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An alternative to conventional treatments for bacterial infection

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Abstract

While the concept of phage therapy to control bacterial infection and contamination was conceived almost a century ago, it has now become more important to understand the concept of phage therapy since drug resistant bacteria have become more common and the development of new antibiotics has become more difficult. Phage therapy studies have produced both positive and negative results. This article presents the fundamental knowledge of phage infection in bacterial cells and extends it to phage therapy. This article will present and discuss the major issues concerning advantages and disadvantages of phage therapy as an alternative to using antibiotic treatment of bacterial infection and contamination.

Keywords: *bacteriophage, bacterial host, phage therapy, drug resistance bacteria*

1. Introduction

Bacteriophage, or phage, is a general term for an agent that "destroys bacteria" (Ackermann & DuBow, 1987). However, phages are actually a group of viruses that live as a parasite in the bacterial host. As they can cause cell death, they have been considered as a tool to treat bacterial infection in humans and other organisms since their discovery by Frederick Twort in 1915 (Thiel, 2004; Kropinski, 2006). Since 1917, Felix d'Herelle, also honored as a discoverer of bacteriophage, performed many experiments, successfully showing that the application of bacteriophage can treat bacterial infections (Thiel, 2004). However, many other researchers have not been able to confirm this (Dublanchet & Fruciano, 2008). Their safety for use as a therapeutic agent in the human body is also still uncertain.

Historically, the acceptance of phage therapy has been reluctant and with the discovery of penicillin by Alexander Fleming in 1928 (Brown, 2004) and later other antibiotics (Dibner & Richards, 2005), phage therapy was even further disfavored. Thusly, the use of antibiotics has been the main tool to treat bacterial infection in most of the developed countries, subsequently spreading to most of the developing countries as well. As a result, phage therapy has been mostly neglected around the globe (Kropinski, 2006). Nevertheless, research has continued and page therapy accepted in some countries of Eastern Europe, such as the former Soviet Union and Poland (Ho, 2001; Sulakvelidze, Alavidze & Morris, et al., 2001).

The study and application of phage therapy has mainly been limited to monitoring bacterial contamination in food and animal products in order to extend their shelf life (Almeida et al., 2009; Atterbury et al., 2007). Many of these studies gave promising results among researchers in Eastern Europe but not in America and Western Europe. Over the last decade, with the appearance of drug resistant bacteria and the challenges in discovering new antibiotics to treat such bacteria (Duran & Marshall, 2005), the concept of phage therapy has been revisited as an alternative to antibiotic therapy (Górski et al., 2009). As a result, phage therapy is now approved by the FDA (Food and Drug Administration of the USA) (Merril, Scholl & Adhya, 2003).

2. How phages affect bacteria

As shown in Figure 1, there are two kinds of phages, virulent and temperate. Virulent phage infects bacterial cell by the mechanism of lytic infection only, so it is called a lytic phage. Lytic infection is synonymous with acute infection which causes bacterial cell death via cell lysis as a result of the viral replication process (Dimmock, Easton & Leppard, 2001; Cairns et al., 2009). On the other hand, temperate phage can infect bacterial cells not only by lytic infection but also by lysogenic infection. Lysogenic infection closely resembles chronic or persistent infection, as observed in animals (Dimmock, Easton & Leppard, 2001). Most temperate phage cause lysogenic infection by

integrating their genome into the bacterial chromosome, co-existing as a dormant parasite but do not kill the bacterial host (Dimmock et al., 2001; Pasharawipas et al., 2005). However, during the later stage of temperate phage infection, the virus can switch to the lysogenic mode of infection. (Dimmock, Easton & Leppard, 2001). While there is no clear explanation of this phenomenon, we know that the expression of two sets of genes in temperate phages are involved in the change between lytic and lysogenic infection (Stahl, 1998). While the first set of genes, *CI* genes, play a role in initiating the lysogenic infection, a second set of genes, *Cro* genes, initiate the lytic infection, (Stahl, 1998; Dimmock, Easton & Leppard, 2001; Oppenheim et al., 2005). The principle factors that activate the *CI* and *Cro* genes are not well understood. However, ultraviolet radiation and organic substances such as mitomycin-C are reported to induce the lytic mode of temperate phages (d'Ari, 1985).

