RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY (2004) 7:163–171
www.im.microbios.org

INTERNATIONAL MICROBIOLOGY

Rubens López

Department of Molecular Microbiology, Biological Research Center, CSIC, Madrid, Spain

Streptococcus pneumoniae and its bacteriophages: one long argument

Summary. Infectious diseases currently kill more than 15 million people annually, and the WHO estimates that every year 1.6 million people die from pneumococcal diseases. *Streptococcus pneumoniae* (pneumococcus), a bacterium with a long biological pedigree, best illustrates the rapid evolution of antibiotic resistance, which has led to major public health concern. This article discusses the molecular basis of the two main virulence factors of pneumococcus, the capsule and cell-wall hydrolases, as well as new approaches to developing medicinal weapons for preventing pneumococcal infections. In addition, current knowledge regarding pneumococcal phages as potential contributors to virulence and the use of lytic enzymes encoded by these phages as therapeutic tools is reviewed. [**Int Microbiol** 2004; 7(3):163–171]

Key words: *Streptococcus pneumoniae* · pneumococcus · capsule · pneumococcal bacteriophages · lytic enzymes

Received 4 May 2004
Accepted 30 May 2004

Address for correspondence:
Departamento de Microbiología Molecular
Centro de Investigaciones Biológicas, CSIC
Ramiro de Maeztu, 9
28040 Madrid, Spain
Tel. +34-918373112. Fax +34-915360432
E-mail: ruben@cib.csic.es

Historical periods are dominated by distinct sets of ideas which, taken together, form a well defined Zeitgeist. *Greek philosophy, Christianity, Renaissance thought, the Scientific Revolution, and the Enlightenment are examples of sets of ideas that dominated their historical period. The changes from one period to the next are usually rather gradual: other changes—more abrupt—are often referred to as revolutions.*

Ernst Mayr, *One Long Argument*, 1991

Introduction

Pneumococcus (*Streptococcus pneumoniae*) has been a bacterium of utmost clinical significance since Louis Pasteur (1822–1895) and George Miller Sternberg (1838–1915) discovered it independently in 1881. Its pedigree is indeed among the most notorious in the microbial world. Until the 1940s, when the discovery of antibiotics brought much-needed relief to humanity, pneumococcus killed more human beings than cancer and heart diseases together. At that time, this regular colonizer of the human pharynx, then called *Diplococcus pneumoniae* because the cells are often in pairs, had been used as an experimental vehicle to demonstrate that DNA was the carrying material of genetic inheritance. Oswald T. Avery (1877–1955), Colin M. Macleod (1909–1972) and Maclyn McCarty (born 1911), based their studies of genetic transformation on a unique phenotypic characteristic of this microorganism—the polysaccharide capsule that surrounds the bacterial cell [2].

Over the last decade, *S. pneumoniae* has joined the ranks of so-called reemerging infectious agents, due in large part to its genetic plasticity, which endows it with resistance to many antibiotics thereby making it a major cause of death throughout the world. Currently, *S. pneumoniae* infects around 100 million people every year, with a fatality rate of more than 10%. In addition, pneumococcus is responsible for 10–25% of pneumonia cases, 10% of meningitis infections (with 30% mortality), and over 50% of cases of otitis media. It has also been reported to be the cause of disease in $10–20 \times 10^5$ inhabitants/year in four European countries (Denmark, Finland, Spain and the United Kingdom). As surveillance of the disease has been limited to hospitalized patients, this figure is surely an underestimation of the actual incidence [21].

This article is based on the Opening Lecture of the 19th National Congress of the Spanish Society for Microbiology (SEM), given by the author in Santiago de Compostela, Spain, on September 22, 2003.

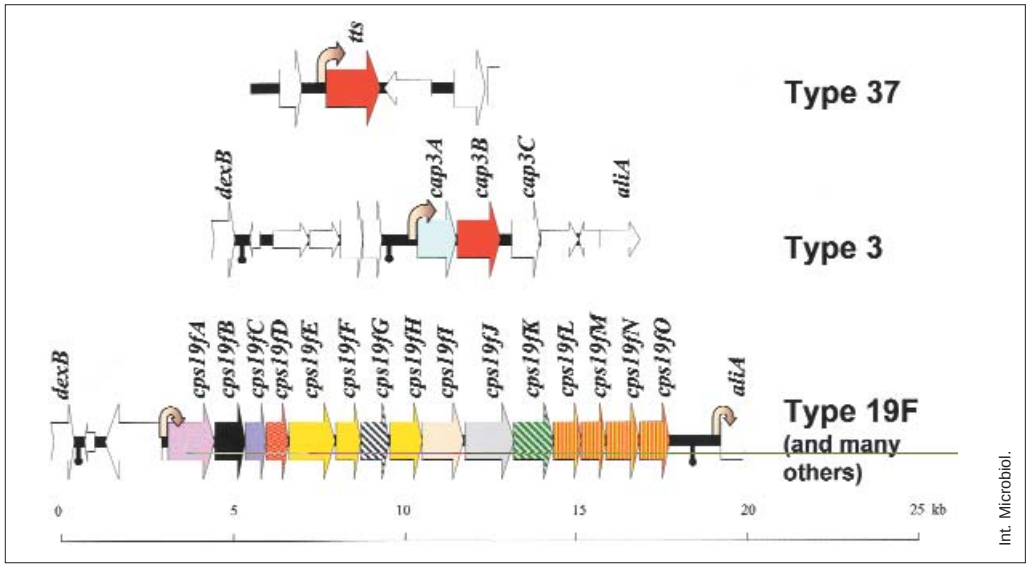

Fig. 1. Genetic organization of the *Streptococcus pneumoniae* region containing the *cap/cps* gene cluster of three representative capsular types. **Large arrows**, complete ORFs; **thin arrows**, interrupted ORFs. Regions with identical color and shading have more than 90% identical nucleotides. **Inverted matchsticks**, putative transcription terminators; **bent arrows**, location of functional promoters. In type 3, the four initial ORFs of the capsular operon are not involved in capsular polysaccharide biosynthesis and are not expressed. Type 19F represents the most common capsular gene cluster organization. Type 37 capsulation is exceptional, since it is due to the presence of a single copy of a gene (*tts*) located far from the *cap* cluster.

