Bacteriophage Pharmacology and Immunology

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Abstract

The discovery of bacterial viruses approximately 100 years ago fairly quickly led to their use as antibacterial agents. For roughly two decades – early 1920s to early 1940s – bacteriophages represented the only means readily available to medicine by which many bacterial infections might be treated and cured. This near monopoly, however, came to a close as antibiotics became generally available. Antibiotics, especially as more broadly specific, selectively toxic antibacterials were both more easily developed and more easily used medicinals than phages. Phage therapy did not disappear from medical practice altogether, however, and increasingly is viewed as a viable alternative to antibiotics under circumstances where bacterial resistance to antibiotics is an issue. In addition are circumstances where a more selectively toxic antibacterial is desired, antibacterials that, for example, have less of a negative impact on nontarget members of a body's microbiome. As for any drug, the successful development of phage therapeutics requires a pharmacological approach, whether implicit or, ideally, explicitly implemented. In this chapter, we consider pharmacokinetic and pharmacodynamic principles, body impact on drugs and drug impact on body, respectively, and both as they may be applied to the development of phage-based antimicrobials. As an important facet of both the pharmacokinetics and pharmacodynamics of phage therapy, we take a close look particularly at phage interactions with the mammalian immune system.

Introduction

While the development of phages as medicinals has been strongly influenced by the parallel development of antibiotics, at the same time antibiotics have generally been studied in terms of a much more rigorous pharmacological tradition than has been the case for phage therapy. There exist perhaps four prominent reasons for that dissimilarity in development approaches.

First is that phages for phage therapy in almost all instances have proven to be mostly safe to use as drug equivalents (Olszowska-Zaremba et al. 2012; McCallin et al. 2013; Abedon 2015d; Pirnay et al. 2015), and particularly so in purified forms (Gill and Hyman 2010; Łobocka et al. 2014; Fish et al. 2016; Schooley et al. 2017; Chan et al. 2018). Low drug toxicity has the effect of reducing the importance of

secondary pharmacodynamic issues, meaning in turn that approaches to improving phage therapy efficacy often can be explored without overriding concern for potential negative impacts of those strategies on patient health. Prominently, the display by a drug of only low levels of toxicity allows for reduced need to rein in drug concentrations, that is, to devote substantial effort towards the avoidance of exceeding a drug's minimum toxic density during the course of dosing. As a result, efforts in phage therapy development have tended to emphasize phage antibacterial effectiveness and delivery strategies, and this is rather than achievement of sufficient densities while simultaneously avoiding toxicity. Generally, that is, reaching bacteria with sufficient numbers of the right kind of phages, and over sufficient time spans, can be the emphasis of phage therapy development even to the point where in fact it can be helpful to remind researchers that explicit monitoring of potential toxicities – especially of treated animals during preclinical development, if only for the sake of building "up in the literature a record of phage toxicity testing" (Abedon 2012b) – can be important as well.

A second reason for pharmacology having played less of a role in phage therapy development, versus development of small molecule antibiotics, stems from the potential for phages to increase in concentration during their action in situ. That is, phages as viruses possess an ability to replicate in the course of their antibacterial action, which in turn has the effect, in a number of clinical circumstances, of increasing the potential for specific phage *delivery* strategies to result in sufficient phage densities to achieve bacterial eradication. Generally, if bacterial infections by phages are robust enough – large enough burst sizes in combination with sufficiently fast virion adsorption and not excessively long latent periods – then simply delivering even relatively small quantities of phages to those bacteria often can result in the generation of sufficient phage densities, over time, to result in eradication of sensitive bacteria (i.e., active treatment). Furthermore, this typically can be achieved, as noted, without substantial concerns over phage-associated toxicity. The need to study the ability of a drug to reach its intended target in sufficient numbers therefore can be alleviated somewhat if an inherent property of the drug is one of increasing to sufficient numbers once it has reached its target.

Third, though there is justified interest during phage therapy development in phage host range (Hyman and Abedon 2010; Chan and Abedon 2012a; Łobocka et al. 2014), in terms of phage spectrum of activity, there tends to be much less interest in what for antibiotics is a major consideration, that of minimum inhibitory concentration (MIC). Though at least in part this lack of interest in MIC determination is a result of conceptual difficulties in defining phage minimum inhibitory concentrations (Abedon 2011a), such as due to the single-hit killing characteristics of phages (Bull and Regoes 2006), the net result nonetheless is that there has been little tradition in phage therapy for studying the underlying bases of efficacy in terms of phage performance except to the extent that should one phage type prove to be ineffective in the clinic then another phage – from among of what typically is a diversity of possible, safe choices – may be employed instead (Pirnay et al. 2011; Chan et al. 2013). This latter point is changing to a degree, however, as researchers have begun to more formally link together more subtle aspects of phage host range, that is, infection performance in vitro, with their effectiveness during experimental

phage therapy (Henry et al. 2013; Bull and Gill 2014; Lindberg et al. 2014); see also (Łobocka et al. 2014). Nevertheless, an important aspect of antibiotic pharmacological study, that of a concentration dependence of antibacterial activity, has played much less of a role in phage therapy development than it has for chemotherapies.

Fourth, especially early development of the practice of phage therapy was a time during which substantial *clinical* experimentation was permissible, for example, Abedon (2015d, 2018a), but also Chanishvili (2012a). When alternative treatments are lacking, and especially when a patient's survival is under threat, then principles of compassionate care can be applied including as emergency investigational new drugs (Międzybrodzki et al. 2012; Kutter et al. 2015; Fish et al. 2016; Schooley et al. 2017; Chan et al. 2018). Treatments thus may commence without consideration of possible subtleties of drug pharmacology beyond following standard protocols of drug application or infection preparation (e.g., wound debridement), and otherwise carefully monitoring patient health, as well as use of informed phage choice and purification (Gill and Hyman 2010; Łobocka et al. 2014). At the same time, additional pharmacological study, versus simply efforts towards efficacy enhancement (e.g., trying different phages or delivery strategies), may not be robust in the course of such care. By contrast, in modern medicine, where an emphasis is usually placed more on regulation and standards of care rather than extensive clinical trial and error, pharmacological considerations can and must have a much more prominent place in drug development. For a historical look at phage use clinically versus preclinically, see the review by Abedon (2015d) on the use of phages to treat lung-associated infections.

Despite the potential for both the study of phage therapy and achievement of efficacy without strong consideration of phage therapy pharmacology, modern norms of drug development nonetheless provide a countering force to this tendency. Related to the previous point is the use of animal models for phage therapy development. Animal models typically do not perfectly mimic human disease. As a consequence, use of animals as an aspect of drug development requires consideration of how pharmacological characteristics may differ between these experimental systems and actual patients. Indeed, patient safety considerations along with the typical expense of clinical trials alone should drive such an interest. Furthermore, there exists an economic as well as moral argument for consideration of phage therapy pharmacology as an aid towards improving treatment protocols before, during, and after animal experimentation since this can lead to reductions in the total number of trials – clinical as well as preclinical – that are required for successful development.

Included among phage properties during phage therapy that warrant such increased consideration – and consistently of interest to those familiarizing themselves with this technology – is the issue of phage virion interactions with mammalian immune systems. These interactions can be distinguished into three somewhat distinct facets (1) the potential for immune systems to inhibit phage therapy efficacy (a pharmacokinetic concern), (2) the potential for immune system reaction to phage presence to result in side effects (as distinct, it should be noted, from the potential for target bacteria to provoke negative immune reactions), and (3) the potential for phages to provide positive immunomodulatory effects, including as may occur independently of bacterial targeting during phage therapy.

With such considerations in mind, in this chapter we walk through the basics of phage therapy pharmacology, pointing the reader to a growing literature on the subject. We begin with discussion of the relative safety that has been observed with phage therapy. We then provide a general discussion of phage therapy pharmacology. Lastly, we consider issues of phage-immune system interaction.

For additional reading, note that Łobocka et al. (2014) provide an excellent primer on the criteria that can be employed in choosing a phage for phage therapy; see equivalently Gill and Hyman (2010). For discussion of routes of phage delivery into bodies, see Ryan et al. (2011). For practical considerations of phage therapy pharmacology, experimentation, and debugging of protocols, see Abedon (2012b, 2017c, 2018b) as well as the Appendix to Abedon (2017a). For consideration of the use of phage cocktails in phage therapy, see Chan and Abedon (2012b) and Chan et al. (2013). For discussion of the distinction between phage-mediated "biocontrol" and phage therapy, see Abedon (2009). For additional consideration of the history of phage therapy, see Summers (2005), Abedon et al. (2011), Chanishvili (2012b), Harper and Morales (2012), Summers (2012), and Abedon (2017b) (chapter "► The Discovery of Bacteriophages and the Historical Context"). And for volumes with a substantial phage therapy component, see Kutter and Sulakvelidze (2005), Sabour and Griffiths (2010), Abedon (2010), Hyman and Abedon (2012), and Borysowski et al. (2014) along with this volume. A glossary of terms relevant to phage therapy pharmacology can be found in Table 1.

Safety Considerations in Phage Choice for Phage Therapy

As noted, a key reason for why phage therapy has tended to be developed without an accompanying robust pharmacological tradition is the relative safety of phages as antibacterials, which includes, historically, the phage therapy of fairly large numbers of people in Europe and the former Soviet Union (Abedon et al. 2011; Kutter et al. 2010; Chanishvili 2012a; Kutter et al. 2014); see also (Abedon 2015d). This safety is the result of a number of factors stemming from a combination of inherent phage properties and well-informed phage choice. In short, an important goal for phage choice in phage therapy is to choose those phages that are inherently safe (Łobocka et al. 2014; Pirnay et al. 2015), and this generally means (1) the avoidance of phages that can display lysogenic cycles (chapter ► "Temperate Phages, Prophages, and Lysogeny"), (2) avoidance as well of phages that may otherwise encode potentially toxic genes (chapter ► "Temperate Phages, Prophages, and Lysogeny,"), and (3) otherwise, at least to some degree, avoiding phages that are able to transduce nonphage genes between bacteria. To a first approximation, the phage potential to display all three of these properties can be reduced via the exclusive use of nontemperate and particularly professionally lytic phages for phage therapy purposes (chapter ► "Phage Infection and Lysis"). In this section, we consider these issues further. Note again the reviews by Łobocka et al. (2014) and Gill and Hyman (2010) covering the subject of phage choice.

Table 1 Glossary of terms relevant to phage therapy pharmacology

Term	Definition
Abortive infection (broadly defined)	Bacterial infections by phages that result in bacterium death in combination with substantially reduced phage-infection productivity; abortive infections can be the result either of incompatibilities between infecting phages and bacteria or instead bacteria-encoded antiphage mechanisms
Abortive infection (narrowly defined)	Bacterial infection by phages that result in bacterial death in combination with inactivation of the infecting phage (i.e., a combination of resulting in neither a phage burst nor a latent phage latent infection)
Absorption	Uptake of medicinals into the blood; relevance during phage therapy is seen either given requirements for systemic phage application or, alternatively, should more localized phage administration result in phage movement into blood; contrast "A*d*sorption," below
Active infection	Equivalent to productive infection
Active penetration	Proposed scenario of phage antibiofilm activity that is dependent on phage-induced lysis of target bacteria, particularly as results either in more effective phage movement to underlying layers of bacteria and/or as allows for more effective phage infection of these underlying bacteria given successful adsorption
Active replication	The consequence of active infection
Active treatment (or therapy)	Phage therapy or phage-mediated biocontrol of bacteria that requires productive phage infections to be successful; particularly, active treatment is required to the extent that abortive/bactericidal infections alone are insufficient to result in successful eradication of target bacteria
Adsorption	Phage-virion acquisition of a new bacterial host; contrast "A*b*sorption," above. The process of phage adsorption generally ends with specific virion interactions with receptor molecules found on bacterial surfaces and is followed by translocation of virion-associated nucleic acid into the bacterial cytoplasm
Auto dosing	In situ generation of new phage particles as a consequence of productive phage infections. Auto dosing involves active phage replication and productive phage infections and is required for active treatment
Bactericidal phage infection	Typically associated with phage lytic cycles though can result from phage abortive infections as well
Distribution	Movement of medicinals out of the blood and (ideally) into target tissues; note that the term "penetration," though not quite identical in meaning, nevertheless is often employed in a phage-therapy context rather than distribution
Excretion	Movement of medicinals out of the body in a chemically intact state; importance to phage therapy is seen especially when such phage movement aids phages in accessing target bacteria, for example, systemic phage dosing towards phage access to the urinary tract

