INT J TUBERC LUNG DIS 3(3):179–184

REVIEW ARTICLE

TB: the return of the phage. A review of fifty years of mycobacteriophage research

R. McNerney

Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

SUMMARY

The first mycobacteriophage was isolated in 1947, and since that time over 250 of these viruses have been identified. Phages have made a significant contribution to our knowledge of mycobacteria over the past fifty years, and following the development of typing techniques in the 1960s and 1970s they were widely used in epidemiological studies of tuberculosis. Unfortunately, attempts to use lytic phages therapeutically during tuberculosis infection have so far failed to elicit cure in experimentally infected animals. During the past decade phages have become important in molecular studies of mycobacteria, both in terms of studying phage biology and as tools in recombinant DNA technology, thus facilitating the investigation of mycobacterial pathogenesis. Today their potential as diagnostics reagents is also being realised with the development of exciting new techniques for rapid bacterial detection and drug susceptibility testing. This review outlines the history of these remarkable organisms, from their discovery fifty years ago to the current developments in rapid diagnostic techniques.

KEY WORDS: mycobacteriophage; phage typing; tuberculosis; therapy; susceptibility testing

IT IS FIFTY YEARS since mycobacteriophages were isolated and identified for the first time during Gardner and Weiser's investigations at the University of Washington in Seattle.[1] They had collected samples of moist leaf compost from around the city, which they enriched with *Mycobacterium smegmatis* and calcium carbonate before incubating at 37°C for several months. They were eventually able to detect organisms that would cause 'clear areas' or 'plaques' in a lawn of *M. smegmatis* growing on nutrient agar. These organisms were identified as bacteriophages that were infecting and destroying the mycobacteria. The phages were specific for *M. smegmatis*, and in their report of 1947 Gardner and Weiser suggested that as bacteriophages for mycobacteria had not previously been reported they might be 'very limited in their distribution'.[1] This, however, proved not to be the case as, using the enrichment technique, researchers soon discovered other phages that would infect the fast-growing environmental species of mycobacteria. In 1954, Froman et al. described phages that were also active against virulent *M. tuberculosis*,[2] while six years later Redmond and Cater isolated phages that were specific for *M. tuberculosis* and *M. bovis* from soil that had been enriched with *M. tuberculosis* $H_{37}Rv$.[3] We now know of over 250 mycobacteriophages that have been extracted from many sources, including environmental, animal and human specimens.[4–6]

Mycobacteriophages are DNA viruses that infect mycobacteria. Following phage replication, lysis of the cell wall occurs, eventually destroying the host bacterium and releasing the progeny phage. Some bacteria, however, were discovered to be resistant to lysis and continued to grow despite the presence of phage. They were found to contain viruses, now classified as temperate phages, that did not cause lysis. It has recently been shown that the temperate mycobacteriophage L5 has an ability to completely shut down expression of its lytic genes during lysogenic growth.[7] Lysogenised strains of bacteria containing temperate phage DNA may exhibit altered biochemical and morphological characteristics.[8]

PHAGE THERAPY

The ability of mycobacteriophages to lyse and destroy mycobacteria led to investigations of their potential as therapeutic agents against tuberculosis. Unfortunately early experiments on phage treatment of experimentally-infected animals not only failed to effect a cure, but in guinea pigs had an adverse effect on their survival![9,10] Although there is renewed interest in phage therapy for the treatment of other bacterial infections, such as those of wounds or the gut,[11,12] during tuberculosis infection the bacteria may be difficult to access by the circulating phages, whilst repeated injection designed to boost the numbers

Correspondence to: Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK. Tel: (+44) 171 927 2391. Fax: (+44) 171 637 4314. e-mail: r.mcnerney@lshtm.ac.uk
Article submitted 27 March 1998. Final version accepted 27 October 1998.