This review focuses on three main aspects of *S. pneumoniae*: (i) molecular analysis of its polysaccharide capsule, (ii) the lytic enzymes of the bacterium and its phages, and (iii) the characteristics of pneumococcal bacteriophages.

The capsule

The polysaccharide capsule that completely envelops *S. pneumoniae* acts as a protective layer, isolating the bacterial cell from its environment, and is the main virulence factor of this bacterium. Genes located within the capsular cluster encode enzymes for the synthesis of more than 90 structural and serological capsular polysaccharides (serotypes) that contribute to the clinical importance of *S. pneumoniae*. Although the biochemistry of some serotypes has long been known, it was not until the early 1990s that the molecular basis of capsule formation in several bacterial species was unraveled. In 1993, the location and isolation of a gene required for the synthesis of serotype-3 capsule was reported by my laboratory [9]. That genes involved in pneumococcal capsule synthesis form a cluster (Fig. 1) has been known since the late 1950s. The localization and isolation of those genes (*cap*/*cps*) showed—as biochemists had suggested—that the greater the complexity of the capsule's composition, the more genes were involved in its formation, and that the cluster was located between *dexB* and *aliA*, which do not participate in capsular formation (Fig. 1). Genes coding specifically for a given serotype or serogroup are found in the central region of the cluster and are flanked by genes encoding functions related to the regulation and transport of the capsule's components. Different capsule types may also have common regions in their clusters [1].

Knowing the location of the cluster for serotype-3 capsule and having observed similarities among the genes from the region bordering *dexB*, we were able to prepare molecular probes for locating other capsular types, including serotypes 1, 8 and 33F. Currently, the organization of 16 serotypes is known. Figure 1 shows the two different archetypes of functional clusters for all the known serotypes, as demonstrated by type 3 and type 19, the latter having the most common form of organization. The figure also depicts the peculiar case of capsule formation of type 37.

In the central region of the serotype 19 cluster, genes that code for the synthesis of each serotype or serogroup are flanked by genes involved in the regulation and transport of the capsule's components.

The modular structure of the pneumococcal capsule facilitates, in a bacterium that undergoes natural genetic transformation, the exchange of specific genes between serotypes by means of recombination between flanking homologous regions. This exchange, evidenced by Coffey et al. [3], is of great

clinical importance, especially when it occurs between antibiotic-resistant strains. Indeed, such exchanges provoke an epidemiological challenge to control the universal expansion of some pneumococcal strains. Moreover, the fact that changes in capsular type by recombination might be relatively frequent among pneumococci has implications for the long-term efficacy of conjugate pneumococcal vaccines, which protect only against a limited number of serotypes [3].

The complexity of pneumococcus biology is evidenced in the case of serotype 37, in which a unique gene (*tts*) is located far from the capsular cluster and enables the bacterium to produce the homopolysaccharide capsule characteristic of this serotype. This gene, first described in my laboratory, is involved in the elaboration of the capsule homopolysaccharide sophorose, which is a characteristic component of this serotype [18]. As a matter of fact, serotype 37 is genetically binary in that it has both an active gene (*tts*) and an inactive cluster almost identical to that of serotype 33. The versatile expression of *tts* (coding for a glucosyltransferase) was demonstrated by introducing the gene into a series of gram-positive species which subsequently became type-37-capsulated [19]. An understanding of these genetically binary strains is therefore of great clinical importance, since this phenomenon further facilitates the perpetuation of pneumococcal pathogenic strains in humans, who are their natural and almost exclusive hosts.

Interestingly, sequencing of the locus *galU* also revealed a gene implicated in the synthesis of all known pneumococcal serotypes. *galU* codes for uridine diphosphoglucose (UDP-Glc) pyrophosphorylase, which has turned out to be an ideal metabolic target for future clinical approaches aimed at blocking the formation of *S. pneumoniae*'s capsule using a new generation of conjugate vaccines [26].