(continued)

Table 1 (continued)

Term	Definition
Immunomodulation	Regulation of elements of immune response by an external factor (here: phage), thus changing the mode of action of the immune system in the presence of this factor
Inundation therapy (or inundative treatment[a])	Equivalent to passive treatment, so named because phage numbers in excess of bacteria numbers must be supplied via standard dosing for successful bacterial eradication to occur
Lysis from without	Abortive interaction between certain phages and their hosts that strictly is associated with a combination of high multiplicity phage adsorption, truncated infections, and bacterial lysis; so far as is known, lysis from without is associated with only a relatively small subset of phage types, that is, T-even-type phages so should not be invoked indiscriminately
Lysogen	Prophage-containing bacterium
Lysogenic conversion	Expression of prophage-encoded genes that results in modification of the phenotype of a lysogenized bacterium
Lysogenic cycle (or infection)	Latent phage infection that does not, without induction, involve production of progeny phage virions and during which the phage genome exists as a prophage
Lytic phage	Bacterial virus capable of infecting lytically. Use of this descriptor ideally has no bearing on a phage's potential to infect lysogenically, and while all virulent phages are lytic phages, not all lytic phages are virulent phages.
Metabolism	Chemical modification of medicinals; for phages this includes activation of bactericidal activity, associated bacterial lysis, and production of virion progeny
Mixed passive-active treatment (or therapy)	Phage therapy or phage-mediated biocontrol of bacteria that does not explicitly require productive phage infections to be successful, that does require bactericidal phage infections, but which nevertheless is enhanced in its effectiveness as a consequence of infection production of new phage virions
Neutralizing antibody	Specific antibody that is capable of blocking the activity of its target
Obligately lytic	Phage that is unable to display lysogenic cycles; equivalent to strictly lytic but see also professionally lytic
Parenteral	Nonalimentary application of medicinals especially for the sake of systemic distribution
Passive treatment (or therapy)	Phage therapy or phage-mediated biocontrol of bacteria that does not require productive phage infections for treatments to be efficacious, but does require that phage infections are bactericidal
Penetration	Physical movement of phage virions especially within poorly mixed environments towards target bacteria, for example, as combining the pharmacokinetic aspects of absorption and distribution (and, in certain cases, also excretion), as well as phage movement into the matrix of bacterial biofilms
Per os	Oral application of medicinals, for example, as can be followed by absorption into the blood and then movement (distribution) out of the blood

(continued)

Table 1 (continued)

Term	Definition
Phage therapy	Application of bacterial viruses especially to bodies as a means of reducing numbers of unwanted bacteria, and particularly as observed within clinical, medicinal, or veterinary contexts
Phage-mediated bacterial biocontrol	Application of bacterial viruses especially other than bacteria-infected organisms as a means of reducing numbers of unwanted bacteria
Pharmacodynamics	Impact of drugs on a body, that is, how drugs affect bodies, with bodies defined to include not just body tissues but also a body's microbiome. Pharmacodynamics thus considers both the positive and negative impacts of drugs, on bodies, but not factors specifically affecting drug concentrations over time in the body – see instead pharmacokinetics for the latter. Pharmacodynamics can be distinguished into primary versus secondary effects
Pharmacodynamics, primary	Intended impact of drugs on a body
Pharmacodynamics, secondary	Unintended impact of drugs on a body, including though not limited to in terms of toxicities and side effects
Pharmacokinetics	Impact of a body on drugs, that is, how bodies chemically or spatially influence drugs following dosing; the pharmacokinetics of a drug helps to determine a drug's concentration within the vicinity of drug targets within the body over time, where drug concentrations within the vicinity of drug targets within the body in turn will tend to determine the magnitude of pharmacodynamic effects; see also absorption, distribution, excretion, and metabolism as pharmacokinetic processes
Pharmacologically emergent property	Ability of medicinals to display especially unexpected toxicities during drug preclinical as well as clinical development
Primary infection (of individual cells)	Phage infection of a previously phage-uninfected bacterium, though the concept can be applied to the active infection of a previously latently phage-infected bacteria as well, for example, phage adsorption followed by productive infection of a bacterial lysogen
Primary infection (epidemiological sense)	Phage infection of a previously not actively phage-infected bacterium where the infecting virion was *not* generated in situ but rather was supplied via dosing, that is, an infection that occurs by a virion after that virion has entered into a new environment
Productive infection	Phage infection that directly results in the production as well as release of progeny phage virions
Professionally lytic (as used here)	Lytic phage that is both unable to display lysogenic cycles, that is, is obligately lytic, and is not otherwise closely related to or recently descended from phages that can display lysogenic cycles (i.e., not similar genetically to temperate phages)
Prophage	Term used to describe latently infecting phage genomes during lysogenic cycles
Restrictive infection or restricted infection	Phage infection that results in phage inactivation in conjunction with survival of the host bacterium, for example, as mediated by bacterial restriction-modification systems or CRISPR-Cas systems

(continued)

Table 1 (continued)

Term	Definition
Reticuloendothelial system	Mechanism of phagocytic removal of particles such as viruses from blood without requirement for antibody or complement, a.k.a., mononuclear phagocyte system
Secondary infection (of individual cells)	Phage infection of a previously actively phage-infected bacterium (though the concept can be applied to the secondary infection of previously latently phage-infected bacteria as well); many authors use the phrase superinfection instead
Secondary infection (epidemiological sense)	Phage infection of a previously not actively phage-infected bacterium where the infecting virion had been generated in situ rather than supplied via dosing; secondary infections in this sense are the hallmark of active treatments
Single-hit killing characteristics	Phage potential to effect bactericidal effects on bacteria given the adsorption of only one phage; contrast with the multihit killing kinetics of bactericidal small-molecule antibiotics
Strictly lytic	Equivalent to obligately lytic
Superinfection immunity	Prophage-expressed means by which phages of equivalent immunity type are prevented from successfully infecting (i.e.,, prevented from superinfecting), including prevented from displaying bactericidal activity
Temperate phage	Bacterial virus that is capable of infecting lysogenically; often incorrectly referred to instead as lysogenic phages
Transduction	Movement by phages especially of bacterial DNA from one bacterial host to another
Translocation (phage infection)	Movement of phage DNA into a bacterium's cytoplasm following virion adsorption; more generally, this is nucleic acid translocation
Translocation (pharmacokinetics)	Movement of phage virions especially across the wall of the gastrointestinal tract into systemic circulation, as more or less equivalent to absorption; often referred to as phage translocation

[a]Note that the while the proper adjectival form of "inundate" in fact is "inundatory," Payne et al. (2000) as well as Payne and Jansen (2003) refer to an "inundative dose" or "inundative doses" while "inundative biological control" is a legitimate term, of which 'inundative treatment," a form of biological control using phages as the control agent, could be viewed as an example ("inundatory biological control," by contrast, is not a legitimate term). Though Payne et al. (2000) also speak of an "inundatory dose," in fact, as determined via Google as well as Google Scholar, the use of "inundative treatment" or "inundative dose" is far more prevalent than the equivalent use of "inundatory treatment" or "inundatory dose"

Avoiding Temperate Phages

Bacteriophages come in a variety of types. For purposes of phage therapy, or phage-mediated biocontrol of bacteria, the vast majority are both *lytic* and *tailed*. Lytic refers to the means by which a phage is released from its bacterial host, involving the destruction of the bacterial cell envelope (chapter ► "Phage Infection and Lysis"). In the phage life cycle, this lysis allows the intracellularly produced phage progeny virions to exit the host cell. In terms of phage therapy, lysis also represents a prominent aspect of the phage bactericidal nature, though in fact bacterial genetic

death, if not explicitly bacterial metabolic death, generally precedes phage-induced bacterial lysis. The phage tail by contrast is not explicitly required for phage therapy but nonetheless is very common among lytic phages (chapter "► Structure and Function of Bacteriophages") and, as a consequence, is very common as well among those phages that are employed in phage therapy.

Among tailed phages there are temperate phages versus those that are not temperate. The latter also are known as virulent or what has been variously described as "obligately lytic," "professionally lytic," or "strictly lytic" (Hobbs and Abedon 2016). Many authors also, though incorrectly, use simply the term "lytic" as a synonym for not temperate, though this latter practice should be discouraged since the productive cycle of all tailed phages is what is known as a lytic cycle. Temperate phages – which are common among tailed phages, for example, perhaps half (Ackermann 2005) – also with relatively few exceptions employ lytic cycles towards phage virion production but in addition are able to display lysogenic cycles (chapter ► "Temperate Phages, Prophages, and Lysogeny,"). The resulting lysogens tend to be resistant to hosting infections by subsequently adsorbing phages, particularly phages that are of the same type as those already lysogenically infecting the bacterium. This impact of phages displaying lysogenic cycles on subsequent infections by phages of the same type is described as superinfection immunity, homoimmunity, or simply immunity (Campbell 2006; Casjens and Hendrix 2015). As a consequence of this phage-mediated immunity, use of temperate phages in phage therapy can directly result – even in the absence of bacterial mutation – in the generation of bacterial pathogens that are resistant to the very phages employed as antibacterials to combat them. This is one reason that the use of temperate phages for phage therapy is frowned upon, and this is so even to the extent that temperate phages, upon bacterial infection, often will enter into lytic cycles rather than lysogenic cycles.

Avoiding Phages Encoding Virulence Factors

An important second reason for avoiding the use of temperate phages in phage therapy is that, among phages, the encoding of bacterial virulence factors is almost if not always associated with temperate phages (Christie et al. 2012; Kuhl et al. 2012). Obligately lytic phages – or more explicitly, professionally lytic phages, defined as lytic phages that are not immediate descendants of temperate phages (Hobbs and Abedon 2016) – are as a consequence preferentially used for phage therapy purposes. For safety reasons, and even though the expectation is that professionally lytic phages will not carry potentially dangerous bacterial virulence factor genes, nevertheless it is common practice to fully sequence and then do bioinformatic analysis (chapter "► Genetics and Genomics of Bacteriophages") on phages prior to their use for phage therapy purposes (Łobocka et al. 2014). If nothing else, such analysis can be helpful in determining whether or not such phages truly are professionally lytic, that is, not containing lysogeny-associated gene sequences, versus being temperate phages that under the conditions tested simply happen to not display lysogenic cycles.

Despite the potential utility of fully sequencing and annotating phages prior to clinical use, in practice the utility and safety of phage therapy substantially predates the development of such bioinformatic analysis. As the costs of sequencing continue to decline, however, arguments against including routine bioinformatic analysis in phage characterization will become weaker. An additional issue that is related to phage bioinformatic analysis prior to use stems from potential regulatory approaches which, in principle, could favor the approval of general strategies of phage product development rather than the development simply of specific phages, for example, strategies as one sees with the yearly influenza vaccine (Sulakvelidze and Kutter 2005), a comparison which to date has been pointed out by a number of authors, that is, as listed in Abedon (2017b). To the extent that full-genome sequencing and bioinformatic analysis becomes incorporated into approved protocols, then such analysis would become inherent to the ongoing development of phage therapy schemes.

Avoiding Transducing Phages

As noted, an additional issue is that of transduction (Łobocka et al. 2014) (chapter "► Bacteriophage-Mediated Horizontal Gene Transfer: Transduction"). Two types of phage-mediated transduction of bacterial genes exist, which are described as specialized transduction versus generalized transduction. Specialized transduction, as usually defined, explicitly is the carriage of bacterial genes by partially intact *temperate* phages. Specialized transduction as a concern to phage therapy consequently can be avoided via the exclusive use of nontemperate and especially professionally lytic phages for phage therapy. Generalized transduction is the carriage of bacterial genes by phage virions that have failed also to package phage genes. This property is seen especially in phages whose DNA packaging does not involve specific genome-packaging sequences and also for phages whose life cycles do not involve substantial destruction of the genome of the bacterial host in the course of infection. Notwithstanding the frequently expressed concern with the ability of some phages to readily transduce bacterial DNA, not all authors agree that this is of substantial concern regarding phage choice for phage therapy (chapter "► Bacteriophage-Mediated Horizontal Gene Transfer: Transduction").