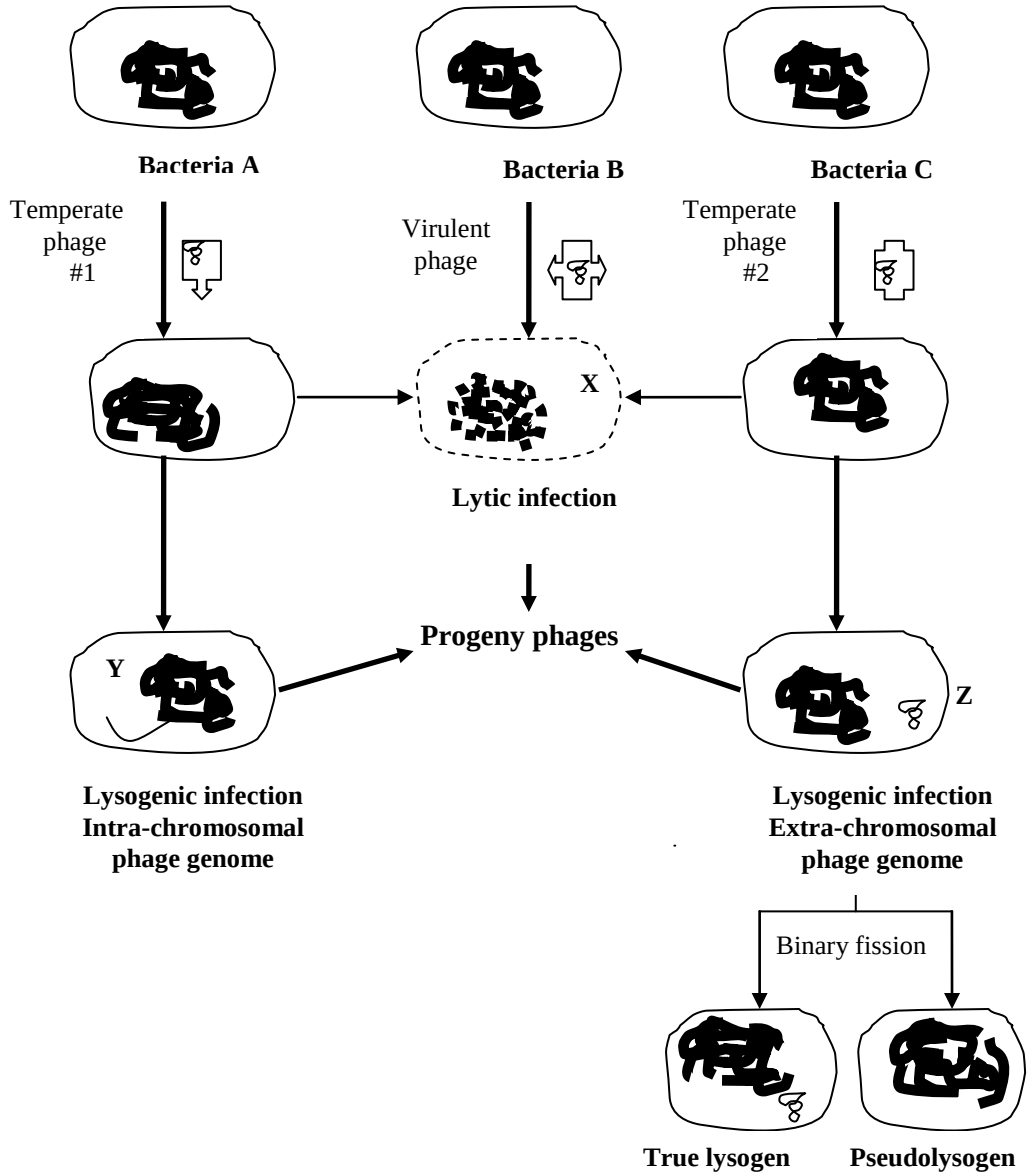

Figure 1 Schematic representation of virulent and temperate phage infection in bacterial host cells to produce progeny phages. Virulent phage causes only lytic infection (X) while temperate phage can cause either lytic or lysogenic infection. There are two kinds of temperate phages. The first (#1) inserts its genome, as shown with a thin line, into bacterial chromosome (Y) while the other

In contrast to the main class of temperate phages, there is another kind of temperate phage in which its genome locates in the bacterial cell like a plasmid. This means the phage genome of some temperate phage do not intercalate into the bacterial chromosome but localizes extra-chromosomally (Kawakami & Landman, 1968; Khemayan et al., 2006; Pasharawipas, Wetchakit & Sriurairatana, 2008). Khemayan et al. (2006) called the lysogen with an extra-chromosomal phage genome a true lysogen (TL). They showed that sub-culture colonies of TL can produce another kind of lysogen, called a pseudolysogen (PL). The PL, which do not contain a phage genome in its cytoplasm, was shown to be generated from TL (Khemayan et al., 2006). It is hypothesized that PL is derived from the TL during host cell replication due to the unequal rates of host and phage genome replication. Thus, some daughter cells do not receive a copy of the phage genome. These cured daughter cells, are called pseudolysogens (Pashrawipas et al., 2008).