induces a neutralising immune response from the animal.[10] In 1981 Sula et al. reported positive indicators with a reduction in the observed lesions in the spleen, lungs and livers of guinea pigs following therapy with DS-6A.[13] More recent work demonstrated that phage therapy could have a beneficial effect in guinea pigs with disseminated tuberculosis, but that its action was considerably less pronounced than that of isoniazid monotherapy.[14] Interestingly, while tuberculoid inflammation within the granulomas decreased, features typical of sarciodosis were seen to develop. As early as 1962 Mankiewicz and co-workers had suggested an association between lysogenised *M. tuberculosis* bacteria and sarcoidosis,[15] but other workers failed to confirm this when using experimentally-infected animals,[16] and the clinical significance of lysogenised mycobacteria has yet to be fully elucidated. Recent studies using molecular techniques to detect nucleic acid from *M. tuberculosis* in sarcoid lesions have also proved inconclusive, as while some workers found a positive association others could find no evidence of *M. tuberculosis* involvement.[17–19]

The recent upsurge of drug-resistant bacterial infection has prompted fresh interest in the field of phage therapy, with investigation of novel strategies to facilitate the delivery of the phages to their target bacteria.[11,20] Whilst it is clearly rather premature to speculate on a role for mycobacteriophages in the treatment of tuberculosis, the emergence of multidrug-resistant strains, coupled with the current human immunodeficiency virus (HIV) epidemic, surely make this an area worthy of further consideration.

TYPING

As the family of mycobacteriophages continued to grow, consideration was given to their utility for identifying mycobacterial species and consequently to the development of phage typing. It was found, however, that the wide host range of many phages limited their value in taxonomic studies,[21] and interest was concentrated on studies of the pathogenic strains and the phages that could differentiate strains of *M. tuberculosis*.[22,23] In 1965, following an initiative by the Tuberculosis Division of the World Health Organization (WHO), a working group was established to facilitate the development and standardisation of methods for typing mycobacteria. Ten years later a report was published detailing a panel of 12 mycobacteriophages and the standard methodology to be used for typing of *M. tuberculosis*.[24] Several excellent reviews of this subject have previously been published, and detailed consideration of this technology is not included in this article.[21,25,26] Phage typing rapidly became a valuable epidemiological tool and numerous studies were undertaken using these techniques, both on a micro-scale during investigations of transmission and as part of large international surveys where striking geographic variations in the phage susceptibility of *M. tuberculosis* were uncovered.[7,27] Strains were classified as type A, type B, type C or 'intermediate'—having a pattern of phage susceptibility between types A and B.[21] It was found that while type A strains predominated in Hong Kong, type B strains were more common in northern Europe, and of the strains from Madras in the south of India, over forty per cent were of the intermediate type.[28] Although phage typing yielded much interesting data, it has remained a crude tool, and far greater discrimination can now be achieved using molecular methods.[29] DNA fingerprinting techniques such as restriction fragment length polymorphism[30] and spoligotyping[31] have now become the methods of choice for studies on the transmission of tuberculosis.

MOLECULAR STUDIES

While in recent years the role of phage typing has been eclipsed by the emergence of DNA fingerprinting, mycobacteriophages have emerged as major players in the field of molecular genetics.[6,32] Recombinant DNA technology for the mycobacteria was slow to develop in comparison to that of other organisms, as methods used for introducing genetic material into bacteria such as *Escherichia coli* were ineffective when applied to the slow-growing mycobacteria. This was partly due to difficulties in transporting DNA through the lipid rich cell wall, and also because of failure to achieve stable integration of foreign DNA into the genome.