Summary: Safety Considerations

To avoid negative secondary pharmacodynamic issues during phage therapy – that is, preventing phage-based antibacterials from displaying toxicity and side effects – it is thus crucial under most circumstances to show with reasonably high certainty that a phage is professionally lytic, that it lacks genes that could potentially encode bacterial virulence factors, and also, as some argue, it can be helpful as well to avoid for therapy purposes phages that are able to support generalized transduction. In addition to these issues of phage choice, and especially for parenteral delivery

(Speck and Smithyman 2016), it can be crucial to purify phages away from lysis-associated bacterial toxins such as endotoxin (Boratynski et al. 2004; Gill and Hyman 2010; Łobocka et al. 2014; Pirnay et al. 2015; Szermer-Olearnik and Boratynski 2015). Thus, once phages have been deemed to be obligately lytic – and ideally professionally lytic – as well as free of bacterial virulence factors genes, unable to readily effect the transduction of bacterial genes, and have been appropriately purified, then they generally can be deemed to be safe for phage therapy use (Olszowska-Zaremba et al. 2012). Phages, contrasting novel chemotherapeutics, tend also to be relatively lacking in pharmacologically emergent properties (Curtright and Abedon 2011). Thus, once characterized in vitro there tend to be few emergent safety issues observed following the introduction of new phage types into either animals or the clinic.

Phage Therapy Pharmacology Basics

In pharmacology, the body is considered to consist of both its own tissues *and* associated microbiota. Thus, in the course of treating an infection, the normal functioning of an antimicrobial agent is to affect the body by causing the elimination of a microbial parasite or pathogen. This action can be described as the antimicrobial's primary pharmacodynamic behavior. Secondary pharmacodynamic behavior of an antimicrobial agent, that is, abnormal or at least unintended or undesired functioning, instead is associated with the disruption of normal body tissues, of body metabolism generally, or of normal microbiota, with the latter consisting of commensal or mutualistic microorganisms that contribute to the maintenance of body homeostasis. A major advantage of properly chosen phages as antibacterial agents – especially as delivered topically, in a purified form, or both – is that there is a *low* tendency for these agents to give rise to substantial disruptions of normal body tissues, metabolism, or normal microbiota beyond as caused by the bacterial infection itself. See Fig. 1 for an overview of these and additional pharmacological concepts.

Pharmacokinetics and Pharmacodynamics

The body's impact *on* a drug (pharmacokinetics) generally affects a drug's impact on the body (pharmacodynamics), rather than the other way around. Specifically, a drug needs to reach its target before it can act, and the more drug that reaches its target then typically the stronger its effect, either positive or negative (primary or secondary). From a pharmacological perspective, then, the factors controlling a drug's concentration in the vicinity of its target within a body are a function of the body's impact on that drug. Pharmacokinetics thus impacts pharmacodynamics by impacting what a drug's density will be within the immediate vicinity of target tissues or within the immediate vicinity of microorganisms. Thus: dosing ➔ pharmacokinetics ➔ local drug densities ➔ pharmacodynamics ➔ efficacy or toxicity

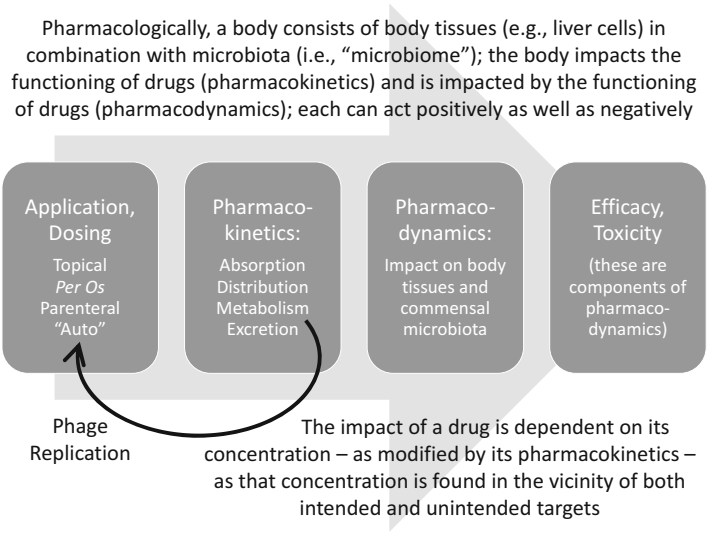

Fig. 1 Overview of pharmacological basics with small emphasis on phage therapy. The latter is found in the lower-left of the figure, that is, "Phage Replication," but the other factors discussed are applicable to phage therapy pharmacology as well as pharmacology more generally. "Application" and "Dosing" are to and of the body, while "Auto" refers to dosing that is generated automatically by the drug in the course of interaction with the body, that is, such as resulting from phage replication (auto dosing). These, along with pharmacokinetics, control medicinal concentrations in specific locations of the body, which in turn affects pharmacodynamics. Generally larger pharmacodynamic effects are observed, both positive and negative, the greater a drug's concentration. In addition, as a general rule we can expect greater positive effects the higher that drug concentrations are found in the immediate vicinity of desired targets and greater negative effects the higher that drug concentrations are in the vicinity of undesired targets. Vis-à-vis "Phage Replication" in the figure, along with the curved arrow, we are explicitly describing "metabolism" as an aspect of phage therapy pharmacokinetics, that is, the chemical modification of a medicinal (phage interactions with host bacteria, in other words, results in chemical modifications of phages otherwise known as a phage infection)

(for the latter, positive/primary pharmacodynamics effects and negative/secondary pharmacodynamic effects, respectively).

Towards distinguishing "pharmacodynamics" from "pharmacokinetics" mnemonically, consider that "dynamics" refers to "change" within a system. Thus, in pharmacology "dynamics" refers to the extent to which a drug impacts, that is, changes a body. Kinetics, by contrast, refers to rates. Pharmacokinetics thus addresses the rate at which effective drug concentrations are reached or, alternatively, are removed, where in pharmacology generally these rates are controlled by the action of the body on a drug.

In the progression, dosing ➔ pharmacokinetics ➔ local drug densities ➔ pharmacodynamics ➔ efficacy or toxicity, the last entry reflects that pharmacodynamics can be differentiated into intended drug modifications of the body, that is, the above-noted primary (i.e., "principal") pharmacodynamics, versus a drug's less

intended and occasionally toxic impact on the body, or secondary ("incidental") pharmacodynamics. In either case, the degree of pharmacodynamic effects is a function of local drug densities and thus is dependent on a drug's pharmacokinetics. It is important to keep in mind, however, that primary effects and secondary effects can result from drug densities that have built up in different locations within the body, with primary pharmacodynamic effects taking place in one location and secondary pharmacodynamic effects potentially taking place in another. The underlying physiological basis for a drug's efficacy thus need not be identical to the underlying physiological basis for a drug's toxicity. This is a situation which can "reward" high drug specificity since the result can be inherently lower occurrences of secondary pharmacodynamic effects, that is, to the extent that a drug interacts physiologically with as few targets within a body as possible and/or builds up in concentration in a minimal number of locations within the body.

Pharmacokinetics in More Detail

Note that drug pharmacokinetics can be distinguished further into what traditionally are described as absorption (with a "b," that is, not "adsorption" with a "d"), distribution, metabolism, and excretion. These, respectively, are drug entrance into the blood (where "absorb" means for something to move into something else), drug movement out of the blood into body tissues (i.e., "distribute" throughout the body), drug chemical modification (recall that "metabolism" refers to chemical reactions), and drug removal from the body ("excretion" meaning to expel or eliminate something as waste). These are important general pharmacological concepts. In the actual practice of phage therapy, however, they typically are referenced slightly differently, as we will consider. For additional discussion of pharmacokinetics and its application to phage therapy, see Abedon and Thomas-Abedon (2010) and Abedon (2014a, 2014b). See Table 2 for general summary of pharmacokinetics.

Conceptualizing Pharmacology

Various concepts of pharmacokinetics (PK), pharmacodynamics (PD), and the impact of pharmacokinetics on pharmacodynamics we represent in a general, simplified form in Fig. 2. Indicated explicitly is the impact of pharmacokinetics on pharmacodynamics. This occurs via the various processes involved in pharmacokinetics, that is, absorption, distribution, metabolism, and excretion (Table 2). The arrows can be interpreted as indicating the PK impact on drug concentrations within specific regions of the body such that various PD effects may be realized. These PD effects can be primary (positive or efficacy) or, instead, secondary (generally negative).

In the following subsections, we elaborate on Fig. 2 towards discussing phage therapy passive treatment, active penetration, and active treatment in greater pharmacological detail. Generally not considered will be specific dosing strategies including topical (which includes directly into the lungs), per os for treatment within the

Table 2 Overview of different facets of pharmacokinetics[a]

PK aspect	General effects	Positive effects	Negative effects
Absorption	Increase in drug concentrations within the blood	Access of drugs to systemic circulation, thereby increasing drug concentrations both within the blood and systemically	Exposure of body systemically to drugs; dilution of drug from that of original dose
Distribution	Increase in drug concentrations outside of the blood (typically as reached from the blood)	Access of drugs to targets as found beyond systemic circulation, thereby increasing drug concentrations within the body outside of the blood, though this can occur with varying degrees of specificity as well as efficiency depending on the drug, target tissues, and circumstances	Exposure of tissues generally to potential drug toxicity; further dilution of drug concentration
Metabolism	Change in drug concentrations due to chemical modification of the drug; for phages this includes aspects of virion adsorption, gene expression, and also bactericidal, bacteriolytic, and reproduction activity	Though positive effects of metabolism on traditional drugs tend to be relatively rare, in certain cases drug activation within the body occurs through chemical modification, thereby increasing drug in situ concentrations; note, for example, phage reproduction	Inactivation of drugs through chemical modification explicitly reduces drug quantities and thereby drug concentration; note, for example, consequences of phage-immune system interactions
Excretion	Change in drug concentrations within the body due to removal of intact drug from the body	Though rare as a positive effect, excretion can result, in certain cases, in drug movement to excretory organs as drug targets, for example, the urinary tract, thereby increasing local drug concentrations	Removal of chemically intact drugs from the body explicitly reduces drug quantities within the body and thereby decreases drug concentration
Penetration	Combining phage-virion absorption, distribution, and to some extent metabolism; this term is not traditionally used as an aspect of pharmacokinetics but nevertheless is useful for discussion of phage therapy pharmacology	As an endpoint, penetration results in phage genome presence within the cytoplasm of target bacteria, with virion adsorption and genome translocation requiring phage metabolism; this is all towards initiation of further phage metabolism	Generally this would be due to movement (penetration) away from target bacteria, that is, such that phage concentrations in the vicinity of target bacteria are not as large as they otherwise could be

[a]Recall that pharmacokinetics refers to the body's impact on a drug, which can be viewed as affecting the rate at which effective drug concentrations are reached within the vicinity of drug targets – both primary and secondary – as well as the rate at which these concentrations will then tend to decline, both as following dosing

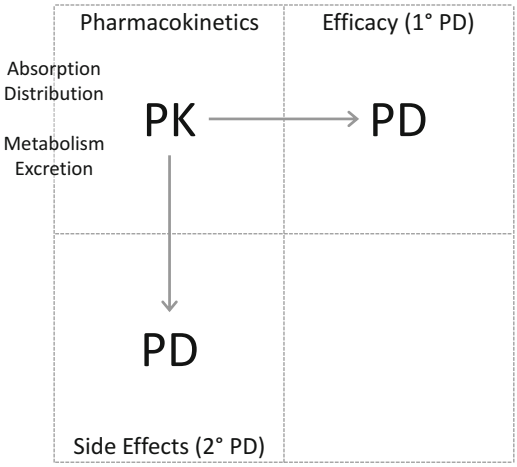

Fig. 2 Pharmacology basics. Pharmacokinetics is abbreviated as "PK" and pharmacodynamics as "PD." Pharmacokinetics is distinguished into absorption, distribution, metabolism, and excretion, as defined in the main text as well as Table 2. Pharmacodynamics are distinguished further into primary (1°) or efficacy-associated pharmacodynamic effects (i.e., intended consequences of drug treatment) and secondary (2°), particularly body-harmful pharmacodynamic effects, that is, especially unintended consequences of drug treatment, i.e., side effects. It is usually the latter which represent pharmacologically emergent properties during drug development – pharmacologically emergent properties are difficult-to-predict properties of drugs that, as a consequence, tend to come to light only in the course of animal or clinical testing of drugs for safety. Unexpected toxicity can derail the development of a drug, though so too can in vitro or animal-testing-associated efficacy that fails to translate, for pharmacokinetic or pharmacodynamic reasons, into sufficient efficacy during clinical trials

gastrointestinal track, per os for systemic treatment, and various more direct means of introducing phages, or drugs, systemically including parenteral (directly into body tissue), where the latter includes intraperitoneally (IP, or into the peritoneum, that is, the abdominal cavity), intramuscular (IM), and subcutaneous (immediately below the skin). For further discussion of such details, see Abedon (2014a). An additional consideration is the potential to arm phages with, for example, homing peptides to enable their localization, following more systemic delivery, to infected tissues in densities which can better ensure efficient eradication of target bacteria (Górski et al. 2015).

