system following their intranasal application (Rogers et al., 2005). This report also confirms earlier suggestions that upper respiratory microorganisms use a mechanism similar to that of enteric ones in the intestine to gain access to underlying tissue, lymphatics and blood (Cleary et al., 2004).
In conclusion, there are suggestions that phages can circulate in the blood of normal animals and healthy and diseased humans. It is important to perform more systematic studies in animals to determine the pharmacokinetics of phages, particularly those relevant in phage therapy (e.g., phages against Staphylococcus aureus including MRSA phages, Pseudomonas, Enterococcus, Escherichia coli). Such studies should examine their detection in blood at different times after oral administration in increasing doses and using different schemes (e.g., with and without neutralization of gastric juice). Similar studies should be done in healthy volunteers. In addition, patients orally treated with phages should be monitored for phage presence in blood. As phage migration from the gut could be increased in various pathological conditions (similar to increased bacterial translocation), basic pharmacokinetics studies should also be performed in a subgroup of patients scheduled to receive phage therapy. Furthermore, the fundamental problem of possible natural occurrence of phages in blood of normal and diseased human individuals should be addressed by culture and electron microscopic studies in normal volunteers as well as patients likely to have increased penetration of naturally occurring coliphages in their intestinal tract (intestinal and liver diseases, burns, immunosuppressive therapy, etc.). Studies concentrating on phage presence in the urine (both natural phages and those possibly penetrating to there as a result of phage therapy) are also indicated, as urinary tract infections pose a great challenge in medicine, and the literature on the role of phages in this clinical setting is virtually nonexistent.

**Possible immunobiological significance of phage translocation**

The presence of phages within the gastrointestinal tract, often in significant numbers, raises the important possibility of their interactions with enterocytes and gut-associated lymphoid tissue (GALT), an immunological network which comprises the majority of T cells and a significant B cell compartment (Acheson & Luccioli, 2004). Such interaction may be even more relevant in view of the possibility that phages may translocate, which obviously facilitates and enhances their interactions with cells present in the intestinal wall. Human intestinal epithelial cells constitutively express the key elements for antigen processing and the production of exosomes, suggesting an important role for them in the processing and presentation of antigen. These cells also express αIIbβ3 (CD41a) and β3 (CD61), which may be implicated in T4 phage binding (Górski & Weber-Dąbrowska, 2005). Interestingly, bacterial internalization and translocation seem to be fairly independent of enterocyte integrin expression, while integrins (e.g., the β3 family) may be relevant in the translocation of bacteria mediated by M cells, located in the follicle-associated epithelium of Peyer’s patches (Martin-Villa et al., 1997; Schulte et al., 2000; Hess et al., 2001; Le Gat et al., 2003; Acheson & Luccioli, 2004).

In recent years there has been a greatly revived interest in microbial gut interactions in health and disease and in unraveling the mechanisms which protect the host from potentially harmful gut pathogens. The possibly countless numbers of foreign antigens and the involvement of the gastrointestinal system in a variety of pathologies, including autoimmune disorders and heart failure, have been emphasized (Acheson & Luccioli, 2004; Krack et al., 2005). It is believed that interactions between dendritic cells and bacteria are instrumental in the regulation of intestinal immunity (Stagg et al., 2004). Dendritic cells can reach into the intestinal lumen to sample bacteria, which may then gain entry to the intestinal mucosa (Rimoldi et al., 2005); one could therefore expect that dendritic cells could also sample intestinal phages. Interestingly, dendritic cells rapidly and abundantly phagocytose T4 phage, in contrast to, for example, latex particles (Barfoot et al., 1989), and the phage causes inhibition of the phagocytosis of subsequently provided particles (Jalil, Jakobiak and Górski, unpublished observations). Gut phages, both endogenous and exogenous (administered for therapeutic purposes), could downregulate the processing abilities of intestinal dendritic cells and thereby prevent their proinflammatory action which can cause local gut injury. Our studies have revealed that T4 phages inhibit specific antibody responses in mice (a process dependent on dendritic cell-mediated antigen processing and presentation) (Kniotek et al., 2004b) and extend skin allograft survival in mice concomitantly with a significant reduction of transplant infiltration by mononuclear cells. Furthermore, phages may inhibit in vitro CD3-triggered T cell activation and proliferation as well as activation of the transcription factor nuclear factor (NF)-κB in response to viral pathogens (Górski et al., in press) and reactive oxygen species production (Przerwa et al., 2006). Moreover, our recent data indicate that phages may also inhibit interleukin (IL)-2, tumor necrosis factor (TNF) and, to some extent, interferon (IFN) gamma production by human leukocytes (Przerwa et al., 2005). The newly available data strongly suggest that phages may have immunoregulatory activities and, by their ability to downregulate specific and nonspecific immune reactions, can help maintain local immune tolerance to foreign antigens derived from gut microorganisms. Phages could limit bacterial translocation by directly eliminating sensitive bacteria and reducing gut inflammation, including that caused by local immune responses to bacterial translocation, thereby improving the ‘leaky gut’ syndrome. In other ways, phages may have probiotic functions, acting in a similar manner to probiotic bacteria, which...
may function in part by modulating intestinal immunity, including dendritic cell functions (Stagg et al., 2004).