Transfection using mycobacteriophage DNA was first demonstrated in 1964 with the introduction of DNA from D29 into *M. smegmatis*,[33] and early investigations using mutant D29 phages provided tentative insight into the genetic structure of this phage.[34] Transduction was demonstrated in 1970, following the isolation of phage I3 from soil samples enriched with *M. smegmatis*.[35] This advance initiated the study of basic functional genetics, including investigation of the chromosomal material responsible for resistance to the anti-tuberculosis drugs isoniazid and streptomycin.[36] Genomic studies of mycobacteria were further enhanced when, in 1987, Jacobs and co-workers successfully introduced foreign DNA into *M. smegmatis* via cloning vectors which they had constructed by inserting *E. coli* plasmid DNA into the genome of mycobacteriophage TM4.[37] A year later, stable integration and replication of foreign DNA in mycobacteria was demonstrated using shuttle phasmids constructed from the phage L1,[38] while efficient and stable transformation has since been reported using L5.[39] A gene from L5 which was discovered to be involved in regulation of lysogeny has been utilised as a selectable marker for genetic transformation in mycobacteria, avoiding the need for antibiotic resistance genes.[40] Transposition, a powerful technique

for the study of gene function, was difficult to achieve in *M. tuberculosis* until recently, when efficient transposon mutagenesis was demonstrated using a phage delivery system.[41]

While advances in our understanding of the structure and function of phages have led to the development of important new research tools, there remain several areas yet to be elucidated, including those mechanisms which determine the host-range of a mycobacteriophage. The ability to replicate within a host-cell will depend on compatibility of the phage for host restriction and modification systems which may vary between mycobacterial species.[42,43] Phage infection also depends on the availability of specific receptors on the cell surface, and investigation of the structure and biosynthesis of the cell wall may be undertaken using phage-resistant mutants.[44] It has been suggested that infection by D29 may be affected by changes in bacterial glycolipid composition,[45] while more recently a gene was discovered in *M. smegmatis* which when overexpressed was found to induce resistance to infection by L5 and D29.[46] This area of research has now been greatly enriched by the sequencing of the complete genomes of both L5 and the closely related lytic phage D29.[47,48]

The new phage-based tools enable investigation of the structure, expression and regulation of mycobacterial genes, including those which may be associated with drug resistance[49,50] or virulence,[51,52] and have an important role to play in the search for new drug targets. With the recent completion of the sequencing of the *M. tuberculosis* genome,[53] mycobacteriophages look set to remain a vital instrument in the elucidation of the pathogenesis of *M. tuberculosis*.

DIAGNOSIS AND SUSCEPTIBILITY TESTING

Despite the recent advances in molecular technology, tuberculosis remains a major public health problem, and it has been estimated that currently more people are dying each year from tuberculosis than at any previous time in history.[54] Prompt and accurate diagnosis is fundamental to the control of pulmonary tuberculosis, and in some areas of the world there is also a requirement for rapid identification of drug-resistant strains. There is particularly a need for simple, inexpensive tests that are appropriate for use in those low-income countries where the majority of tuberculosis patients are to be found. This challenge has prompted scientists to reconsider the use of mycobacteriophages as tools in diagnosis and drug susceptibility testing.

The effect of anti-tuberculosis drugs on mycobacteriophages was previously investigated by Sellers et al., who in 1965 demonstrated that streptomycin blocked phage replication in susceptible *M. smegmatis* whilst not affecting replication or the subsequent release of progeny phage in a drug-resistant strain.[55] The effects of other drugs on phage synthesis were also examined,[56] and in 1980 David et al. demonstrated inhibition of phage D29 replication by clofazimine, colistin, rifampicin, streptomycin and, to a lesser extent, with dapsone and isoniazid, whereas the addition of ethambutol following infection failed to block replication.[57] D29 phages are able to infect both slow-growing pathogenic mycobacteria and fast-growing environmental strains, and will form visible plaques following overnight incubation on a lawn of fast-growing *M. smegmatis*. This enabled the rapid detection of any progeny phage released following infection of the target mycobacteria, where an increase in the numbers of phage indicated the presence of viable bacteria.[58] They concluded that this simple, rapid method could be used to screen for anti-mycobacterial drugs, and that it might also be useful for determining the drug susceptibility of 'difficult-to-grow' mycobacteria.[57]