Passive Treatment Versus Active Treatment

In Figs. 1 and 2 we presented the interplay between (1) phage dosing, (2) phage therapy pharmacokinetics, and (3) phage therapy pharmacodynamics. In Fig. 3 we add the concepts of passive treatment, active treatment, and what can be described as active penetration. As considered in detail in the following subsections, phages often must penetrate through various barriers or into various compartments in order to reach bacteria. Ideally for phage therapy, this penetration is followed at

the very least by bactericidal activity. Bactericidal activity typically is usually (though not necessarily) associated with bacterial lysis as well as the release of additional phage virions from the lysed bacteria. The result is some degree of auto dosing, that is, in situ increases in phage numbers due to in situ phage replication.

Passive treatment depends solely on phage bactericidal effects. In other words, it requires only a combination of phage-virion penetration to bacteria and subsequent phage chemical modification (i.e., chemical modification as occurs in the course of phage adsorption and subsequent infection; these latter processes are aspects of phage metabolic activity and therefore, pharmacokinetically, of metabolism; Table 2). Active penetration, such as into bacterial biofilms, probably depends, minimally, on phage-induced bacterial lysis, another aspect of phage therapy pharmacokinetics and also as follows virion penetration (see chapter ▶ "Biofilm Applications of Bacteriophages"). Active treatment is dependent on the production of new virions, though also follows a combination of phage penetration and subsequent phage chemical modification. These various issues are discussed further in the following subsections.

The terms "active" and "passive" can be semantically confusing (Abedon and Thomas-Abedon 2010). It may be useful therefore to think of these processes in the following terms:

- Passive treatment is entirely dependent on phages that are supplied from outside of the site of treatment, that is, as via traditional dosing procedures such as by a clinician. It requires some degree of phage metabolic activity to achieve bactericidal effects but not so much metabolic activity that new virions necessarily are produced. It is "passive" in the sense that there is less metabolic activity on the part of infecting phages than is required to produce and release new phage virions.
- Active treatment is dependent on the production of new phage virions as well as bactericidal activity. It is "active" in the sense that it requires "active" production of new phage virions as well as "active" lysis of phage-infected bacteria.
- Active penetration is dependent, presumably, on the lysis of phage-infected bacteria but not necessarily dependent on the production of new phages by those infections. It is "active" because we at least assume that it requires "active" lysis of phage-infected bacteria.
- Mixed passive-active treatment is dependent on bactericidal phage infections – which, as noted, can confusingly be thought of metabolically as more "passive" – but as augmented by "active" phage production by phage-infected bacteria.

"Active" thus implies greater metabolic activity on the part of an infecting phage than "passive." See Fig. 3 as well as Table 3 for summary.

Passive Phage Therapy

Figure 4 serves as a modification of Fig. 2. These modifications make Fig. 4 more specifically a description of phage therapy. Here the term "Penetration" has been

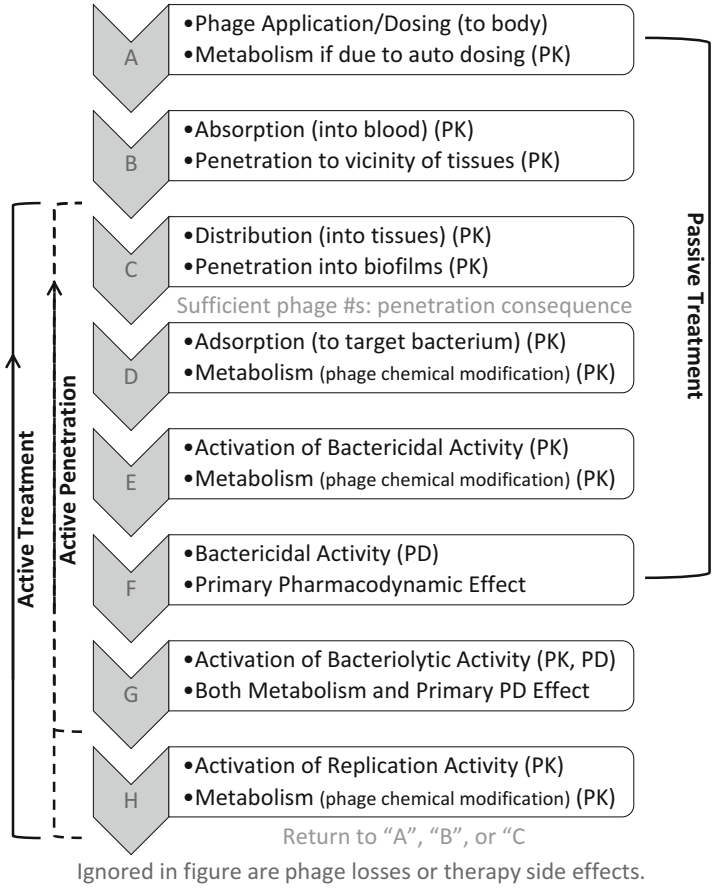

Fig. 3 Overview of phage therapy pharmacokinetics, primary pharmacodynamics, and different treatment approaches. From A through H are various sequential phenomena associated with the phage treatment of bacterial infections. Not all steps are seen in all treatment approaches nor are all steps always necessary for treatment success. Here efficacy is equated with eradication or at least killing of target bacteria and therefore, for phages and phage therapy, is equivalent to bactericidal effects (F). Both penetration (B and C) and metabolism (D and E) refer to various pharmacokinetic phenomena, with some overlap most notably at the point of phage adsorption. Passive treatment, at a minimum, requires only those steps necessary to effect bactericidal activity (A through F). Though in principle passive treatment involves penetration of sufficient numbers (#s) of phages that in situ production of new virions is not required for treatment success (H). Nevertheless, even passive treatment often can benefit from additional dosing (A). Active penetration, which is defined in terms of phage infection-mediated movement, such as into bacterial biofilms, is thought minimally to require bacteriolytic effects (G), but presumably can benefit as well from new virion production and release (H). Active penetration likely can benefit as well from subsequent phage dosing (A). Active treatment is dependent upon active, in situ phage replication (H) to supply sufficient phage numbers to result in treatment success, and it too can benefit from repeated dosing (A). Note that the absorption (B) and distribution (C) steps in particular are thought to contribute to primary pharmacodynamic effects only given systemic rather than more localized phage dosing

Table 3 Summary of phage therapy treatment approaches

Approach	Bactericidal-dependent?	Bacteriolysis-dependent?	Productive[a]-dependent?
Passive treatment	Yes	No	No
Active treatment	Yes	Yes	Yes
Mixed passive-active treatment	Yes	No, but helps	No, but helps
Active penetration	Yes	Yes	Not necessarily

[a]Productive refers to in situ phage-virion production, i.e., auto dosing

used to replace "Absorption" and "Distribution" (Table 2; Fig. 3). This has been done because both of these latter terms refer essentially to the movement of drugs from their point of administration, to patients, to their point of association with target tissues. For phages – whether to a localized bacterial infection, into the midst of a more systemic infection, or instead into bacterial biofilms – such movement often is described in terms that are equivalent to that of penetration, for example, Kutateladze and Adamia (2008). In addition, the term penetration potentially incorporates not just absorption and distribution but also an aspect of the pharmacokinetic concept of metabolism, that is, as associated with virion adsorption since this involves modification of the virion particle. The latter also represents the first step of phage activation towards bactericidal activity. Activation of bactericidal activity typically also involves phage-genome translocation into the bacterial cytoplasm, which at least arguably is also an aspect of phage penetration. Phage gene expression clearly, by contrast, is pharmacokinetically an aspect of metabolism rather than penetration. All three of these steps, however, are typically necessary for phages to effect bactericidal activity – adsorption, translocation, gene expression – and represent chemical or at least physical modification of the adsorbing or infecting phages. In Fig. 4, however, metabolism is not explicitly presented but instead is shown in terms of its consequences, that is, in the form of resulting "Activity," keeping in mind that for passive treatment such activity must by definition be bactericidal.

For a chemotherapeutic, metabolism in combination with excretion typically results in declines in drug presence within bodies. Hence, in Fig. 4 these two pharmacokinetic processes – phage inactivation due to chemical modification and phage removal from the body via excretion – have been replaced simply with "Losses" (note, though, that additional aspects of metabolism in phage therapy are also considered in this figure, as well as subsequent figures). More generally, pharmacokinetic processes control the concentrations of a drug that can reach targets, which is shown here for phages as "Sufficient #s." This is sufficient numbers or densities of phages in the vicinity of target bacteria, particularly phages that have been metabolically "activated" (Abedon 2014b) in the course of phage adsorption of target bacteria in combination with subsequent phage infection of those bacteria. Sufficient numbers of phages is crucial for phage therapy success, or success of phage-mediated bacterial biocontrol more generally (Abedon 2008; Hagens and Loessner 2010; Abedon 2011a, c). Specifically, it is the density of biologically active

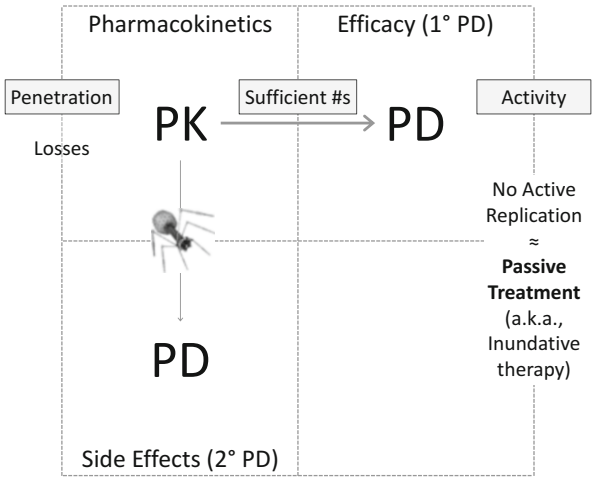

Fig. 4 Phage therapy pharmacology basics. Starting with Fig. 2, four modifications have been made to generate this figure. The first is that generally there is a relatively low association between those phages used in phage therapy and resulting treatment side effects. To explicitly acknowledge this lessening of secondary pharmacodynamic effects, the vertical arrow upon which the phage image is now superposed has been narrowed. More explicitly, this is a description of a lower tendency for well-characterized phages to display pharmacologically emergent properties, a.k.a., unexpected side effects. The second modification is actually a series which includes first the replacement of "Absorption" and "Distribution" simply with "Penetration," which for phages is often a more apt term, and particularly so when referring to topical treatment as well as phage treatment of bacterial biofilms. In addition, "Metabolism" and "Excretion" have been replaced in part with "Losses," that is, *phage* losses. Nonetheless, it is important to keep in mind that phage metabolism also can be associated in a pharmacokinetic sense with gains in phage function. Some of these gains are implicitly associated in the figure with the concept of penetration – as in, not only must phages reach target bacteria to have an impact on those bacteria, but they also must be metabolically activated by those bacteria, in the guise of phage-virion adsorption (which typically requires some degree of virion-particle rearrangement (chapter "► Structure and Function of Bacteriophages")) and also phage-genome translocation into the bacterial cytoplasm. Third, the role of pharmacokinetics in impacting phage concentrations, as required for primary pharmacodynamic effects, is explicitly indicated as contributing to "Sufficient #s," that is sufficient phage numbers, which can be interpreted also as sufficient numbers of bactericidal phage infections (sufficient phage numbers are also required for secondary pharmacodynamic effects, though this is not emphasized in the figure). Lastly, a column for phage "Activity" has been added, referring to the manner of impact of phages on target bacteria, particularly as mediated, pharmacokinetically, by metabolism. In this column, the concept of "passive treatment," that is, "inundative therapy," has been added. This is phage therapy in which phages act equivalently to chemotherapeutic antibacterials in the sense that there is no requirement for any phage *activity* beyond their antibacterial nature, which for lytic phages would inherently be bactericidal. Note nevertheless that phage replication in fact can occur within the context of passive treatment, though if such replication adds to phage antibacterial activity then the result may instead be referred to as a mixed passive-active treatment

agents, that is, titer for phages (Abedon 2016b), that tends to determine the magnitude of pharmacodynamic processes, whether primary or secondary.