Lytic enzymes

Gram-positive bacteria are surrounded by peptidoglycan layers (the glycanic chain made of repetitive units of *N*-acetylglucosamine and *N*-acetylmuramic acid linked by peptide bonds), which give them their special shape. For the bacterial cell to expand, this rigid sacculum must continuously adapt. Cellular restructuring requires the action of murein hydrolases, which are endogenous enzymes capable of degrading peptidoglycan by cleaving covalent bonds of the cell wall. Cell-wall hydrolases (CWH), or lytic enzymes, have been found in all eubacteria studied so far, and are considered to play major roles in the biology of bacteria, including cellular expansion, division, and separation of daughter cells. CWH are crucial in microbial chemotherapy since they are responsible for the irreversible effects caused by β-lactam antibiotics. Based on their bond specificity, lytic enzymes are classified as: (i) glycosidases (lysozymes or muramidases and glucosaminidases), (ii) endopeptidases, and (iii) amidases. Figure 2 shows the bonds cleaved by different pneumococcal CWH. The membrane lipoteichoic acid of pneuococci seems to constrain the activity of some pneumococcal lytic enzymes. Most interestingly, teichoic acids, linked to muramic acids, contain choline in their structures. Höltje and Tomasz [16] showed that this amino alcohol plays a crucial role in the physiology of the pneumococcal cell wall. In fact, it is an essential lig-

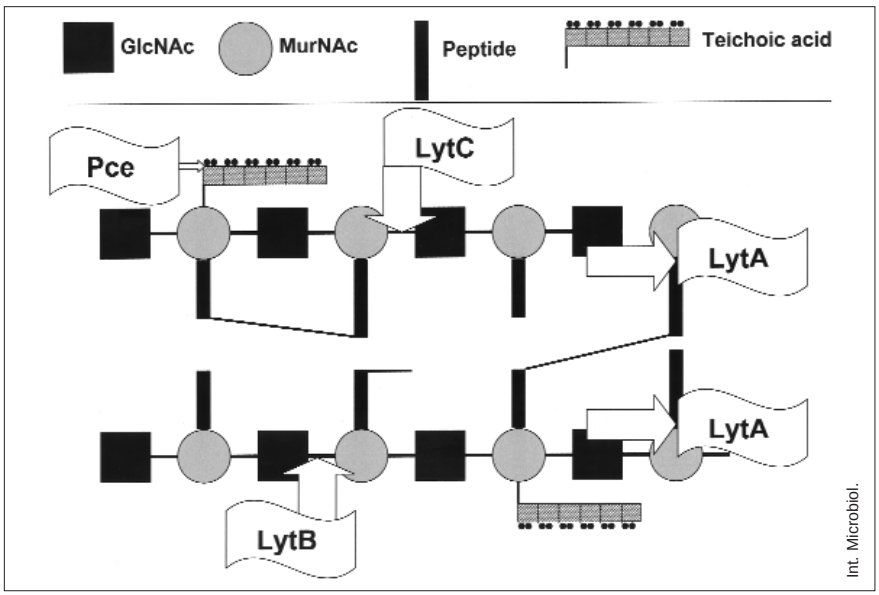

Fig. 2. The pneumococcal cell wall, showing the bonds cleaved by different pneumococcal cell-wall hydrolases (CWH). The upper part of the diagram indicates the different components of the cell wall. For simplicity, only two layers are represented. Transglycosylases and endopeptidases have not been found in *S. pneumoniae*. The small black circles attached to teichoic acids represent choline molecules; GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid; LytA, amidase; LytB, glucosaminidase; LytC, lysozyme; Pce, phosphoryl choline esterase.

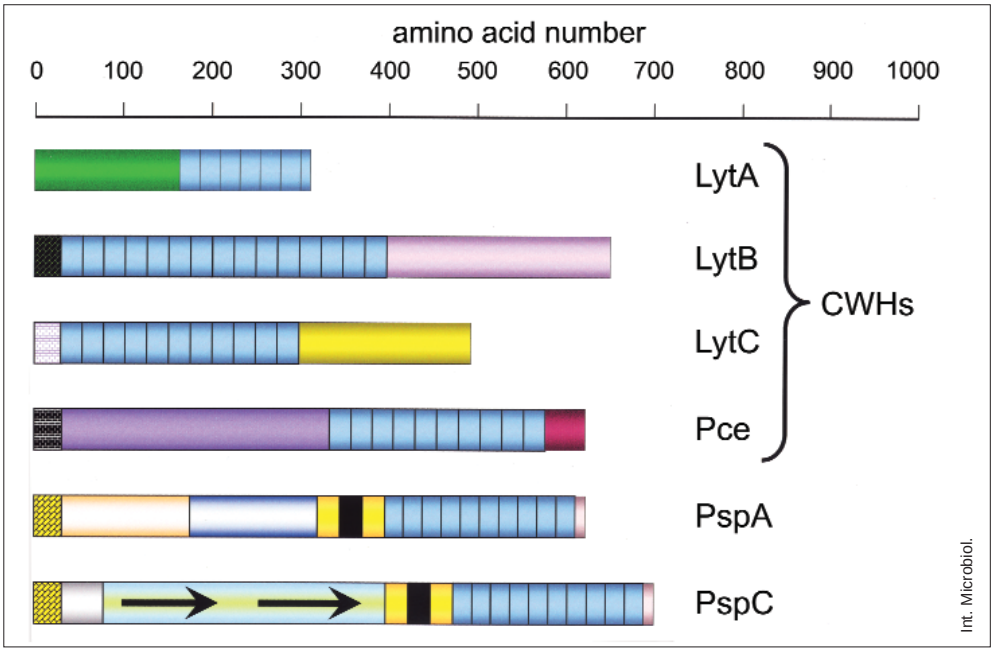

Fig. 3. The structure of the pneumococcal CWH LytA, LytB, LytC, and Pce and of two other choline-binding proteins (PspA and PspC). The domain containing the active center of the enzymes is shown in light green (amidase), yellow (lysozyme), pink (glucosaminidase), or violet (phosphorylcholine esterase). The choline-binding domain (blue) and the choline-binding repeats are also shown.

and for the activity of the major murein hydrolase LytA amidase, which carries out 99% of the lytic activity in the cell. The gene coding for this fundamental enzyme (*lytA*) was cloned and sequence in 1985 [7], providing the first report of the molecular manipulation of a bacterial autolysin. When pneumococci are grown in the presence of the choline analogue ethanolamine, it is incorporated into the cell wall instead of choline, and the cell undergoes dramatic biochemical and morphological changes [16].