Usually, lysogenic infection can prevent super-infection of the same kind of phage in both TL and PL (Dimmock, Easton & Leppard, 2001), the PL inheriting the ability to resist super-infection from its mother TL cell (Pashrawipas et al., 2008). The resistance to phage super-infection observed in lysogenicly infected bacteria is also found in virally infected eukaryotic cells. The mechanism for this has been proposed by various hypotheses such as the appearance of defective phages (Cambell, 1960), the role of cytokines (Sidahmed et al., 2007), gene mutation (Skurnik & Strauch, 2006) and disappearance of viral receptor molecules (Skurnik & Strauch, 2006, Pasharawipas, Wetchakit & Sriurairatana, 2008).

Consequently, the resistance of lysogens, intra and extra-chromosomal phage genome, and PL to re-infection by the same phage is one of problems for the phage therapy. Thus, to make the concept of phage therapy possible, it is more appropriate to use virulent phage for phage therapy to avoid lysogenic infection. This is further discussed below.

3. The concerns for the success of phage therapy

As mentioned above, since the study of Felix d'Herelle, the concept of phage therapy has been reported by many groups of researchers with controversial results. The published results of previous studies, compared here on the basis of multiplicity of infection (MOI) which corresponds to the ratio of phage particles to bacterial cells in the culture, differed greatly among each research group. Soothill, (1992) reported using MOI as low as 10^{-5}, which means 1 phage particle can kill 10^5 bacterial cells. The studies of other groups required higher MOI, as high as 10^3-10^4, which means 10^3-10^4 phage particles were required to kill only one bacterial cell (Abedon & Thomas-Abedon, 2010; Almeida et al., 2009; Skurnik & Strauch, 2006). The vastly different results of these previous studies, which greatly contributed to the controversy over the usage of phage therapy, might be due to the use of virulent versus temperate phage. However, this was not discussed in these reports.

Logically, it is more promising to use virulent phage to treat bacterial infections, especially in animals and humans (Kumari, Harjai & Chhibber, 2009; Wang et al., 2007) and food product contamination (Leverentz et al., 2001; Wall et al., 2010). This is because the virulent phage progeny can attack the bacteria's daughter cells without interference from super-infection. On the other hand, temperate phage can cause the target bacteria to resist the same phage infection, producing either a lysogen or a pseudolysogen.

Phage are infectious only to bacteria, thus it is unlikely that they can be directly pathogenic to animals and humans but no reports have yet been published concerning this. However, regarding the safety of phage therapy, the main concern is the phenomenon of phage conversion. In phage conversion, the temperate phage genome causes the normally non–pathogenic, natural flora bacteria of the infected animal to convert to a pathogenic phenotype. This can occur with the use of temperate phage but not with virulent phage. Therefore, it has been suggested that the application of temperate phage is inappropriate for *in vivo* treatment of humans and animals (Dublanchet & Fruciano, 2008). Phage conversion has been reported in *Vibrio cholerae* (Campos et al., 2010; Hanssan et al., 2010), *Staphylococcus aureus* (Endo et al., 2003) *Salmonella enterica* (Brown et al., 1999), and others (Dobrindt & Reidl, 2000). While page conversion does not readily occur because phage require a specific bacterial host, treating a bacterial infection with temperate phage therapy is nonetheless contraindicated if the infecting bacteria is a strain of a naturally occurring flora already present in the body (e.g., virulent *E. coli*). On the other hand, using temperate phage to treat *Salmonella* species will not cause phage conversion since *Salmonella* is not naturally present in the human body. Thus, we

should consider, case by case, the use of temperate phage in the treatment of *in vivo* bacterial infections. In cases where temperate phage is indicated, it is a better alternative to use two or more kinds of temperate phage which can infect the same strain of bacteria. In this case, we should also determine if each phage should be used simultaneously or on a schedule.

Another issue raised by a previous report is the standard for judging the success of phage therapy. In this report, animal survival rates of phage-treated groups were compared to the control group (Almeida et al., 2009). In our recent report, (Pasharawipas, Manopvisetcharean & Flegel, 2011), we suggested that the success of phage treatment be judged on the basis that the phage kills or completely eliminates the infecting bacterial cells. Otherwise, it cannot be conclusively determined that the phage in question is an effective tool to treat the infection because any surviving bacteria, even if just one cell, can proliferate significantly within 24 hours, causing a relapse.