There are two additional changes going from Fig. 2 to Fig. 4 that involve pharmacodynamics. The simpler is a narrowing of the arrow pointing downward

to secondary pharmacodynamics, indicating that toxicities/side effects tend to not be a substantial concern with phage therapy (to indicate that this is a phage-associated property, we have placed the image of a phage virion, that of phage T4, over this arrow). The second point, as also relevant to phage therapy pharmacodynamics, is that the extent of phage primary pharmacodynamic activity can be a function of the degree to which phages have been pharmacokinetically activated, particularly metabolically activated, hence addition of the term, "Activity," as found in the box to the right in Fig. 4. Under this heading in this figure, the pharmacokinetically activated pharmacodynamic phage action is bactericidal. Phages thus are relatively safe (few secondary pharmacodynamic effects) and in fact that relative safety stems to a fair extent from this requirement that they be metabolically activated, particularly in the course of their intimate as well as irreversible interaction with target bacteria, prior to displaying cytotoxic activity.

A "Productive" phage infection is one that does *not* result in either lysogeny, abortive infection, or lysis from without (Abedon 2011d). Confusingly, vis-à-vis phage therapy pharmacology, a productive phage infection also can be described as "Active" (above). That is, with an "Active" infection, phage progeny will be produced and virions released relatively soon after phage adsorption of a target bacterium. If active or productive phage infections are *not* required for successful phage therapy, then a therapy may be described as an inundative treatment or, equivalently, as a passive treatment (Payne et al. 2000; Payne and Jansen 2001). A complication on this latter idea, however, is that it is possible for passive treatment to take place even given active phage replication. Nevertheless, so long as that replication is not absolutely required to achieve therapeutic success, and in situ production of virions also does not otherwise improve the rapidity or likelihood of treatment success – and even if it is difficult to inundate target bacteria with phages using a single phage dose – then that treatment still can be described as passive/inundative. Passive treatment requires only a minimal degree of pharmacokinetically associated metabolism: activation of phage bactericidal activity.

Active Penetration

In addition to phage action against individual bacteria, we can also consider phage penetration into bacterial biofilms (chapter "► Biofilm Applications of Bacteriophages"). This penetration at a minimum may require phage-induced bacterial lysis versus solely bacteria killing (chapter ► "Phage Infection and Lysis"). Lysis-induced stripping away of bacteria, such as from the surface of bacterial microcolonies or biofilms, can be described as at least one aspect of phage "active penetration" into those structures (Abedon and Thomas-Abedon 2010). This represents a pharmacodynamic impact (bactericidal activity) that is followed by yet another form of pharmacokinetic metabolism, in this case bacterium-associated biochemical modification of the phage "drug" that leads to a phage-induced bacterial lysis. In between these two pharmacokinetic processes is the pharmacodynamic phage killing of target bacteria. This phage-induced bacterial lysis is presented as

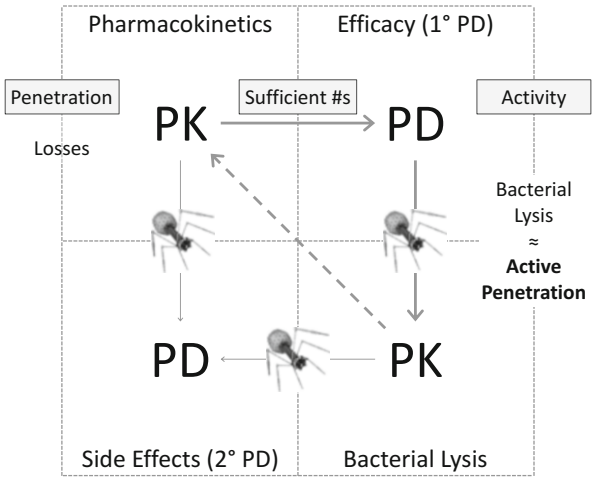

Fig. 5 Pharmacology of phage-therapy active penetration. Modifying Fig. 4, this figure incorporates the pharmacokinetic property of phage-induced bacterial lysis. Pharmacokinetically, this is a consequence of metabolism in that it represents chemical modification of phages as drug equivalents, in this case phage-directed but target bacterium-associated conversion of a bactericidal infection to a bacteriolytic one. The resulting lysis can contribute to what can be described as an active penetration, particularly of phages into bacterial biofilms, a phenomenon which at a minimum likely involves phage-induced bacterial lysis. This lysis might strip away outer layers of biofilm bacteria so that phages can either gain access to underlying bacteria or so that previously underlying bacteria can gain better access to nutrients and thereby change physiologically so that they are better able to support bactericidal and/or bacteriolytic phage infections. The added diagonal line indicates the potential for bacteriolytic phage infections to supply new virions, which could contribute to further phage penetration into biofilms. The diagonal line is dashed, however, to indicate that active penetration in principle may not require in situ phage production as phages might be supplied to previously underlying bacteria instead via standard dosing with additional phage virions. Lastly, bacterial lysis can impact the body more generally, particularly as a consequence of the release of potentially toxic bacterial lysis products. Consequently, a second horizontal arrow has been added (bottom), though for phage therapy purposes the resulting side effects in most instances generally have not been found to be severe, hence the narrow gauge of the resulting arrow

the vertical arrow found on the right of Fig. 5. Thus, pharmacokinetics gives rise to both phage bactericidal and lytic activity, while these processes in turn give rise, respectively, to bacteria killing and potentially also to further phage penetration into bacterial biofilms. The "active" aspect of "active penetration," as noted, refers to phage-induced bacterial lysis as stemming from some approximation of "active" phage replication.

Bacterial lysis may facilitate further phage penetration into biofilms, hence the dashed diagonal arrow added to Fig. 5, connecting the lower-right quadrant with the upper-left. This line is dashed because lysis would contribute only *indirectly* to further phage penetration into biofilms, or into bacterial microcolonies, should phage release happen to not follow phage-induced bacterial lysis, for example, as associated with abortive infections rather than phage-productive ones. Following bacterial

death, bacteria underlying the biofilm surface may become more physically available for virion adsorption, and/or nutrients (or oxygen) may become more physically accessible, thereby contributing to improvement in the physiological potential for those bacteria to support phage infections. Nonetheless, unless lysed bacteria supply new phages then phage infections themselves are not contributing *directly* to subsequent phage penetration. That is, theoretically subsequently penetrating phage virions might instead be supplied exogenously via further phage dosing rather than supplied in situ via auto dosing. Thus, this process of active penetration can be viewed as requiring greater antibacterial action than solely bactericidal activity, but at a minimum this *additional* activity must be phage-induced bacterial lysis – we suggest – rather than explicitly requiring virion production by those same infections as well. Purely passive treatment mediated by bactericidal but not replication-competent phages, in other words, could conceivably be employed to destroy bacterial microcolonies or biofilms, but we speculate that this destruction may be more efficiently achieved if resulting phage infections are associated not just with bacterial killing but with lysis of targeted bacteria as well. To our knowledge, however, that hypothesis has not yet been tested.

It is important to emphasize that phage induced bacterial lysis in the absence of virion release, though possible (e.g., as a form of abortive phage infection), in fact is probably not typical for phage infections during phage therapy. Instead we consider this possibility here predominately to illustrate the point that in principle phages during phage therapy may be able to actively penetrate into bacterial biofilms without necessarily productively infecting the bacteria that they are attacking (i.e., producing new phage virions). Successful active penetration in the absence of active virion production presumably could occur, however, only given further phage application, that is, repeated dosing in the course of treating biofilm-associated bacterial infections. Such repeated dosing may be necessary even with in situ phage production if burst sizes are small or, instead, if phages have a low potential to immediately productively infect underlying bacteria, that is, such as if those bacteria in fact are in a stationary phase-like state (Abedon 2015c, 2017d; Bryan et al. 2016).

Phage-induced bacterial lysis could potentially result in side effects that are in addition to phage application alone. As a consequence, we have added the lower horizontal arrow to Fig. 5. In practice, however, substantial side effects have not been observed with modern phage therapy, hence the narrowness of that arrow as well as the superimposed image to indicate that this low potential for side effects is relatively phage specific. It is important as well to point out that phages are not the only bacteriolytic antibacterial agents that can be employed against bacterial infections, as cell wall disrupting and therefore lysis-inducing antibiotics are common. It is possible, however, that phages are better equipped in terms of their ecological properties to penetrate into biofilms than necessarily are naturally occurring antibiotics (Abedon 2015b), though phages likely still are limited in that ability (Abedon 2016a, 2017d), thereby necessitating multiple dosing in the actual practice of phage therapy against biofilms and/or the use of biofilm-disrupting agents (such as extracellular polymeric substance-disrupting depolymerase enzymes). For more on *phage*

interactions with bacterial biofilms, microcolonies, or cellular arrangements including within a phage therapy context, see Abedon (2011b, 2012c), (2015c), (2016a), (2017a), Brussow (2013), Fan et al. (2013), Harper et al. (2014), Parasion et al. (2014), Sillankorva and Azeredo (2014), Chan and Abedon (2015), Gutierrez et al. (2016), Khalifa et al. (2016), and Motlagh et al. (2016) as well as (chapter "▶ Biofilm Applications of Bacteriophages").

Active Phage Therapy

Active penetration of phages into bacterial biofilms or microcolonies potentially involves – or indeed requires – phage-induced bacterial lysis, as presented in Fig. 5. In addition, an active *treatment*, as presented in Fig. 6, is also possible. With passive treatment, the necessary metabolism aspect of pharmacokinetics is limited to the transformation of a relatively inert phage particle into a bactericidal phage infection. With active penetration, metabolism at a minimum likely must involve the transformation of a phage virion into an infection possessing both bactericidal and bacteriolytic activities. With active treatment, by contrast, phage association with target bacteria must result not only in the metabolic development of both bactericidal and bacteriolytic activity, but productive phage infection as well, that is, the production of new phage virions. Active treatment, in other words, is phage treatment that requires in situ amplification of phage numbers, that is, auto dosing. This activity, pharmacokinetically, is also a consequence of metabolism, that is, further chemical modification of the initially dosed phage "Drug."

This aspect of phage therapy pharmacokinetics feeds back into penetrative aspects as well as to subsequent phage losses. This is indicated in Fig. 6 as a diagonal arrow, now solid as well as overlain with a phage image because by necessity, with active treatment, new phage virions are being supplied in situ. So long as the distances virions must travel to reach new target bacteria are *small*, however, then neither phage losses nor phage penetration, particularly in terms of absorption and distribution, may play large, further roles in impacting "Sufficient #s," this despite auto dosing having a positive impact on phage densities and thereby on phage therapy efficacy at more local scales. In other words, active treatment, once it is initiated locally, will tend to continue to act predominantly locally, with local production of additional phages resulting in further local reductions in numbers of target bacteria, which in turn will give rise to further local phage production (Abedon 2017a). As in situ amplification of phage numbers is not thought to directly result in substantial increases in phage therapy side effects, the bottom, horizontal arrow in Fig. 6 remains thin in this figure as well.