It has been also observed that phages release their progeny using their own lytic enzymes, which, in the pneumococcal system, have features common to the lytic enzymes of the bacterium, such as the dependence of the lytic enzymes of both the host and its phage on the presence of choline in teichoic acids for activity. By using *lytA* as a probe in hybridization experiments, we detected lytic genes in phage that are similar to *lytA*. Sequence comparison of *lytA* and the gene coding for a choline-dependent lysozyme (Cpl-1) from the pneumococcal bacteriophage Cp-1 revealed that the C-terminal region of LytA and Cpl-1 are almost identical and share a modular organization [8,11]. The N-terminal moieties, however, are completely different (Fig. 3) while the C-terminal region consists of a series of repeated motifs. Using a molecular approach, we found six motifs, made up of 20 amino acids, for LytA and Cpl-1. Further genetic experiments confirmed that the C-terminal domain of both proteins with repeated motifs was involved in recognizing the substrate to be degraded, whereas the N-terminal domain harbored the region responsible for catalytic activity [21]. Biochemical studies revealed that the mechanisms of anchorage and exposure of these and other proteins to the pneumococcal cell surface are highly varied and unusual [22]. Nevertheless, this structural organization was later found in many modular proteins in several bacterial systems.

Crystal structure analyses revealed that the pneumococcal C-terminal LytA domain (C-LytA) is a solenoid structure consisting exclusively of multiple β-hairpins that together form a left-handed superhelix [5]. Every hairpin corresponds to the motif that we had defined by analyzing the protein's primary structure (Fig. 3). For the enzyme to achieve its structure, each choline molecule must locate in the hydrophobic interphase formed by the consecutive hairpins. In addition, the active form of the enzyme requires the formation of a boomerang-shaped dimer.

Preparation of deleted LytA mutants lacking the main lytic enzyme (M31) of pneumococcus revealed that this enzyme was crucial for lysis of cultures at the end of stationary phase (autolysis) (Fig. 4A). Bacteria that lacked LytA were clinically tolerant because the bacteriolytic effect induced by β-lactam antibiotics was eliminated, leading to a bacteriostatic response to the drug [32].

The coordinated use of immunomarking techniques with colloidal gold and electron microscopy showed that LytA is located mainly in the equatorial region of the cell (Fig. 4A). The lack in M31 of the major amidase unmasked the presence of other minor proteins that are strongly bound—probably through choline—to peripheral structures globally called choline-binding proteins (ChBPs). Identification of the

