domain (IID) can recognize RNA, including those with overhangs. Mimicry of cellular RIG-I like receptors is part of the arsenal of Ebola virus immune evasion tactics [10].

Unique replication products defined by Deflubé *et al.* are the latest findings that shed light on the unusual replication initiation mechanism utilized by Ebola viruses, which, not too long ago, was considered to be similar to that of other NNSVs.

Acknowledgments

The Leung and Amarasinghe laboratories are partially supported by NIH grants P01AI120943, R01AI114654, R01AI123926, and R01AI143292.

[1]Department of Pathology and Immunology, Washington University School of Medicine in St Louis, St Louis, MO 63110, USA
[2]Department of Medicine, Washington University School of Medicine in St Louis, St Louis, MO 63110, USA
[3]Co-first authors.

*Correspondence:
dwleung@wustl.edu (D.W. Leung).

https://doi.org/10.1016/j.tim.2019.05.004

References

1. Messaoudi, I. *et al.* (2015) Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus. *Nat. Rev. Microbiol.* 13, 663–676
2. Lanini, S. *et al.* (2015) Blood kinetics of Ebola virus in survivors and nonsurvivors. *J. Clin. Invest.* 125, 4692–4698
3. Noton, S.L. and Fearns, R. (2015) Initiation and regulation of paramyxovirus transcription and replication. *Virology* 479-480, 545–554
4. Morin, B. *et al.* (2012) Mechanism of RNA synthesis initiation by the vesicular stomatitis virus polymerase. *EMBO J.* 31, 1320–1329
5. Deflubé, L.R. *et al.* (2019) Ebolavirus polymerase uses an unconventional genome replication mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 116, 8535–8543
6. Poch, O. *et al.* (1990) Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. *J. Gen. Virol.* 71, 1153–1162
7. Wang, Z. *et al.* (2013) Characterization of a nodavirus replicase revealed a de novo initiation mechanism of RNA synthesis and terminal nucleotidyltransferase activity. *J. Biol. Chem.* 288, 30785–30801
8. Morin, B. *et al.* (2013) The polymerase of negative-stranded RNA viruses. *Curr. Opin. Virol.* 3, 103–110
9. Marques, J.T. *et al.* (2006) A structural basis for discriminating between self and nonself double-stranded RNAs in mammalian cells. *Nat. Biotechnol.* 24, 559
10. Leung, D.W. *et al.* (2010) Ebolavirus VP35 is a multifunctional virulence factor. *Virulence* 1, 526–531

Spotlight

Bacteriophage's Dualism in Therapy

V.N. Krylov,[1,*]
M.V. Bourkaltseva,[1] and
E.A. Pleteneva[1]

Careful selection of bacteriophages for phage therapy is needed to avoid undesirable consequences. Different approaches to phage therapy are compared: from the use of multispecies industrially produced phage mixtures with wide range of antibacterial activity to the 'magistral phage' approach in which bacteriophages are selected for treating individual patients.

Prolonged use of new antibiotics, consistently administered for therapy, inevitably leads to the emergence of resistant variants of pathogenic bacteria; this necessitates a search for alternative approaches to antibacterial therapy. One such approach is the use of phage therapy, that is, the use of viruses that infect bacteria; these viruses are called bacteriophages (phages). The initiator of phage therapy was the French scientist F. D'Herelle, one of the discoverers of phages. His collaboration with Georgian researchers in the 1930s promoted the introduction of phage therapy into clinical practice and led to the beginning of the industrial production of phage as a medical preparation. Since then, phage therapy has been successfully applied in Russia and in some countries of Eastern Europe, being used mostly against surface infections – for example, in the treatment of infected wounds. Now several industrial enterprises in Russia (e.g., 'Microgen') produce preparatons of therapeutic phages which display high-level activity against several of the bacterial species most frequently encountered in clinical practice. As an example, the phage mixture 'Pyophage' includes viruses of different species that are active against strains of bacteria such as *Staphylococcus, Streptococcus, Proteus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* – which are the most common pathogens. Maintaining the lytic activity of commercial phage preparations is achieved by continually updating their composition by the introduction of new phages that are active against the newly emerged phage-resistant bacterial variants. As a result, phage preparations released under the same name at different times, or by different enterprises, may differ significantly in activity with respect to a given set of strains isolated from a particular patient.

At the same time, phages of some species should be considered as obviously unacceptable for therapy. This would include those that are a powerful factor in imparting pathogenicity and virulence to bacteria or that may cause undesirable evolutionary changes to pathogenic bacteria.

An example of a phage that is unacceptable for therapy is the filamentous phage Pf, which is active on *P. aeruginosa.* In a series of studies, the results of which were summarized in a recent study [1], a critical role of this bacteriophage was shown to be suppression of immunity against bacterial infection; the presence of Pf was frequently associated with chronic nonhealing wound infections involving *P. aeruginosa*. When phage Pf is internalized by human and murine immune cells it stimulates maladaptive viral pattern-recognition receptors and suppresses bacterial clearance from infected wounds. Accordingly, the authors hypothesized that vaccination against Pf phage virions could be a potential strategy to prevent at least some *P. aeruginosa* infections. It would be interesting to assess the possible effect of Pf phage on the course of bacterial infection of the lungs in an animal model.