Summarizing Phage Therapy Pharmacological Phenomena

In terms of activity, a phage infection can, for instance, be *not* bactericidal. One example of nonbactericidal phage infections is restrictive infections, such as

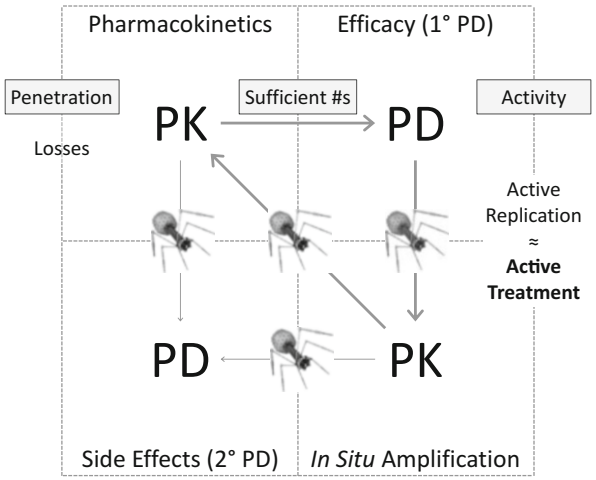

Fig. 6 Pharmacology of active phage therapy. With active treatment, the "PK" found in the lower-right quadrant refers not just to the metabolic conversion (metabolism) of a particle to a lytic infection but to a phage-productive one as well. The resulting phage particles, as represented by the diagonal arrow, are then subject to distribution, to further metabolism (both activation and inactivation), and potentially also to excretion. Ideally, so far as phage therapy is concerned, this in situ amplification results in an increase in phage density that, in turn, results in a greater likelihood of phage adsorption to target bacteria and thereby greater phage therapy efficacy. This process is termed active treatment or active therapy and contrasts with passive treatment (Fig. 4), but can also work in conjunction with lysis towards an active penetration into bacterial biofilms (Fig. 5)

resulting from the action of restriction endonucleases or, alternatively, as mediated by bacterial CRISPR-Cas systems (Hyman and Abedon 2010; Labrie et al. 2010; Abedon 2012a; Dy et al. 2014; Seed 2015). In this case, phage therapy can fail not because there is insufficient phage penetration to target bacteria, or insufficient absolute phage numbers, but instead due to insufficient phage bactericidal activity. Alternatively, it is possible for phage infections to be nonproductive but still bactericidal, which can be described as forms of abortive infections. Such abortive infections, as they result in bacterial death, can support passive therapy/inundative treatment (Fig. 4). Abortive infections as so defined may or may not also result in bacterial lysis or, alternatively, can result in bacterial lysis without substantial infection, that is, as seen with the phenomenon known as lysis from without (Abedon 2011d). These infections, if they are bacteriolytic but not necessarily phage productive, may be able to support an active penetration of phages into biofilms (Fig. 5), though if so constrained then active penetration may occur only if additional phages are supplied via repeated phage dosing. An ability of phage infections to both lyse bacteria and release phage progeny, however, may result in more robust penetration into bacterial biofilms. Such a process, to the extent that it is dependent on in situ phage production, would qualify as both an active penetration and an active treatment (Fig. 6).

We summarize in Fig. 7 these three levels of increasing phage activity that can play important though distinct roles in phage therapy success. Thus, there are bactericidal infections (a minimum level of activity necessary to achieve some degree of efficacy), bacteriolytic infections (a minimum level of activity necessary to achieve active penetration), and productive infections (a minimum level of activity necessary to support active treatment). To achieve any of these ends, insufficiencies in phage penetration, as phage movement towards target bacteria,

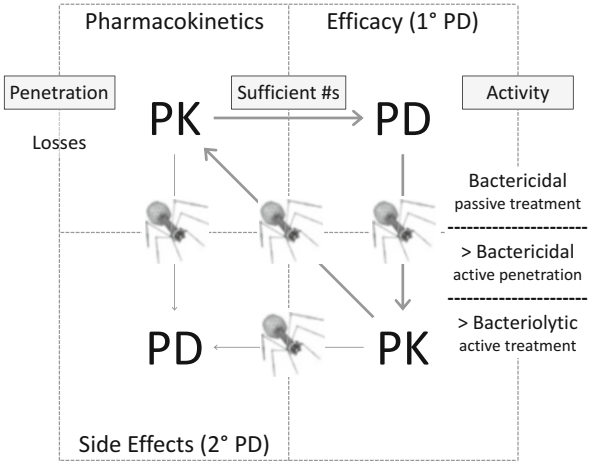

Fig. 7 Comparing passive treatment, active penetration, and active treatment in terms of phage-infection activity. Bactericidal but not bacteriolytic phage activity ("Bactericidal" but not "> Bactericidal") corresponds to passive treatment (see also Fig. 4; the ">" symbol in this figure means "greater than," for example, "greater than just bactericidal activity" or "greater than *just* bacteriolytic activity"). Bacteriolytic but not phage-productive activity ("> bactericidal" but not "> Bacteriolytic") corresponds to what can represent a minimum of activity necessary for phages to effect an active penetration into bacterial biofilms (see also Fig. 5). For bactericidal along with bacteriolytic activity to be effective in the course of phage therapy, in the absence of productive phage infection (i.e., lacking in active infection/auto dosing/secondary infection), then the phages required to infect and kill additional bacteria must be supplied from outside of the area under treatment, particularly via traditional approaches to dosing. That is, in the absence of in situ amplification in phage numbers or instead given only weak amplification, then multiple, repeated phage dosing may be required to achieve eradication of target bacteria. Fully phage productive infections ("> Bacteriolytic," meaning more than simply bacteriolytic) form the basis for active treatment, which explicitly involves the generation of secondary phage infections, that is, phage infections by phage particles that have been generated in situ, i.e., as following active phage replication (auto dosing; see also Fig. 6). This generation of phage particles in active treatment leads directly to secondary infections, which in the figure are indicated via the diagonal arrow superimposed with a phage particle. Note that active penetration and even otherwise passive therapy, that is, inundative treatment, may still be aided by in situ phage amplification/secondary infection, so-called mixed passive-active treatment. Furthermore, active treatment is absolutely dependent on sufficient in situ phage amplification for treatment success to occur. Such treatment thus can fail if phage burst sizes are insufficient, if phage inactivation rates are too high, or instead if bacterial targets are not sufficiently plentiful to support in situ phage population growth to what essentially must be inundatory phage densities for phage therapy to be successful

and otherwise insufficiencies in delivery of adequate phage numbers to target bacteria may potentially be augmented via multiple, repeated phage dosing. Note that phage infections that are a direct consequence of such dosing can be described as primary phage infections, whereas phage infections that occur only following in situ phage replication, a.k.a., active replication or auto dosing, instead can be described as *secondary* phage infections (Abedon 2015a). With secondary infection defined explicitly in this latter, epidemiological sense (Payne et al. 2000; Payne and Jansen 2001; Wei and Krone 2005), then active treatment in phage therapy can be defined as a form of treatment that, as a consequence of dosing with fewer phages than would be necessary to achieve passive treatment, therefore happens to require secondary phage infection.

Phage Interactions with Immune Systems

Bacteriophage interactions with mammalian immune systems have both pharmacokinetic and pharmacodynamic consequences for phage therapy. There are two major reasons for this. First, in terms of pharmacokinetics, with therapeutic application of phages into a living organism, the immune system can serve as a crucial component of a phage's environment (Górski et al. 2012; Hodyra-Stefaniak et al. 2015). Indeed, for the phage as essentially an "invading" microorganism, the immune system represents the primary means by which body tissues, contrasting microflora, impact phages – the "immune systems" of target bacteria are also relevant, though beyond the scope of this chapter; see instead Abedon (2012a) for discussion of the bacteria "immunity" against phages. These interactions with the body's immune system, from a pharmacokinetic perspective, largely result in phage losses, leading to virion inactivation (pharmacokinetically a consequence of metabolism). Phage elimination from the body as whole virions, such as via the kidneys, represents by contrast a pharmacokinetic mechanism of excretion, but generally this occurs absent immune system function. The phage, in situ, thus is constantly in contact with elements that compose the body environment, and these elements, in our own bodies, are dominated – in terms of body-phage interactions – especially by the actions of the mammalian immune system.

The second impact of phage interactions with immune systems during phage therapy stems from the importance of immunity for the health and homeostasis of organisms. This role involves multiple components and requires substantial flexibility to a wide range of possible reactions and interactions. The responses of immune systems, as a consequence, are not solely directed against foreign objects, but also towards the immune system itself, resulting in modifications of its own functions, so-called immune system modulation (immunomodulation). The immune system in particular can react to stimuli, including of the body's own making, that can have positive as well as negative effects on homeostasis. Such stimuli can be provided by bacteriophages as well, thereby contributing to phage-associated pharmacodynamics. As with phage antibacterial activity, these pharmacodynamic effects can be both positive and negative with regard to overall body health, but explicitly are

pharmacodynamic because they represent phage impact on the body rather than (strictly) body impact on the phage. These effects can also occur independently of phage interaction with target bacteria and thereby are to a degree independent of phage auto dosing, though phage amplification in situ can result in enhanced phage immunomodulatory effects, as too can phage modification of target bacteria, particularly in terms of bacterial lysis.

Overview of Immunity

Although the first studies on the interactions between bacteriophages and the immune system were conducted by Felix d'Hérelle shortly after the discovery of bacteriophages (Górski et al. 2012), we still do not have a complete picture of all elements and factors that play roles in phage-mediated modulation of immunity. The incompleteness of this picture stems in part from immune responses to phages resulting from the action of a variety of elements of the immune system as well as both intraspecific and interspecific variation in immune systems functions, but also because different phages will interact with the different components of immune systems in different ways. The most general classification of those immune system elements, however, comprise innate versus adaptive immune responses, that is, innate immunity and adaptive immunity, with major differences existing between these two types both generally and in terms of phage-immune system interactions.

Innate immunity represents nonspecific reactions to foreign objects that are usually recognized by universal molecular patterns associated with pathogens (Pathogen-Associated Molecular Patterns, or PAMPs), for example, bacterial DNA (CpG), peptidoglycan, or lipopolysaccharide. Innate immunity reaction to detected pathogens is related to inflammation, which is one of the first responses of the immune system to infection. This type of response engages a wide collection of leukocyte types, that is, white blood cells. These are mainly the phagocytes: monocytes, macrophages, neutrophils, tissue dendritic cells, and mast cells, but also eosinophils, basophils, and natural killer (NK) cells. Collectively, these cells are capable of recognizing and eliminating pathogens. They are also important mediators in the activation of the adaptive immune system. Acellular and therefore humoral elements of innate immunity are represented mainly by the serum complement system (Rus et al. 2005; Kawai and Akira 2006; Medzhitov 2007).

Adaptive immunity responses are executed by specific elements of the immune system that are distinct from but nevertheless communicate extensively with innate aspects of the immune system. "Adaptive" generally refers to the requirement of adaptive immunity that it must first be "trained" in order to gain an ability to robustly recognize and, especially, respond to "foreign" invaders of the body. The adaptive immune system further asserts immunological memory, that is, retention of enhanced abilities to recognize and respond to these invaders. Adaptive immunity is specifically directed to selected objects, often involving what can be described as at least an approximation of lock-and-key matching. This matching involves spatial

and chemical complementarity between the antigens (more precisely, the epitopes) of foreign substances, on the one hand, and specific immune system molecules on the other. The latter consist particularly of various highly diverse receptors associated with lymphocytes, which are the key adaptive-immunity effecting leukocytes, along with antibodies. As a consequence of the resulting specificity of interactions between antigens and these immune system molecules, adaptive immunity is also described as specific immunity.

Major types of lymphocytes are B cells and T cells. B cells are involved in the specific humoral immune responses, that is, in the production of antibodies. T cells are involved in cell-mediated specific immune response (Janeway et al. 2005; Pancer and Cooper 2006). Importantly, innate immunity involving nonlymphocyte leukocytes and adaptive immunity elements involving B and T lymphocytes are tightly linked into one, consistent system: they cooperate, they stimulate, and/or they control each other, depending on individual situation and needs. Here we consider these complementary interactions with regard to the impact of bacteriophages on immune system functioning, especially with regard to phage virions.

Adaptive Immunity

Specific antibodies to virions have been by far the most often investigated and acknowledged part of mammalian immunity engaged in immune reactions to phages, with studies dating back to 1920s, for example, Muckenfuss (1928). Serological cross-reaction represented the earliest criteria for bacteriophage classification, beside host range, particularly of phages into serologically related groups (Stent 1963). Since the intensity of immunological reactions decreases in correlation with morphological and biological differences between phages, serological classification greatly improved upon the host range-based classification of bacteriophages. Those early studies were based on a passive agglutination test for determination of neutralizing activity of bacteriophages by serum. The ability of bacteriophages to induce specific antibodies was also one of the earlier useful properties of these viruses. More recently, phage immunogenicity has been employed in medicine as a test for the immunocompetence of otherwise immunodeficient patients. In this test, HIV-infected patients can be monitored for the ability of T cells to provide help to B cells in antibody production, amplification, and isotype switching after phiX174 phage immunization (Fogelman et al. 2000).