Recent modification of David's method has enabled both very sensitive detection of viable mycobacteria and rapid drug susceptibility testing of *M. tuberculosis* isolates.[59,60] Following infection of target mycobacteria with phages, the samples are treated to destroy any excess phages that have not infected, whilst the bacteria and the phages replicating within them remain unaffected. The effective removal of all extraneous phages is a key step in this procedure, as it means that all phages detected have been produced from a living mycobacterium during the test. A simple and robust method of phage inactivation has been developed following the discovery that ferrous compounds will inactivate D29 phages at concentrations which do not harm host mycobacteria.[61] Using this technology, very sensitive detection of mycobacteria is possible, and susceptibility of *M. tuberculosis* isolates growing in liquid culture to fast-acting anti-tuberculosis drugs such as rifampicin and streptomycin can be demonstrated within 24 hours. Susceptibility to drugs which do not directly block phage replication can be determined following a pre-incubation step when the bactericidal effect of the drug can be assessed. This technology is also being applied to the detection of mycobacteria in clinical samples, and has the considerable advantage that it requires no specialist equipment other than that already available in a routine culture laboratory. In addition, both the phages and the *M. smegmatis* indicator cells can be produced 'in-house' at low cost, which greatly facilitates the transfer of this technology to laboratories in low-income countries, although it should be noted that, as this method includes the culture of *M. tuberculosis* in liquid media, highly stringent laboratory safety procedures are still necessary. Although at this early stage of development it is not possible to assess the utility of this technology for the routine diagnostic laboratory, the preliminary results give cause for optimism that phages may have a new role to play, this time in the diagnosis and control of tuberculosis.

Another novel rapid assay for sensitivity testing has been developed using recombinant DNA technology to produce luciferase reporter phages. In 1993, Jacobs and colleagues reported the construction of a phage which incorporated the gene for luciferase, an enzyme normally found in fireflies.[62] This recombinant phage has the ability to express the luciferase gene whilst infecting a mycobacterium. When the substrate luciferin is added in the presence of adenosine triphosphate (ATP), light is emitted that can be detected with photosensitive film or measured with a luminometer. The first luciferase reporter phage was based on TM4, a lytic phage, and subsequently reporter phages based on D29 and the temperate L5 have been constructed.[63,64] As phage replication is only supported in viable cells, light will only be produced when live bacteria are present in a sample, and the assay can be used for drug susceptibility testing and in the screening of potential anti-tuberculosis drugs. This exciting new method is extremely rapid as the effect of drugs such as rifampicin and streptomycin on isolates of *M. tuberculosis* can be measured within hours instead of the weeks required by conventional methods, whilst slow acting drugs such as ethambutol, isoniazid and ciprofloxacin can be tested in two to three days.[65,66] The assay can be performed in 96 well plates and the emitted light detected by a luminometer, a system which lends itself to automation, and thus would be convenient for large-scale screening programmes. For diagnostic purposes confirmation of the presence of *M. tuberculosis* complex bacteria is desirable as these reporter phages are able to infect mycobacteria other than tuberculosis (MOTT), which could result in misdiagnosis. This can be achieved using ρ-nitro-α-acetylamino-β-hydroxy propiophenone (NAP), a substance which selectively inhibits growth of *M. tuberculosis* complex bacteria, thus while NAP does not prevent luminescence in MOTT it will inhibit the production of light in tuberculosis strains.[67] Again, whilst this novel technology awaits further evaluation before introduction into the routine laboratory it is clear that we can expect to hear a lot more about luciferase reporter phages over the next few years.

CONCLUSION

In the fifty years since their discovery, the mycobacteriophages have contributed widely to our knowledge of mycobacteria and tuberculosis disease. Although their role in epidemiological studies has diminished in recent years, their utility in the molecular research laboratory is now well established. Current developments promise to keep mycobacteriophages in the forefront of mycobacterial research into the next millennium, while novel diagnostic techniques may offer a new role for phages in the control of tuberculosis disease.