Another group of phages that are unacceptable for phage therapy is a large group of temperate phages – transposable phages. One of the possible scenarios of phage evolution during phage therapy, which can affect human health, may be associated with the activity of transposable phages. The first phage transposon active on *P. aeruginosa* – phage D3112 – was described long ago [2], but the study of phages of this species still reveals unexpected effects, including an amazing ability to integrate into its own genome [3]. Another feature of this phage is the peculiarity of its expression during interspecies transfer. When transferring the D3112 genome in the hybrid plasmid RP4 :: D3112 to *E. coli* (strain B), the *E. coli* exconjugants which received RP4::D3112 retained viability only at 42°C (at subfibril temperature), and when the incubation temperature was lowered to 30°C these cells died, forming filamentous bacteria [4,5]. It is difficult to predict how the properties of the clinical *P. aeruginosa* variants or other bacterial species could change and what effects may result from the interspecies migration of a hybrid plasmid with the transposable phage during a real bacterial infection. With the loss of the RP4 plasmid, *E. coli* clones persistently retain the D3112 genes, as a result of prophage transposition into a bacterial chromosome.

The possibility of interactions of phages of different species and different bacteria, as well as the long-term evolutionary consequences of this, have not yet been investigated. In this regard, it is necessary to be extremely cautious in treating humans with some unusual lytic variants of transposable phages. Thus, the proposed use of phage PA10 [6] – which is closely related to phage D3112 (90% of similarity) and exhibits lytic activity against pathogens of several different species – could lead to unexpected and potentially dangerous results.

From our point of view, ensuring mandatory security in phage therapy remains the most important challenge. Therefore, it is necessary to exercise some caution in the use of multispecies phage mixtures. In phage therapy, we think that it is more secure to use sequential applications of monopecies phage preparations [7].

Work is currently under way in Western Europe to introduce the 'magistral phage' system for the therapeutic use of phages in medical practice [8]. One of the obvious and fair objectives of this system is to make phage therapy more accessible for the individual treatment of patients. The technology of the 'magistral phage' therapy differs from the phage therapy model used, for example, in Russia, primarily in that an acceptable phage preparation is selected from the existing set of industrial phage samples (see above) – which does not require a long individual selection of phage and thus significantly reduces the costs.

The previously proposed suggestion that phages can be used by introducing them into the blood stream, using modified phage λ (active on *E. coli* strains) [9], also requires further detailed studies – in particular, concerning the need to expand the spectrum of lytic activity in relation to other species of pathogenic bacteria.

Since the efficacy of phage therapy depends on the conditions under which phages are used, and the type(s) of phage(s) used, the acceptability of phages for therapy (from the point of view of patient safety and possible influence on the evolution of pathogenic bacteria) will inevitably involve further research.

[1]I.I. Mechnikov Research Institute for Vaccines and Sera, Moscow 105064, Russia

*Correspondence:
krylov.mech.inst@mail.ru (V.N. Krylov).

https://doi.org/10.1016/j.tim.2019.05.001

References

1. Sweere, J.M. *et al.* (2019) Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* 363, eaat9691
2. Krylov, V.N. *et al.* (1980) Bacteriophages of *Pseudomonas aeruginosa*, the DNA structure of which is similar to the structure of phage Mu1 DNA. I. General description, localization of endonuclease-sensitive sites in DNA, and structure of homoduplexes of phage D3112. *Genetika* 16, 528–535
3. Vergnaud, G. *et al.* (2018) Transposition behavior revealed by high-resolution description of *Pseudomonas aeruginosa Saltovirus* integration sites. *Viruses* 7, E245
4. Krylov, V.N. *et al.* (1982) Integration of genome of Mu-like bacteriophage D3112 of *Pseudomonas aeruginosa* into plasmid RP4 and its introduction within the hybrid plasmid into *Pseudomonas putida* and *Escherichia coli* C600 cells. *Genetika* 18, 1–6
5. Plotnikova, T.G. *et al.* (1982) Expression of genome of Mu-like phage D3112 specific for *Pseudomonas aeruginosa* in *Escherichia coli* and *Pseudomonas putida* cells. *Genetika* 18, 793–801
6. Kim, S. *et al.* (2012) Complete genome sequence of *Pseudomonas aeruginosa* lytic bacteriophage PA1O which resembles temperate bacteriophage D3112. *J. Virol.* 86, 3400–3401
7. Krylov, V. *et al.* (2016) Modular approach to select bacteriophages targeting *Pseudomonas aeruginosa* for their application to children suffering with cystic fibrosis. *Front. Microbiol.* 7, 1631
8. Pirnay, J.P. *et al.* (2018) The magistral phage. *Viruses* 10, E64
9. Vitiello, C.L. *et al.* (2005) An amino acid substitution in a capsid protein enhances phage survival in mouse circulatory system more than a 1000-fold. *Virus Res.* 114, 101–103

Forum

Early-Life Microbiota Perturbations and Behavioral Effects

Antonia P. Francis[1] and Maria Gloria Dominguez-Bello[2,*]

The maternal environment, during the prenatal and postnatal periods, is a determinant of offspring development and health. Perturbations during these periods can affect maternal behaviors and maternal–infant bonding, and also impair transmission of maternal microbiota to the offspring. Impaired microbiota has been associated with alterations of offspring cognitive development and behavior.

Microbiota Functions in Human Health

Microbiota refers, collectively, to the microbes that have coevolved with a host; their