Antibody titer is anticipated to grow as a result of repeated medical application of phages, for example, as during phage therapy. The presence of antibodies within an individual's serum against a specific bacteriophage, however, may result from previous, natural contact. These are so-called natural antibodies (Hajek 1967). Since phage-specific antibodies can cross-react with related phages, natural antibodies do not need to be induced by exactly the same phage strain but instead can result from previous contact with one or more related, especially serologically similar phage. In practical terms, natural antiphage antibodies may indicate that an individual has undergone a bacterial infection caused by a given host bacterium, for

example, patients with staphylococcal infections have been found to demonstrate higher titers of antibodies specific to staphylococcal phages in comparison to healthy blood donors (Kucharewicz-Krukowska and Slopek 1987). The other inducers of natural antibodies can potentially be environmental phages that enter the body with food, water, etc., and/or which propagate on symbiotic bacteria such as those present in the gut or other parts of the body. In most cases, however, the true stimulator of the production of antiphage natural antibodies remains obscure. The frequency of natural antibodies in human populations is also difficult to estimate and most probably depends on many factors, including phage type and individual characteristics of investigated patients, including their individual microbiome. The frequency of antibodies specific to staphylococcal phages in a group of patients before phage therapy, that is, suffering from staphylococcal infections, was reported as approximately 23% (Kucharewicz-Krukowska and Slopek 1987). This high frequency suggests a role as well for gut-associated phages in stimulating antibody responses, and indeed the frequency of antibodies specific to T4 coliphages reached 81% in populations of healthy volunteers (Dąbrowska et al. 2014).

Animal models have demonstrated that systemic administration of phages can effectively induce specific antibodies (Huff et al. 2010; Smith et al. 1987). Among the main classes of antibodies – IgM, IgG, IgA, IgE, and IgD – only IgM, IgG, and IgA were demonstrated to be induced by bacteriophages. The schema of antibody production in response to a challenge with a phage, however, seems very typical for antigen challenge generally: maximum IgM production can be observed within 5–10 days after the challenge and this is followed by the increase of IgG that tends to persist for a longer time (Hodyra-Stefaniak et al. 2015), see Fig. 8. Though not so far demonstrated to be associated with humoral responses to phage virions, one should not exclude the possibility that phage-specific IgE and IgD will be detected in future studies, particularly under conditions appropriate to induce these classes of antibodies.

Not surprising, given their diversity, individual phage types differ in their antigenicity. It is unclear to what degree differences in phage antigenicity "map" onto measures of phage diversity, however, as not even moderately comprehensive analyses have been undertaken. Nevertheless, individual structural proteins forming phage particles can differ strongly in their ability to induce specific antibodies (Dąbrowska et al. 2014). This is consistent with the expectation that the molecular composition of individual phage capsid proteins should determine much of phage reactivity with the immune system. It may have further implications for the possibility to select, or even to construct phages with the molecular composition of a desired immune reactivity, such as construction of phages that are less "visible" to the immune system so as to be less efficient in induction of antibodies specific to the phage and thus neutralized less rapidly (note that this idea is distinct from modification of phage interaction with innate immunity, which is covered below). Decrease in the pace of neutralization can also be achieved by a chemical "cover," for example, PEGylation (Goodridge 2010). Alternatively, otherwise similar phages with dissimilar serological properties in principle may be constructed to allow phage

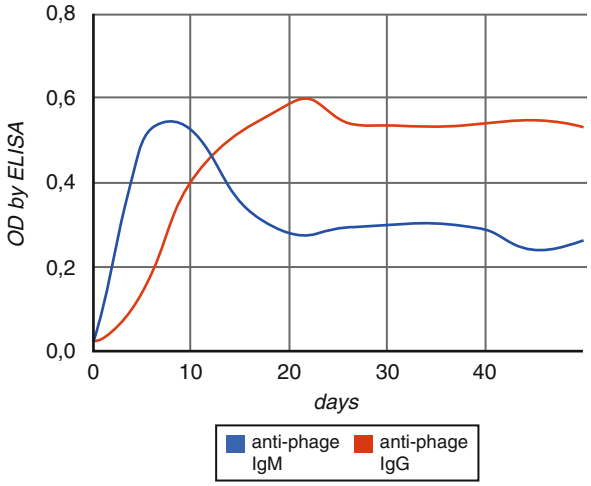

Fig. 8 Induction of specific IgM and IgG antibodies in mice challenged with phage parenterally. Mice were injected subcutaneously with a Myoviridae phage (F8), 10^{10} plaque-forming units (chapter ▶ "Detection of Bacteriophages: Phage Plaques") per mouse on day 0 and again on day 22. Antibodies specific to the phage were detected in blood by ELISA. Observed is a typical increase of IgM as a primary response which is followed by a typical increase of IgG (secondary response). Figure provided by K.D as based on unpublished data

switching during treatments should phage interaction within immune systems come to interfere with treatment success.

There have been many antiphage antibody studies over the decades, but other types of specific immune responses to phages have been much less explored or appreciated. Specific cellular response to phages would engage T lymphocytes, since they play a central role in cell-mediated immunity. These lymphocytes are distinguished from other immunological cells by the presence of a T-cell receptor (TCR) on their surfaces, which is responsible for recognizing antigens bound to major histocompatibility complex molecules as found on vertebrate animal cell surfaces. Interestingly, the interaction between TCR and an antigen is of relatively low affinity and low specificity compared to antibodies produced by B cells; one TCR can recognize many antigens and conversely, T cells capable of recognizing foreign antigens develop via clonal selection before they can act as specific immunological cells. Langbeheim et al. (1978) provides some indication of the ability of a phage (MS-2) to induce a cellular response. It was evaluated in presensitized mice, by intradermal injection of the test antigens, resulting in local erythema and induration. Strong in vivo reactions to the injected phages were reported. Induction of a cellular response was also determined in vitro by measuring proliferative responses of lymph node cells to the test antigens. Srivastava et al. (2004), by contrast, demonstrated that the clearance of phages in the blood was similar in normal (control) and T-cell-deficient mouse strain C57BL/6 J-Hfh11^{nu}, implying that T cells in fact did not play a significant role in the inactivation of these phages in vivo.

Innate Immunity

Contrasting the predominant role of humoral immunity via antibodies in adaptive immune responses to phages, phagocytes are the major players in innate immunity to phages. Very early studies showed that the reticuloendothelial system of the liver and, predominantly, the spleen – a.k.a., the mononuclear phagocyte system – filters bacteriophages from circulation. Bacteriophages are phagocytized by Kupffer cells which are stellate macrophages present in the liver, splenocytes (mainly macrophages), or by peritoneal macrophages. Neutrophils have also been shown to be capable of engulfing phages (Kantoch 1958; Inchley 1969; Geier et al. 1973; Górski et al. 2012).

It has been demonstrated that individual characteristics of phage capsids may determine phage reactivity not just with adaptive immunity but also with nonspecific, that is, innate parts of the mammalian immunity. Merril et al. (1996) used a serial passage scheme to isolate phage mutants possessing reduced sensitivity to filtration and elimination from the blood. These mutants were able to remain in the circulatory system for longer periods of time, and it was shown that substitution of a single amino acid in the major capsid protein was enough to achieve this "long circulating" phenotype. Sokoloff et al. (2000) engineered phage particles to achieve equivalent long-circulating phenotypes by introducing peptides with C-terminal lysine (rat model), arginine (rat model), or tyrosine (humans); these modifications resulted in lower sensitivity to antibody-dependent complement-mediated neutralization. These examples suggest again that phage reactivity with the immune system can be modified by changes in the molecular composition of phage particles.

Phages have been shown to interact with nonadaptive humoral elements of innate immunity as well. The complement system, for example, consists of a number of small proteins that form a cascade leading to the activation of cell-killing membrane attack complexes as well as other antipathogen functions. This complex is targeted to invasive bacteria as well as to viruses (Perreau et al. 2007). Although bacteriophage particles are *substantially* unlike most eukaryotic viruses, particularly in that most phages lack a phospholipid envelope derived from host cells, these "naked" phage virions nevertheless seem to be sensitive to actions stemming from the complement system. The most probable antiphage action of complement therefore likely requires antigen-antibody complexes (immune complexes) for activation, implying a link between nonspecific and specific immune response to phages (Sokoloff et al. 2000; Dąbrowska et al. 2014; Hodyra-Stefaniak et al. 2015).

As noted, the typical mechanisms of nonspecific immunity center on inflammation, which is a complex response that involves immune cells, blood vessels, and molecular mediators (cytokines). Cytokines are small signaling proteins and some of them are typical markers of inflammation, for example, IFN-gamma, TNF-alpha, Il-1, and Il-6. These markers rapidly increase in prevalence in response to the presence of PAMPs as indicators of pathogen invasion. In contrast to pathogens, including pathogenic viruses, phages do not induce inflammation markers in vivo. This corresponds to the fact that phages do not induce production of reactive oxygen species (ROS), which is a characteristic marker of activation of phagocytes

(Miernikiewicz et al. 2013; Park et al. 2014). In that respect, bacteriophages should be discriminated from crude phage lysates that contain multiple PAMPs released during bacterial lysis. These PAMPs include peptidoglycan, lipopolysaccharide, and other bacteria-associated molecules and have strong pro-inflammatory activity. Since these PAMPs are typical bacterial lysis products, highly purified phage particles would lack such inflammatory characteristics (Miernikiewicz et al. 2013). It should be noted, however, that inflammatory markers (CRP, sedimentation rate, leukocytosis) in patients treated with phage lysates may decrease significantly during the treatment (Międzybrodzki et al. 2009).

Effects of Immune Response on Phage Pharmacokinetics

In the case of organisms and materials that are foreign to the body, such as phages, the major role of the mammalian immune response is neutralization and/or removal. Thus, immune responses against phage particles generally will have a limiting effect on phage activity in vivo. Considering phage therapy pharmacokinetics in mammals, the relationship of variables representing concentrations of active phages tends to be inversely associated with variables representing the intensity of antiphage immune responses (Dąbrowska et al. 2014). These are specific antibodies, phagocyte activity, and production or activation of other immunological elements that are able to neutralize phage particles. These elements may differ in their individual contribution to the intensity of the neutralization, and this contribution may also significantly differ for different phages or circumstances.

Confirmation of the ability of specific antibodies to negatively impact phage functionality comes from their impact on phage antibacterial potential in vivo (Dąbrowska et al. 2014, Hodyra-Stefaniak et al. 2015). This is in line with the role of antibody-producing B cells in phage-T7 clearance from the blood of mice as was highlighted by Srivastava et al. (2004). The presence of antiphage antibodies in serum has been postulated to be an important factor potentially limiting phage therapy effectiveness (Carlton 1999; Sulakvelidze et al. 2001). Often the fact that antibodies are demonstrably capable of blocking phage antibacterial activity in vitro is interpreted as a *strong* argument that they can neutralize phages in vivo. Accordingly, animal models usually show that preimmunization with a phage can block phage therapeutic activity (Smith et al. 1987; Huff et al. 2010).

Carlton (1999) suggested that negative impacts of immune-system inactivation of phage virions might be overcome by applying more phages. A recent report of Łusiak-Szelachowska et al. (2014), however, shows that simple conclusions on the effect of immunization on the efficacy of phage therapy may be overly simplistic. Antiphage activity in patients' sera depended on the route of phage administration and phage type, and high antiphage activity of sera during phage therapy was observed in only 12.3% patients receiving phages. Moreover, the induction of antiphage activity in sera during or after phage therapy did not interfere with the antibacterial efficacy of treatments. Also, the first small safety test of T4 phages applied orally to humans as reported by Bruttin and Brüssow (2005) revealed

no T4-specific antibodies in the serum of subjects. Comparison of phage therapy studies in animals – which often conclude with significant increases in antiphage antibodies – to human clinical studies reveals that in most cases phage doses (total plaque-forming units per kg body weight) as applied to animals were much higher than those tested in humans (Letarov et al. 2010; Majewska et al. 2015). Notably, the routes of phage administration were also usually different in humans versus animals: oral or topical in humans, but parenteral (intraperitoneal, intravenous, subcutaneous, and intramuscular injections) in animals. These differences may result in discouraging conclusions from animal studies that, in fact, do not fully correspond to reports from human studies (Międzybrodzki et al. 2012; Łusiak-Szelachowska et al. 2014).