Acknowledgements

The author is grateful to Dr Neil Stoker for the helpful comments during the preparation of this manuscript, to Dr Silvia Bino for assistance with translation and to the Department for International Development, UK, for supporting this study.

References

1 Gardner G M, Weiser R S. A bacteriophage for *Mycobacterium smegmatis*. Proc Soc Exper Biol Med 1947; 66: 205–206.
2 Froman S, Will D W, Bogen E. Bacteriophage active against virulent *Mycobacterium tuberculosis*. I. Isolation and activity. Am J Public Health 1954; 44: 1326–1333.
3 Redmond W B, Cater J C. A bacteriophage specific for *Mycobacterium tuberculosis*, varieties *hominis* and *bovis*. Am Rev Respir Dis 1960; 82: 781–786.
4 Takeya K, Yoshimura T, Yamaura K, Toda T. Studies on the biologic properties of mycobacteriophage. Am Rev Respir Dis 1959; 80: 543–553.
5 Mankiewicz E. Mycobacteriophages isolated from persons with tuberculosis and non-tuberculosis conditions. Nature 196; 191: 1416–1417.
6 Hatfull G F, Jacobs W R. Mycobacteriophages: cornerstones of mycobacterial research. In: Bloom B R, ed. Tuberculosis: pathogenesis, protection and control. Washington, DC; American Society for Microbiology, 1994; pp 165–183.
7 Brown K L, Sarkis G J, Wadsworth C, Hatfull G F. Transcriptional silencing by the mycobacteriophage L5 repressor. EMBO Journal 1997; 16: 5914–5921.
8 Grange J M, Redmond W B. Host-phage relationships in the genus *Mycobacterium* and their clinical significance. Tubercle 1978; 59: 203–225.
9 Hauduroy P, Rosset W. Tentative de traitement des hamsters inoculés avec le BCG par un bactériophage. Annales de l'Institut Pasteur 1963; 104: 419–420.
10 Mankiewicz E, Beland J. The role of mycobacteriophages and of cortisone in experimental tuberculosis and sarcoidosis. Am Rev Respir Dis 1963; 89: 707–720.
11 Barrow P A, Soothill J S. Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. Trends Microbiol 1997; 7: 268–271.
12 Alisky J, Iczkowski K, Rapoport A, Troitsky N. Bacteriophages show promise as antimicrobial agents. J Infect 1998; 36: 5–15.
13 Sula L, Sulova J, Stolcpartova M. Therapy of experimental tuberculosis in guinea pigs with mycobacterial phages DS-6A, GR-21T, My-327. Czech Med 1981; 4: 209–214.
14 Zemskova Z S, Dorozhkova I R. Pathomorphological evaluation of therapeutic effect of mycobacteriophages in tuberculosis. Probl Tuberk 1991; 11: 63–66.
15 Mankiewicz E, Van Walbeek M. Mycobacteriophages: their role in tuberculosis and sarcoidosis. Arch Environ Health 1962; 5: 122–128.
16 Bowman B U, Amos W T, Geer J C. Failure to produce experimental sarcoidosis in guinea pigs with *Mycobacterium tuberculosis* and mycobacteriophage DS6A. Am Rev Respir Dis 1972; 105: 85–94.
17 Saboor S A, Johnson N M, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 1992; 336: 1012–1015.
18 Mitchell I C, Turk J L, Mitchell D N. Detection of mycobacterial rRNA in sarcoidosis with liquid-phase hybridisation. Lancet 1992; 336: 1015–1017.
19 Vokurka M, Lecossier D, du Bois R M, et al. Absence of DNA from mycobacteria of the *M. tuberculosis* complex in sarcoidosis. Am J Respir Crit Care Med 1997; 156: 1000–1003.
20 Merril C R, Biswas B, Carlton R, et al. Long-circulating bacteriophages as antibacterial agents. Proc Natl Acad Sci USA 1996; 93: 3188–3192.