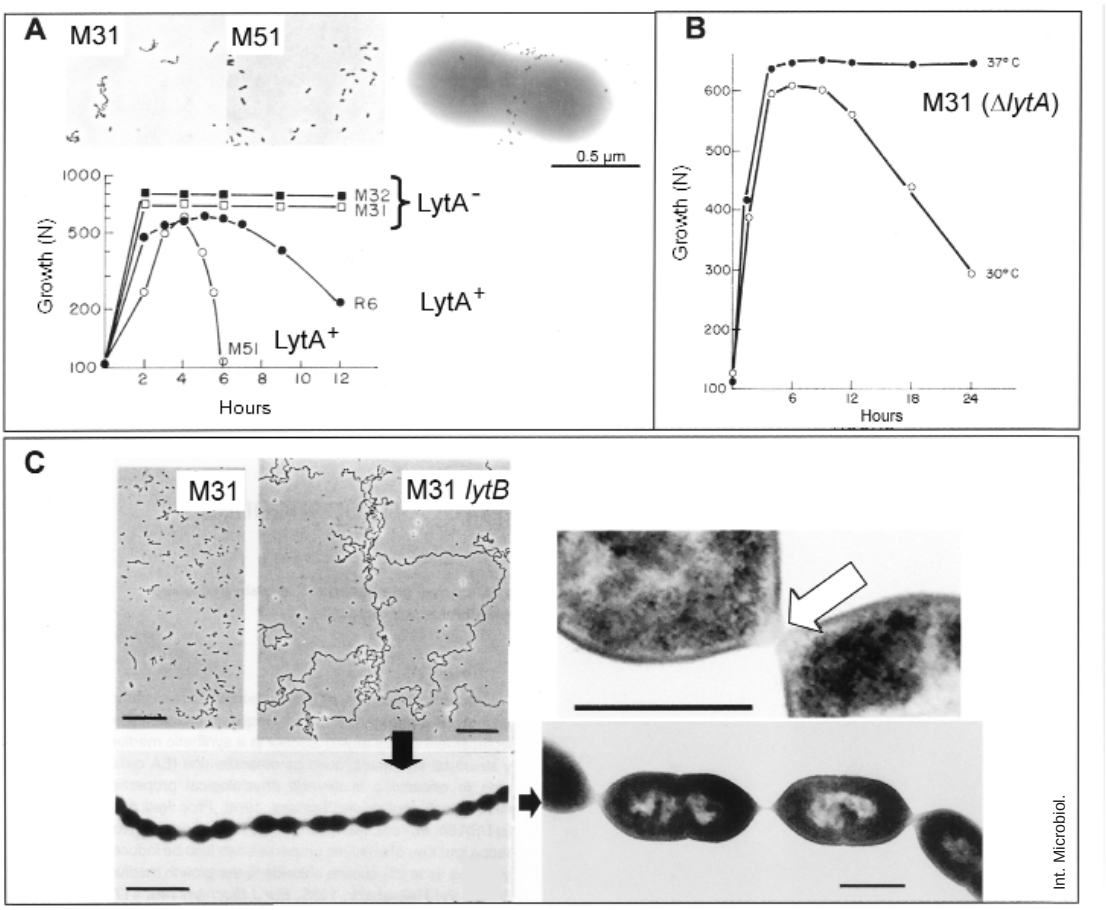

Fig. 4. Biological roles of the pneumococcal LytA (amidase), LytC (lysozyme) and LytB (glucosaminidase). (**A**) Curves of growth of the pneumococcal *lytA* mutants (M31 and M32), M51 (a strain that overexpresses LytA), and R6, the wild-type strain of *S. pneumoniae*. The inset at the upper left shows the morphology of pneumococcus in the absence (M31) or presence (M51) of LytA. The inset at the right shows the immunochemical localization, using gold-labeled antibodies and electron microscopy, of LytA in intact cells. (**B**) Growth curves of the pneumococcal *lytA* mutants incubated at 30 and 37ºC. The activity responsible for lysis at this temperature has been ascribed to LytC. (**C**) Dispersion of M31B chains by the activity of purified LytB. At the top left the morphological alteration of a *S. pneumoniae* strain that lacks *lytA* (M31) and of the *lytA*, *lytB* mutants as examined by phase-contrast microscopy (bar = 25 µm). **Black arrows**, morphology of M31 *lytB* mutants examined by transmission electron microscopy (bar = 2 µm). The bottom right inset shows thin sectioning and electron microscopy of R6B cells untreated (bottom, bar = 1 µm) or treated (top, bar = 0.5 µm) with purified LytB. Note that neighboring cells in the chain are separated by cross walls of normal dimensions and normal depth that are disrupted upon treatment with LytB (**white arrow**).

N-terminal domains of these purified proteins along with the availability of the pneumococcus genome sequence enabled us to isolate and clone the genes that code for two other pneumococcal murein hydrolases, LytC and LytB. LytC and LytB have similar structural organizations, in which, unlike LytA, the region for choline recognition is located at the N-terminal domain and consists of 11 and 18 repeated motifs, respectively. Furthermore, in contrast to LytA, both enzymes also have a signal peptide (see Fig. 3). Inactivation of *lytB* insertion, by using suicidal plasmids, led to the formation of long cell chains (Fig. 4C) [4].

LytC, the first pneumococcal lysozyme described, can be easily isolated on a DEAE-cellulose column, since DEAE is a choline analogue. This technique, developed by my laboratory, selectively retains ChBPs proteins [34]. Biologically, LytC functions as an autolysin (Fig. 4B) when cultures are incubated at 30ºC [12]. As the carrier state of pneumococcus is located in the upper respiratory tract, usually a well-ventilated region of the body (ca. 34ºC), this enzyme might play a role in the natural transformation processes that are carried out in this location. Likewise, we have observed that cells lacking this enzyme tend to form clusters.

We have purified LytB and identified it as a glucosaminidase. Addition of the purified enzyme to cultures of pneumococcus mutants R6B or M31B, which form characteristic long chains, promoted their dispersion into diplococci or