The second powerful way that phages can be inactivated within bodies, as noted above, is via phagocytosis, which has been demonstrated as playing an important role in affecting circulating bacteriophages in vivo. Phagocytes engulf phage particles as foreign objects and the process seems to be rapid, that is, within minutes of phage delivery to the blood. Phage disruption inside phagocytic cells usually takes place within 15 min to 2 h (Aronow et al. 1964; Kantoch 1958). Two studies reported that neither macrophages nor neutrophils are substantially involved in the clearance of phages from blood after systemic administration (mouse models) (Srivastava et al. 2004; Uchiyama et al. 2009; Górski et al. 2012), but other studies suggest that phagocytes may have greater capabilities to neutralize phages when these cells are activated, for example, by typical PAMPs during bacterial infection (Hodyra-Stefaniak et al. 2015). Phage therapy also has been found to not impair the killing ability of patients' granulocytes and monocytes and may even upregulate this ability (Jończyk-Matysiak et al. 2015).

In conclusion, with regard to immune system impact on phage pharmacokinetics, that is, the ability of phages to reach and sustain sufficient densities within the vicinity of target bacteria, phage sensitivity to immune system action probably also depends on specific conditions, for example, particularly phage dose. For T2 phages an experimental "cut-off value" of 10^7 plaque-forming units/ml was observed, that is, under this value the effect of phagocytosis on phage concentration was not detected (Kantoch 1961). Other important factors can be the immune status of patients (or model animals), which can be of key importance particularly in terms of understanding immune system impact on phage prevalence and persistence within animals. Differences in properties are likely to be seen between dissimilar phage types, however, greatly complicating the study of phage-immune system interaction.

Relation of Immune Response to Phage Pharmacodynamics

Nonbactericidal pharmacodynamic effects of bacteriophages in mammals can be related to immunomodulatory effects of phage preparations. There are a few distinct aspects that need to be considered. First, "phage effects" should be separated from "phage preparation effects" since phage lysates can exert important effects that are mediated by bacterial components only, with no direct role of associated phage virions. Second, some effects can be truly attributed to phages, but they are not

caused by the direct impact of virions on immunity but instead are associated with phage antibacterial activity. Phages, by killing bacteria, can normalize immune system stimulation, which has otherwise been boosted by bacterial infection (Górski et al. 2012). Phages also can interact with components of bacteria that have been released into the larger organism. Coliphages and their proteins, for instance, can be capable of binding to LPS found in solution, not only that incorporated into bacterial outer membrane (Miernikiewicz et al. 2016). Only very few effects can be conclusively attributed to phages and their direct interaction with body tissues, however, with no infectivity or toxic effects on mammalian cells reported (Merril 2008). This fact is crucial for tolerance by the body to phages, that is, with secondary pharmacodynamics effects (toxicities, side effects) that are attributable to phage virions themselves at worse only negligible in nature (Bruttin and Brüssow 2005; Kutter et al. 2010; Abedon et al. 2011; Abedon 2015d); see also (Curtright and Abedon 2011).

As noted, mammalian phagocytes may engulf phage virions. During a bacterial infection, phagocytes also are expected to engulf and effectively kill infecting bacteria, which is an important part of innate immune defenses. We therefore may ask whether phages might interfere with the intracellular killing of bacteria by phagocytes, hypothetically, by modifying phagocyte activity, particularly cytokine release and endocytic capabilities. Intracellular killing of bacteria is one of the fundamental immune responses against invading pathogens, and thus, possible effects of phages on this mechanism would be of importance to therapeutic approaches (Jończyk-Matysiak et al. 2015). According to recent observations, however, phage therapy did not reduce the ability of patient phagocytes to kill bacteria and phagocytosis of phages does not affect the activity of phagocytes in patients who initially displayed a reduced ability to kill bacteria intracellularly. These observations support observations that experimental phage therapy seems to have no significant adverse effects on patient immune system functions (Jończyk-Matysiak et al. 2015).

Nonbactericidal phage pharmacodynamic effects that can be observed are usually indirect, that is, they result from the tripartite interactions of mammalian immunity, phages, and especially target bacteria. A good example of this complex effect is inhibition of respiratory burst (ROS production) in peripheral blood polymorphonuclear leukocytes (PMNs) as stimulated by LPS serving as a standard activator. Significant reductions in such leukocyte activation were observed that probably were due not only to phage-PMN interactions but also to phage-LPS interactions (Międzybrodzki et al. 2008). Interestingly, some antiviral effects mediated by phages have also been described, as T4 phage was shown to inhibit attachment and replication of adenovirus (Przybylski et al. 2015).

In general, positive results of phage treatment should be related to the normalization of immunological parameters through decreases in inflammation. Such anti-inflammatory effects of phages have been reported earlier in animals as well as patients receiving phage therapy (Górski et al. 2006a; Międzybrodzki et al. 2009). Phage lysates that have not been purified prior to application to animals, that is, animals without experimental infection, in principle may cause significant increases

in inflammatory markers indicating activation of the immune system, where such activation as mediated directly by medicinals generally is considered to be an undesired effect (Pincus et al. 2015). Phage preparations, including lysates used as antibacterials for the treatment of experimental infections in animals, nevertheless can reduce levels of pro-inflammatory cytokines (Międzybrodzki et al. 2008; Zimecki et al. 2009; Kumari et al. 2010; Górski et al. 2012; Międzybrodzki et al. 2012). Importantly, extensive analysis of immunological parameters in human patients presented by Górski et al. (2012) have shown that no significant over-stimulation of the immune system by phage lysates is observed during phage therapy. Some authors suggest that phage therapy success may depend on phages altering bacterial sensitivity to the immune system, for example, as may be seen with phages capable of removing protective surfaces from bacteria (Mushtaq et al. 2004; Schmerer et al. 2014). All these observations illustrate the importance of the dynamic interactions between mammalian organism, infecting bacteria, and phages as three elements that together can contribute to the pharmacodynamic effects displayed by phages during phage therapy.

Conclusion

To summarize pharmacology, from the perspectives considered here, one can differentiate the body's impact on drugs from a drug's impact on the body, which are described as pharmacokinetics and pharmacodynamics, respectively. The body's impact on a drug – especially the pharmacokinetic aspects described as absorption, distribution, metabolism, and excretion, all in combination with drug dosing strategies – controls the potential to raise drug concentrations within the immediate vicinity of target tissues or, in the case of antimicrobial agents, within the vicinity of target microorganisms. The higher a drug's density within a body then generally the greater a drug's impact on that body, for better or for worse. To the extent that drug densities can be higher especially in association with target tissues, that is, as due to specificity in targeting, then typically the greater the potential for positive efficacy effects (primary pharmacodynamic effects), and the lower the potential for drug side effects (i.e., as a component of secondary pharmacodynamic effects).

With bacteriophages, pharmacokinetics often can be considered to involve some form of phage penetration to target bacteria in combination with phage evasion of immune-system-mediated inactivation, though in certain circumstances excretion is relevant as well, for example, as in the treatment of urinary tract infections by systemic phage delivery (Abedon 2014a). Dosing can be topical, parenteral (along with other means of directly supplying phages systemically), or, at least potentially, per os delivery (Ryan et al. 2011), with generally a goal of achieving at least relatively high phage virion densities within the immediate vicinity of target bacteria, that is, within micrometers such that adsorption is highly likely. Over time, phages must adsorb to and then bactericidally infect a large fraction of targeted bacterial cells in order to successfully clear bacterial infections, or other instances of biocontrol of nuisance bacteria. Over macroscopic dimensions, such clearance likely

requires phage densities of at least 10^7/ml sustained over long periods (many hours, days, or even weeks) or instead higher densities (e.g., 10^8/ml) sustained if treatments are expected to take place over shorter timescales (Abedon 2014a).

With passive treatment, achievement of relatively high virion densities depends entirely on traditional methods of dosing. With active treatment, such dosing is assisted by what can be described instead as auto dosing, where phage replication during infection of target bacteria results especially in highly localized increases in virion densities. This potential can allow application of lower phage numbers topically or, instead, allow for less direct, usually systemic phage application, that is, given circumstances where direct delivery of high phage densities to target bacteria is not easily achieved. With mixed passive-active treatment, a concept otherwise not considered to any great extent in this chapter, an assumption is made that phage in situ replication can to some degree enhance phage therapy efficacy even when high phage densities nevertheless may be directly applied (Abedon 2014b). For instance, auto dosing in this case might achieve not so much relevant enhancements in overall phage densities but instead enhancements in phage penetration to otherwise difficult-to-reach bacteria. By increasing phage virion densities but in an extremely localized manner, especially in the vicinity of immediately adjacent bacteria, such highly localized enhancement of virion densities thus might contribute to phage active penetration into bacterial biofilms as well.

There exists a possibility that applying extremely high phage densities may not always be ideal for achieving bacterial eradication, and one scenario in which this could be the case also is when active penetration is required for successful bacterial eradication. Here the idea is that with bacteria existing within clumps, such as microcolonies or biofilms, the outermost bacteria may be able to shield underlying bacteria from phage attack. In this case, should infection with higher multiplicities result in abortive infections that do not result in lysis (Abedon 2011d), then something other than extremely high phage densities (e.g., 10^9/ml) delivered to the immediate vicinity of target bacteria may be warranted. Such delivery of lower phage doses, for example, 10^7/ml, may be accomplished via repeated dosing, auto dosing, or some combination of the two. Indeed, repeated dosing can be warranted in circumstances where phage penetration ability either changes over time or is positively impacted by previous phage activity, or in which phage in situ amplification is insufficiently robust to sustain phage densities within the vicinity of target bacteria over relevant time frames. Theory aside, there is no substitute for empiricism, so if phage treatments are less efficacious than desired, use of higher or perhaps even lower phage densities might be explored as possible solutions. See Table 4 for summary of passive treatment, active treatment, and passive-active treatment as well as active penetration.

The body's immune system can negatively impact protein-based drug densities, for example, such as phage virion densities. Immune systems therefore can have limiting effects on phage concentrations in target tissues and organs and thereby, at least in principle, can interfere with efficacy (that is, with primary pharmacodynamic effects). Activation of immune responses nevertheless tends to be more

Table 4 Comparing passive and active variations on phage therapy treatment

	Size of phage dose	Requires direct, e.g., topical application or injection directly into an abscess	Requires phage-induced bacterial lysis	Requires in situ production of new phage virions	Ultimately requires phage densities to locally exceed densities of target bacteria[a]
Passive treatment	Much greater than numbers of to-be adsorbed bacteria	Yes	No	No	Yes
Active penetration	Potentially approx. same as numbers of to-be adsorbed bacteria	Potentially	Yes	Potentially	Yes
Active treatment	Not substantially greater than or even lower numbers of to-be adsorbed bacteria	No	Yes	Yes	Yes
Passive-active treatment	Much greater than numbers of to-be adsorbed bacteria	Yes	Yes	Yes	Yes

[a]Phage densities result either from traditional dosing or instead from in situ phage replication and "local" means in the immediate vicinity of target bacteria, e.g., within micrometers

bacterial infection-associated rather than strictly phage-virion related. No direct toxic effect of phage particles applied to mammals, that is, secondary pharmacodynamic effects associated with purified phage particles, have been reported so far. Phages as one element of the phage-bacteria-mammalian immunity balance can give rise to positive immune system modulatory (immunomodulatory) effects, which mainly relates to normalization of pro-inflammatory mediators after boosting by bacterial infection. This means that phages are capable of impacting immune systems, thus affecting the reactivity of immunological factors. On the other hand, intestinal phages have been shown to mediate immunosuppressive effects that can be also relevant at other body sites due to the phenomenon of phage translocation (Górski and Weber-Dąbrowska 2005; Górski et al. 2006b; Barr 2017; Nguyen et al. 2017). These factors shape phage pharmacokinetics and phage fate in vivo. By extension, immunomodulatory capabilities of phage are a weighty determining factor of phage pharmacokinetics.

Cross-References

- ▶ Bacteriophage as Biocontrol Agents
- ▶ Bacteriophage Utilization in Animal Hygiene
- ▶ Clinical Trials of Bacteriophage Therapeutics
- ▶ Early Therapeutic and Prophylactic Uses of Bacteriophages
- ▶ The Use of Bacteriophages in Veterinary Therapy

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