21 Redmond W B, Bates J H, Engel H W B. Methods for bacteriophage typing of mycobacteria. In: Bergan T, ed. Methods in microbiology Vol 13. London, UK; Academic Press 1979: pp 345–375.
22 Baess I. A bacteriophage for subdividing the species *M. tuberculosis*. Am Rev Respir Dis 1966; 93: 622–623.
23 Bates J H, Fitzhugh J K. Subdivision of the species *M. tuberculosis* by mycobacteriophage typing. Am Rev Respir Dis 1967; 96: 7–10.
24 Rado T A, Bates J H, Engel H W B, et al. World Health Organisation studies bacteriophage typing of mycobacteria. Subdivision of the species *Mycobacterium tuberculosis*. Am Rev Respir Dis 1975; 111: 459–468.
25 Crawford J, Bates J H. Phage typing of mycobacteria. In: Kubica G P, Wayne L G, eds. The mycobacteria: a sourcebook, part A. New York, NY; Marcel Dekker Inc. 1984: pp 123–132.
26 Engel H W B. Mycobacteriophage and phage typing. Ann Microbiol 1978; 129A: 75–90.
27 Snider D E Jr, Jones W D, Good R C. The usefulness of phage typing *Mycobacterium tuberculosis* isolates. Am Rev Respir Dis 1984; 130: 1095–1099.
28 Bates J H, Mitchison D A. Geographic distribution of bacteriophage types of *Mycobacterium tuberculosis*. Am Rev Respir Dis 1969; 100: 189–193.
29 Butcher P D, Hutchinson N A, Doran T J, Dale J W. The application of molecular techniques to the diagnosis and epidemiology of mycobacterial diseases. J App Bacteriol 1996; 81: S53–S71.
30 Van Embden J D A, Cave M D, Crawford J T, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993; 31: 406–409.
31 Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997; 35: 907–914.
32 Hatfull G F. The molecular genetics of *Mycobacterium tuberculosis*. Curr Topics Microbiol Immunol 1996; 215: 29–47.
33 Tokunaga T, Sellers M I. Infection of *Mycobacterium smegmatis* and D29 phage DNA. J Exp Med 1964; 119: 139–149.
34 Mizuguchi Y, Sellers M I. Isolation and characterization of temperature-sensitive mutants of mycobacteriophage D29. In: Juhasz S E, Plummer G, eds. Host-virus relationships in mycobacterium, nocardia and actinomyces. Springfield, IL; Charles C Thomas 1970: pp 65–79.
35 Sundar Raj C V, Ramakrishnan T. Transduction in *Mycobacterium smegmatis*. Nature 1970; 228: 280–281.
36 Saroja D, Gopinathan K P. Transduction of isoniazid susceptibility-resistance and streptomycin resistance in mycobacteria. Antimicrob Agents Chemother 1973; 4: 643–645.
37 Jacobs W R Jr, Tuckman M, Bloom B R. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. Nature 1987; 327: 532–536.
38 Snapper S B, Lugosi L, Jekkel A, et al. Lysogeny and transformation of mycobacteria: stable expression of foreign genes. Proc Natl Acad Sci USA 1988; 85: 6987–6991.
39 Lee M H, Pascopella L, Jacobs W R, Hatfull G F. Site-specific integration of mycobacteriophage L5: integration-proficient vectors for *Mycobacterium smegmatis*, *Mycobacterium tuberculosis* and bacille Calmette-Guerin. Proc Natl Acad Sci USA 1991; 88: 3111–3115.
40 Donnelly-Wu M K, Jacobs W R, Hatfull G F. Superinfection immunity of mycobacteriophage L5: applications for genetic transformation of mycobacteria. Mol Microbiol 1993; 7: 407–417.
41 Bardarov S, Kriakov J, Carriere C, et al. Conditionally replicating mycobacteriophages: a system for transposon delivery to *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 1997; 94: 10961–10966.
42 Rado T A, Bates J H, Fitzhugh J K. Evidence for host-dependent modification and restriction of bacteriophage DNA in *Mycobacterium tuberculosis*. J Gen Virol 1976; 30: 91–97.