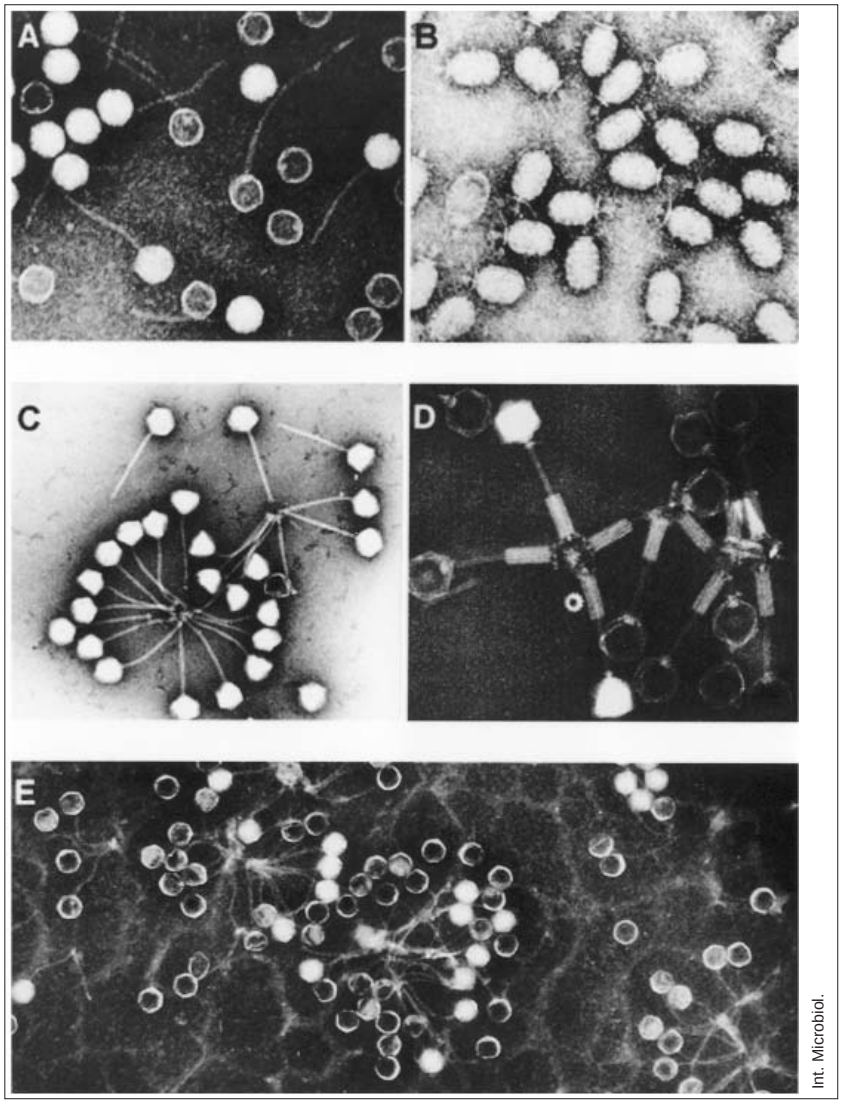

Fig. 5. Electron micrographs of negatively stained preparations of purified pneumococcal bacteriophages. (**A**) Dp-1; (**B**) Cp-1; (**C**) HB-3; (**D**) EJ-1, (**E**) MM1.

short chains, the typical morphology of R6 and M31 strains, respectively (Fig. 4C). In addition, the preparation of chimeric enzymes by means of a translational fusion between the gene coding for green fluorescent protein (GFP), *gfp*, and *lytB* showed that LytB accumulates at the cell poles, where it might very selectively lyse the cell wall (not shown). Variations in the composition of choline motifs might account for the selective recognition of LytB; for example, there could be specific receptors for the enzyme at the polar region of the cell surface, where peptidoglycan hydrolysis takes place. This could explain why LytB, unlike LytA and LytC, does not behave as an autolytic enzyme. Since cellular dispersion might be a major factor in virulence, the lack of LytB should make pneumoccal dissemination during the infectious process more difficult.

Pneumococcal bacteriophages

The lack of a simple culture medium and reproducible techniques to visualize phage plaques on pneumococcal lawns delayed the isolation and identification of phages in this system. In fact, it was not until 1975 that a phage was first isolated, independently by C. Ronda and M. McDonnell (Dp-1), in A. Tomasz's laboratory [24], and by J.G. Tiraby, in M. Fox's laboratory (ω1) [37]. Later on, in 1981, our laboratory isolated the Cp (Complutense phage) family [30]. Since then we have analyzed a series of lytic and temperate phages [21]. Their morphologies are shown in Fig. 5.

Using this system, we also carried out transfection experiments employing pneumococcal phage Cp-1 DNA as donor

material and intact competent cells as receptors. While transfecting activity disappeared when Cp-1 DNA was treated with proteolytic enzymes, transfection with bacteriophage Dp-4 DNA was not affected [31]. Salas and Viñuela had previously shown that the genomes of several small *Bacillus* phages are associated with proteins [33]. It was also reported that a protein covalently linked to the 5´ end of phage DNA, through a phosphodiester bond between serine and dAMP, was essential for replication of ϕ29 phage DNA in vivo [14]. As in the case of *Bacillus subtilis* ϕ29 phage, Cp-1 DNA has a protein covalently bound to DNA (in this case through a phosphothreonine bond) [10,13] that serves as the initiator of its DNA replication by a "sliding-back" mechanism, similar to that described for ϕ29 phage [25]. These observations explain the loss of transfecting ability of the Cp-1 DNA-protein complex treated with proteolytic enzymes.

Our laboratory also showed that the presence of choline in cell-wall receptors was essential for the adherence of certain phages, e.g. Dp-1. In fact, when choline was replaced by ethanolamine, pneumococcal cells could not adsorb to the cell wall. In addition, the cells were resistant to lysis by bacterial or phage-encoded lysins [21]. More recently, sequencing of the complete genomes of two lytic and two temperate phages [23,27–29], Cp-1, Dp-1, MM1 and EJ-1, respectively, enabled us to determine their functional organization. Several of the genes in these clusters are the focus of our current research, for example, a gene in the genome of Dp-1 (*orf55*, coding for the anti-receptor) that codes for a protein exhibiting motifs similar to those in ChBPs for recognition of choline in the cell wall. This observation explains the choline requirement of Dp-1 phage receptors for adsorption of the phage Dp-1 [21]. We have also described the structural organization of four ChBPs, identified as CWHs, that are responsible for the specific recognition of choline units and which liberate progeny from infected *S. pneumoniae.* These pneumococcal lytic enzymes have great intrinsic flexibility, which enables them to change the recognition units from the C-terminal the N-terminal region (Fig. 4). This ability fulfills the functional exchanging properties that Doolittle attributed to a well-defined domain [22]**.** We also observed that the murein hydrolase of phage Cp-7, which is very similar to Cp-1, was an exception to the choline dependency of phage lytic enzymes, since the enzyme degrades the pneumococcal wall in the absence of the amino alcohol. These features are reflected in the primary structure of the protein, as the motifs for choline recognition are replaced by three large, identical 48-amino-acid motifs [11]**.**

To obtain direct experimental evidence for the modular organization of pneumococcal enzymes, we constructed chimeric functional phage-bacterial lytic enzymes [6]. The functional characteristics of these proteins differed from those of the parental enzymes. Furthermore, we produced intergeneric chimeric enzymes with *Clostridium acetobutylicum* [21]**.** The results allowed us to postulate that this kind of exchange can endow the pneumococcal system with great plasticity, in that new enzymatic combinations, by means of simple genetic recombination, are possible. Experiments based on the isolation, cloning and purification of the Dp-1 phage lytic enzyme Pal supported this working hypothesis. This enzyme, which is an amidase, has an N-terminal region very similar to that of a lactococcus phage amidase, whereas the C-terminal domain is highly similar to those recognizing choline-containing substrates. This means that Pal is a natural intergeneric chimera [36] and suggests that the catalytic efficiency of a primigenic enzyme lacking a recognition unit can be improved by joining it with such a unit.