It is also possible that phages can interact with the CD40–CD40L system, which is believed to play a central role in major immune and inflammatory reactions, also involving the gastrointestinal tract (Danese & Fiocchi, 2005). CD40L, a primary platelet agonist, activates the receptor function of the αIIbβ3 integrin receptor as measured by fibrinogen binding and the formation of platelet microparticles. Platelet stimulation is induced by the binding of the KGD domain of soluble CD40L to αIIbβ3 (Prasad et al., 2003); importantly, this domain is also present within the gp24 head corner protein of the T4 phage (Dąbrowska et al., 2006). It is believed that many potent biological activities mediated through the D40/CD40L pathway by immune and nonimmune cells are exerted by activated platelets and their products (e.g. platelet-derived microparticles, which can coat other cells and thereby confer on them new functions dependent on platelet-derived molecules (e.g. β3 integrins), including the ability to induce metastasis and angiogenesis) (Janowska-Wieczorek et al., 2005). These activities include inflammatory, immunoregulatory and hemostatic functions, which are relevant in atherosclerosis, diabetes and, importantly, inflammatory bowel disease. Platelet interactions with human intestinal microvascular endothelial cells may critically contribute to the activation of the CD40–CD40L pathway, leading to inflammatory bowel disease. What is more, CD40L expression is markedly higher in platelets from inflammatory bowel disease patients (Danese & Fiocchi, 2005). Platelet activation antagonists may improve intestinal dysfunction in pancreatitis, diminish local leukocyte recruitment, and restore gut barrier function.

It is also possible that phages can interact with the CD40–CD40L system, which is believed to play a central role in major immune and inflammatory reactions, also involving the gastrointestinal tract (Danese & Fiocchi, 2005). CD40L, a primary platelet agonist, activates the receptor function of the αIIbβ3 integrin receptor as measured by fibrinogen binding and the formation of platelet microparticles. Platelet stimulation is induced by the binding of the KGD domain of soluble CD40L to αIIbβ3 (Prasad et al., 2003); importantly, this domain is also present within the gp24 head corner protein of the T4 phage (Dąbrowska et al., 2006). It is believed that many potent biological activities mediated through the D40/CD40L pathway by immune and nonimmune cells are exerted by activated platelets and their products (e.g. platelet-derived microparticles, which can coat other cells and thereby confer on them new functions dependent on platelet-derived molecules (e.g. β3 integrins), including the ability to induce metastasis and angiogenesis) (Janowska-Wieczorek et al., 2005). These activities include inflammatory, immunoregulatory and hemostatic functions, which are relevant in atherosclerosis, diabetes and, importantly, inflammatory bowel disease. Platelet interactions with human intestinal microvascular endothelial cells may critically contribute to the activation of the CD40–CD40L pathway, leading to inflammatory bowel disease. What is more, CD40L expression is markedly higher in platelets from inflammatory bowel disease patients (Danese & Fiocchi, 2005). Platelet activation antagonists may improve intestinal dysfunction in pancreatitis, diminish local leukocyte recruitment, and restore gut barrier function (Leveau et al., 2005). We have shown adhesive interactions between human platelets and T4 phages and the ability of these phages to inhibit platelet adhesion to their natural ligand, fibrinogen (Kniotek et al., 2004a). Thus, KGD-expressing phages (phages of the T4 family and possibly other phages present in the intestines) could bind platelet αIIbβ3 integrin and thereby block the stimulatory effects of CD40L on platelets mediated by the same integrin receptor.

Gut coliphages may also have some anticancer properties. We have shown that T4 phage may be active against the growth and metastasis formation of murine melanoma. Its substrain, HAP1, binds cancer cells more strongly, having much higher anticancer activity (Dąbrowska et al., 2004). At least some immunomodulatory and anticancer properties of these phages may be mediated by Hoc protein (highly immunogenic outer capsid protein), a component of the T4 bacteriophage head related to proteins of the immunoglobulin superfamily. We believe that Hoc protein may be one of the molecules predicted to interact with mammalian organisms and/or modulate these interactions (and may perhaps be responsible for some of the immunomodulatory effects of the phages) (Dąbrowska et al., 2006).

Conclusions

The available data suggest that phages may not only reside within the gut lumen but also pass the intestinal wall in a process similar to bacterial translocation. The precise conditions enabling this remain obscure, but it is likely that phage passage is determined by a number of factors: phage concentration; specific sequences within the phage capsid proteins interacting with enterocyte receptors; and phage interactions with gut immune cells (dendritic cells in particular). It could be expected that factors known to affect the gut barrier should also enhance phage passage. Moreover, factors causing prophage induction may also be relevant. In this regard it would be interesting to study the effect of different cells/tissues. Phage migration and circulation in the mammalian body may not only be a newly described and interesting biological phenomenon, but also have an important role in the body’s defenses.

Acknowledgements

Supported by Ministry of Science grant No PBZ-MIN-007/P04/2003 and The Medical University of Warsaw intramural grant 1MG/W1/2005.

References


Dąbrowska A, Głowacka S, Probst PG & Petricciani JC (1972) The bacteriophage T4 head related to proteins of the immunoglobulin superfamily. We believe that Hoc protein may be one of the molecules predicted to interact with mammalian organisms and/or modulate these interactions (and may perhaps be responsible for some of the immunomodulatory effects of the phages) (Dąbrowska et al., 2006).

Conclusions

The available data suggest that phages may not only reside within the gut lumen but also pass the intestinal wall in a process similar to bacterial translocation. The precise conditions enabling this remain obscure, but it is likely that phage passage is determined by a number of factors: phage concentration; specific sequences within the phage capsid proteins interacting with enterocyte receptors; and phage interactions with gut immune cells (dendritic cells in particular). It could be expected that factors known to affect the gut barrier should also enhance phage passage. Moreover, factors causing prophage induction may also be relevant. In this regard it would be interesting to study the effect of different cells/tissues. Phage migration and circulation in the mammalian body may not only be a newly described and interesting biological phenomenon, but also have an important role in the body’s defenses.

Acknowledgements

Supported by Ministry of Science grant No PBZ-MIN-007/P04/2003 and The Medical University of Warsaw intramural grant 1MG/W1/2005.

References