43 Jones W D Jr , Greensburg J. Host modification and restriction with a mycobacteriophage isolated from a pseudolysogenic *Mycobacterium chelonei*. J Gen Microbiol 1977; 99: 389–395.
44 Parish T, Liu J, Nikaido H, Stoker N G. A *Mycobacterium smegmatis* mutant with a defective inositol monophosphate phosphatase gene homolog has altered cell envelope permeability. J Bacteriol 1997; 179: 7827–7833.
45 Bersa G S, Khoo K H, Belisle J T, et al. New pyruvylated, glycosylated acyltrehaloses from *Mycobacterium smegmatis* strains, and their implications for phage resistance in mycobacteria. Carbohydr Res 1994; 251: 99–114.
46 Barsom E K, Hatfull G F. Characterisation of a *Mycobacterium smegmatis* gene that confers resistance to phages L5 and D29 when overexpressed. Mol Microbiol 1996; 21: 159–170.
47 Hatfull G F, Sarkis G. DNA sequence, structure and gene expression of mycobacteriophage L5: a phage system for mycobacterial genetics. Mol Microbiol 1993; 7: 395–405.
48 Ford M E, Sarkis G J, Belanger A E, Hendrix R W, Hatfull G F. Genome structure of mycobacteriophage D29: implications for phage evolution. J Mol Biol 1998; 279: 143–164.
49 Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature 1992; 358: 591–593.
50 Banerjee A, Dubnau E, Quemard A, et al. inhA, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. Science 1994; 263: 227–230.
51 Pascopella L, Collins F M, Martin J M, et al. Use of in vivo complementation in *Mycobacterium tuberculosis* to identify a genomic fragment associated with virulence. Infect Immun 1994; 62: 1313–1320.
52 Wren B W, Stabler R A, Das S S, et al. Characterization of a haemolysin from *Mycobacterium tuberculosis* with homology to a virulence factor of *Serpulina hyodysenteriae*. Microbiol 1998; 144: 1205–1211.
53 Cole S T, Brosch R, Parkhill J, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 1998; 393: 537–544.
54 Reichman L B. Tuberculosis elimination—what's to stop us? Int J Tuberc Lung Dis 1997; 1: 3–11.
55 Tokunaga T, Sellers M I. Streptomycin induction of premature lysis of bacteriophage-infected mycobacteria. J Bacteriol 1965; 89: 537–539.
56 Phillips L M, Sellers M I. Effects of ethambutol, actinomycin D and mitomycin C on the biosynthesis of D29-infected *Mycobacterium smegmatis*. In: Juhasz S E, Plummer G, eds. Host-virus relationships in mycobacterium, nocardia and actinomyces. Springfield, IL; Charles C Thomas, 1970: pp 80–120.
57 David H L, Clavel S, Clement F, Moniz-Pereira J. Effects of antituberculosis and antileprosy drugs on mycobacteriophage D29 growth. Antimicrob Agents Chemother 1980; 18: 357–359.
58 David H L, Clavel S, Clement F. Adsorption and growth of the bacteriophage D29 in selected mycobacteria. Ann Virol 1980; 131E: 167–184.
59 McNerney R. Diagnosis: present difficulties and prospects for the future. Africa Health 1996; 19: 22–23.
60 Wilson S M, Al Suwaidi Z, McNerney R, Porter J, Drobniewski F. Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of *Mycobacterium tuberculosis*. Nature Med 1997; 34: 465–468.
61 McNerney R, Wilson S M, Sidhu A M, et al. Inactivation of mycobacteriophage D29 using ferrous ammonium sulphate as a tool for the detection of viable *Mycobacteria smegmatis* and *Mycobacterium tuberculosis*. Res Microbiol 1998; 149: 487–495.
62 Jacobs W R Jr, Barletta R, Udani R, et al. Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. Science 1993; 260: 819–822.