Since 1927 it has been known that temperate phages possess genes that, in the lysogenic state, are capable of expressing toxins associated with bacterial virulence. For example, in the case of scarlet fever, it was previously observed that filtered supernatants of toxigenic streptococcal cultures contained scarlatinal toxin. Although poorly understood at the time, this phenomenon was suggested to be due to transduction—the transfer of genetic material to a bacterial cell via phage infection [38]. The hypothesis that bacteria acquire virulence properties from phages has since been widely accepted. In fact, it has been shown that many virulence genes are transferred among bacteria by phages (via transduction) and other mobile genetic elements, such as plasmids (via conjugation), as well as by incorporation of the phage genome into the bacterial chromosome. This type of mechanism was later ascribed to cholera, diphtheria and several other bacterial diseases, and is most likely the case in *S. pneumoniae*. In fact, 70% of pneumoccocal genomes from clinical isolates have temperate phages. How these phages contribute to pneumococcal virulence is under investigation in our laboratory. It is probable that the causes of virulence also follow other patterns.

Since their discovery by d'Herelle and Twort some 90 years ago, phages have been used as an antibacterial therapy in Eastern Europe [21]. A brilliant experimental variant to phage therapy was developed by Loeffler et al. [20], who used lytic enzymes; in the case of pneumococcus, these assays included the Pal amidase [20]**.** More recently, this experimental approach was extended to *Bacillus anthracis*, with the aim of fighting anthrax spread by bioterrorism [35], and to *Streptococcus pyogenes*.

The lytic enzymes used in such experimental approaches have been designed as *enzybiotics*, and instillations of these purified enzymes have been tested in a murine model of

pharynx infection. In addition, a murine septicemia model of *S. pneumoniae* was developed in collaborative research between my laboratory and the Instituto Carlos III, in Madrid. Our results showed that a single dose of Cpl-1 lysozyme or Pal amidase could protect experimental animals from fatal infection by virulent pneumococcus obtained from a clinical isolate. So far, there have been no adverse reactions arising from the application of these murein hydrolases [17]. Thus, enzybiotics offer a promising new therapeutic approach to lessen the alarming resistances evolved by many bacterial species, especially pneumococcus, against a wide array of antibiotics due to the genetic plasticity of bacteria and to the misuse of these drugs.

Conclusion

The capsular polysaccharide is an absolute requirement for pneumococcal infections and thus serves as the basis for the development of the polysaccharide and conjugate vaccines that are currently available. Elucidation of the wide genetic variability underlying capsular formation requires detailed knowledge of the molecular mechanisms involved in producing the 90 serotypes already known. Moreover, detailed analysis of the unique characteristics of the lytic enzymes of pneumococcus and its phages has provided valuable information on their biological roles while also leading to the therapeutical use of these enzymes in treating infections.

Phages are the most abundant living entities in nature: there are an estimated 10^{31} phage particles on Earth, and they represent a pool of biological agents whose potential for the control of human pathogens has not yet been explored [21]. The detailed knowledge provided by analysis of the crystalline structure of phage lytic enzymes—including Cpl-1 lysozyme (see Cover of this issue), recently established by Hermoso et al. [15]—will surely contribute to establishing a wide range of clinical applications of these proteins.

Seneca, the Roman philosopher born in the Iberian Peninsula, stated that: "Peoples that forget their history are sentenced to repeat it." Although microorganisms have caused more deaths than all wars throughout history (more than 500 million in the twentieth century alone), they have also contributed to the enormous progress in molecular biology and to the improved social welfare of developed countries. Hopefully, microbial-based therapies will also contribute to fighting infectious diseases in less fortunate countries, in which they remain the main cause of mortality.

Acknowledgements. My friends and collaborators E. García, C. Ronda, P. García and J. L. García, who have dedicated many years of effort and commitment to work in pneumococcus and its phages, and A. Tomasz, my mentor at Rockefeller University, deserve special recognition and my long-lasting gratitude. I sincerely thank both my former and current students and the many colleagues who have contributed to enriching the more than 40 years that I have carried out scientific research. I especially thank Mercè Piqueras and Marta León, from the Publication Board of INTERNATIONAL MICROBIOLOGY, for their efficient collaboration in the final form of this article, based on the inaugural lecture of the 19th Congress of the Spanish Society for Microbiology.