4. Proposed phage modification

There are reports that more temperate phages exist in our body than do virulent phages (Furuse, 1987; Chibani-Chennoufi et al., 2004a) but more virulent phages than temperate phages in the natural environment (Chibani-Chennoufi et al, 2004a). We studied a phage of *Vibrio harveyi* (VH), which is a pathogenic bacterium of black tiger shrimp. Although over 20 strains of temperate VH phage have been isolated, we have never been able to isolate a virulent phage for VH from any shrimp pond. According to our report (Pasharawipas, Manopvisetcharean & Flegel, 2011), it is possible to use temperate phage to treat bacterial contamination in shrimp ponds only if sufficient MOI of the temperate phage is applied to kill all the bacteria at once. Insufficient MOI can induce the formation of lysogenic bacteria which can resist phage super-infection. The therapeutic use of wild-type temperate phage is often inappropriate due to the risk of phage conversion. Thus the concept of deletion or modification of any gene(s) that induce the lysogenic pathway should be considered as a way to circumvent this problem. Theoretically, genetic modification techniques may be used to convert a temperate phage into a virulent phage (Wolf & Woodside, 2005). By sequencing the phage genome to identify the involved lysogenic genes and subsequently altering or deleting them should prevent lysogenic infection in the bacterial cell, thus making the temperate phage appropriate for treatment of bacterial infection and food contamination.

The most significant limitation to using phage as a tool to fight bacteria is the specificity of phage infection in bacterial cells. Research shows that phage infection in bacteria is strain specific, not species specific (Nagy, 1974; Rakhuba et al., 2010; Chibani-Chennoufi et al., 2004b). This means that if there is more than one strain of pathogenic bacterium involved, we need to obtain all the strains of phage that can infect each of the various pathogenic bacteria. It is estimated that each species of bacteria may be infected by at least 10 phage species (Chibani-Chennoufi et al., 2004a). Thus, like searching for a needle in a hay stack, we need to isolate all the phages for the particular species of bacterial pathogens and prepare a cocktail of these phage, (Santos et al., 2010). However, there is a report of polyvalent phages which can infect a broad range of bacteria (Kilic et al., 2001; Lu et al., 2003). Additionally, there is a phage that can reorient its receptor-interacting gene to synthesize a new ligand for attachment to a different bacterial cell (Scholl et al., 2001). This information can lead to the possibility that we can develop a common ligand for phage infection, allowing the attack of a broad range of bacterial strains and even species. Thus, one might propose a study to develop only one kind of phage to treat multiple strains or species of bacteria, eliminating the need for preparing various phage cocktails. To make this successful, we, at least, need to thoroughly understand the diverse interactions between bacterial cells and phages.

5. The obstacles to phage administration

In the case of bacterial contamination of food and localized infection in the body, phage therapy can be applied by using phage particles which interact directly with bacterial cells (Leverentz et al., 2001). In the case of systemic infections, however, an issue that we have to consider is the oral administration of phage into the body (Kumari, Harjai & Chhibber, 2009; McVay, Velásquez & Fralick, 2007; Wang et al., 2006). Based on phage structural components, phage therapy is rendered ineffective by physical and chemical barriers, especially in the gastrointestinal (GI) tract (Bruttin & Brüssow, 2005). This is a significant obstacle for phage therapy because the oral route is the most convenient administration pathway, as it also is for

antibiotic treatment. Regardless of route of administration, another problem is the immune response resulting from repeated administration of phage into the body. Memory immune cells will be generated and the immune response can rapidly attack and destroy the phage particle, especially during subsequent administration of phage. Also, the immune complex of phage particle and antibody, including various immunological cytokines, can interfere with other biological functions of the patient. At present, there is no appropriate technology to directly inhibit the body's immune response to phage particles. Despite this, immunotolerance can be induced in the body and can be used as a mechanism to produce tolerance to phage antigens. However, this is a wishful speculation. If the immune reaction can be prevented, the use of phage therapy should be very promising even in systemic infections. So far, however, there is no report of the administration of phage *in vivo* for a long duration. Thus, there is currently no definite evidence to evaluate this paradigm.

6. Conclusion

The main advantages of using virulent phage as a therapy is its specificity to pathogenic bacteria and its inability to harm native flora. However, phage specificity to the bacterial host is disadvantageous as it reduces the number of useful phage to a limited range of bacterial species. While it is more reasonable to use virulent phage as a promising tool for treatment of bacterial infections, virulent phage are a scarcity for many bacteria. In this case, temperate phage may be a viable alternative but sufficient amounts of temperate phage must be administered with special care to prevent lysogenic infection. Alternatively, molecular biology techniques can be applied to modify a temperate phage to act as a virulent phage, preventing lysogenic infection. Also, technological advances could produce a phage which attacks a broader range of strains or even species of bacteria. However, present knowledge is still insufficient to overcome the immune response in systemic therapy. In conclusion, while there are still many obstacles limiting the use of phage therapy for the treatment of bacterial infection, phage therapy could be a viable future alternative in the fight against bacterial infection and contamination. Further research in the field of phage therapy is warranted.

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