63 Sarkis G J, Jacobs W R Jr, Hatfull G F. L5 luciferase reporter mycobacteriophages: a sensitive tool for the detection and assay of live mycobacteria. Mol Microbiol 1995; 15: 1055–1067.
64 Pearson R E, Jurgensen S, Sarkis G J, Hatfull G F, Jacobs W R Jr. Construction of D29 shuttle phasmids and luciferase reporter phages for detection of mycobacteria. Gene 1996; 183: 129–136.
65 Carriere C, Riska P F, Zimhony O, et al. Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of *Mycobacterium tuberculosis*. J Clin Microbiol 1997; 35: 3232–3239.
66 Riska P F, Jacobs W R Jr. The use of luciferase reporter phage for antibiotic susceptibility testing of mycobacteria. In: Parish T, Stoker N G, eds. Methods in molecular biology. Vol 101. Mycobacteria protocols. New Jersey, NJ; Humana Press 1998: 431–455.
67 Riska P F, Jacobs W R Jr, Bloom B R, McKitrick J, Chan J. Specific identification of *Mycobacterium tuberculosis* with the luciferase reporter mycobacteriophage: use of ρ-nitro-α-acetylamino-β-hydroxy propiophenone. J Clin Microbiol 1997; 35: 3225–3231.

RÉSUMÉ

Le premier mycobactériophage a été isolé en 1947, et depuis lors, plus de 250 de ces virus ont été identifiés. Les phages ont contribué de manière significative à notre connaissance des mycobactéries au cours des cinquante dernières années ; à la suite du développement des techniques de typage dans les années 1960 et 1970, ils ont été largement utilisés dans les études épidémiologiques sur la tuberculose. Malheureusement, les essais d'utilisation des phages lytiques pour le traitement au cours de l'infection tuberculeuse ont échoué jusqu'ici dans les tentatives de traitement des animaux infectés expérimentalement. Au cours de la dernière décennie, les phages sont devenus importants pour les études moléculaires des mycobactéries, à la fois par l'étude de la biologie des phages et comme instrument de la technologie recombinante de l'ADN qui facilite l'investigation de la pathogénie mycobactérienne. A ce jour, leur potentialité comme réactif de diagnostic est également apparue avec le développement de nouvelles techniques intéressantes pour une détection bactérienne rapide, et pour tester la sensibilité à l'égard des médicaments. Cette revue décrit l'histoire de ces organismes remarquables depuis leur découverte il y a cinquante ans jusqu'au développement récent des techniques de diagnostic rapide.

RESUMEN

El primer micobacteriófago fue aislado en 1947 y desde entonces se han identificado más de 250 de estos virus. Los fagos han hecho una contribución importante a nuestros conocimientos sobre las micobacterias en los últimos 50 años y gracias al desarrollo de las técnicas de tipificación en los años 1960 y 1970 han sido usados ampliamente en estudios epidemiológicos de la tuberculosis. Desgraciadamente las tentativas de usar fagos líticos terapéuticamente en la infección tuberculosa de animales de experimentación ha fracasado. En las últimas décadas los fagos han llegado a ser importantes en los estudios moleculares de las micobacterias, tanto para estudiar la biología fágica como para ser usados como armas en la tecnología del ADN recombinante, facilitando la investigación de la patogenia micobacteriana. En la actualidad se constata su valor como elemento de diagnóstico, con el desarrollo de nuevas técnicas para la detección rápida de bacterias y los tests de sensibilidad a las drogas. Esta revisión destaca la historia de estos extraordinarios organismos desde su descubrimiento hace cincuenta años hasta el actual desarrollo de técnicas para el diagnóstico rápido.