References

1. Arrecubieta C, López R, García E (1996) Type 3-specific synthase of *Streptococcus pneumoniae* (Cap3B) directs type 3 polysaccharide biosynthesis in *Escherichia coli* and in pneumococcal strains of different serotypes. J Exp Med 184:449–455
2. Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med 76:127–158
3. Coffey TJ, Enright MC, Daniels M, Morona JK, Morona R, Hryniewicz W, Paton JC, Spratt BG (1998) Recombinational exchanges at the capsular polysaccharide biosynthetic locus lead to frequent serotype changes among natural isolates of *Streptococcus pneumoniae*. Mol Microbiol 27:73–83
4. De las Rivas B, García JL, López R, García P (2002) Purification and polar localization of pneumococcal LytB, a putative endo-β-*N*-acetilglucosaminidase: the chain dispersing murein hydrolase. J Bacteriol 184:4988–5000
5. Fernández-Tornero C, López R, García E, Giménez-Gallego G, Romero A (2001) A novel solenoid fold in the cell wall anchoring domain of the pneumococcal virulence factor LytA. Nat Struct Biol 8:1020–1024
6. Díaz E, López R, García JL (1990) Chimeric phage-bacterial enzymes: A clue to the modular evolution of genes. Proc Natl Acad Sci USA 87:8125–8129
7. García E, García JL, Ronda C, García P, López R (1985) Cloning and expression of the pneumococcal autolysin gene in *Escherichia coli*. Mol Gen Genet 201:225–230
8. García E, García JL, García P, Arrarás A, Sánchez-Puelles JM, López R (1988) Molecular evolution of lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages. Proc Natl Acad Sci USA 85:914–918
9. García E, García P, López R (1993) Cloning and sequencing of a gene involved in the synthesis of the capsular polysaccharide of *Streptococcus pneumoniae* type 3. Mol Gen Genet 239:188–195
10. García E, Gómez A, Ronda C, Escarmís C, López R (1983) Pneumococcal bacteriophage Cp-1 contains a protein bound to the 5´ termini of its DNA.Virology 128:92–104
11. García P, García JL, García E, Sánchez-Puelles JM, López R (1990) Modular organization of the lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages. Gene 86:81–88
12. García P, González MP, García E, García JL, López R. (1999) The molecular characterization of the first autolytic lysozyme of *Streptococcus pneumoniae* reveals evolutionary mobile domains. Mol Microbiol 33:128–138
13. García P, Hermoso JM, García JA, García E, García JL, López R, Salas M (1986) Formation of a covalent complex between the terminal protein of pneumococcal bacteriophage Cp-1 and 5´-dAMP. J. Virol. 58:31–35
14. Hermoso JM, Salas M (1980) Protein p3 is linked to the DNA of phage ϕ29 through a phosphoester bond between serine and 5´-dAMP. Proc Natl Acad Sci USA 77:6425–6428

15. Hermoso JA, Monterroso B, Albert A, Galan B, Ahrazem O, García P, Martínez-Ripoll M, García JL, Menéndez M (2003) Structural basis for selective recognition of the pneumococcal cell wall by modular endolysin from phage Cp-1. Structure 11:1239–1249
16. Höltje JV, Tomasz A (1975) Specific recognition of choline residues in the cell wall teichoic acid by the *N*-acetylmuramyl-L-alanine amidase of pneumococcus. J Biol Chem 250:6072–6076
17. Jado I, López R, García E, Fenoll A, Casal J, García P, Spanish Pneumococcal Infection Study Network (2003) Phage lytic enzymes as therapy for antibiotic-resistant *Streptococcus pneumoniae* infection in a murine sepsis model. J Antimicrob Chemother 52:967–973
18. Llull D, Muñoz R, López R, García E (1999) A single gene (*tts*) located outside the cap locus directs the formation of *Streptococcus pneumoniae* type 37 capsular polysaccharide. Type 37 pneumococci are natural, genetically binary strains. J Exp Med 190:241–252
19. Llull D, García E, López R (2001) Tts, a processive β-glucosyltransferase of *Streptococcus pneumoniae*, directs the synthesis of the branched type 37 capsular polysaccharide in pneumococcus and other Gram-positive species. J Biol Chem 276:21053–21061
20. Loeffler J, Nelson D, Fischetti VA (2001) Rapid killing of *Streptococcus pneumoniae* with a bacteriophage cell wall hydrolase. Science 294:2170–2172
21. López R, García E (2004) Recent trends on the molecular biology of pneumococcal capsules, lytic enzymes, and bacteriophage. FEMS Microbiol Rev doi:10.1016/j.femsre.2004.05.002
22. López R, García E, García P, García JL (2004) Cell wall hydrolases. In: Tuomanen EI (ed) The Pneumococcus. ASM Press Washington, DC, pp 75-89
23. Martín AC, López R, García P (1996) Analysis of the complete nucleotide sequence and functional organization of the genome of *Streptococcus pneumoniae* bacteriophage Cp-1. J Virol 70:3678-3687
24. McDonnell M, Ronda C, Tomasz A (1975) "Diplophage": a bacteriophage of *Diplococcus pneumoniae*. Virology 63:577–582
25. Meijar WJ, Horcajadas JA, Salas M (2001) ϕ29 Family phages. Microbiol Mol Biol Rev 65:261–287
26. Mollerach M, López R, García E (1998) Characterization of the *galU* gene of *Streptococcus pneumoniae* encoding a uridine diphosphoglucose pyrophosphorylase: a gene essential for capsular polysaccharide biosynthesis. J Exp Med 188:2047–2056
27. Obregón V, García JL, García E, López R, García P (2003) Genome organization and molecular analysis of the temperate bacteriophage MM1 of *Streptococcus pneumoniae*. J Bacteriol 185:2362–2368
28. Obregón V, García JL, García E, López R, García P (2004) Peculiarities of the MM1, a temperate phage of *Streptococcus pneumoniae*. Int Microbiol 7:133–137
29. Romero P, López R, García E (2004) Genomic organization and molecular analysis of the inducible prophage EJ-1, a mosaic myovirus from an atypical pneumococcus. Virology 322:239–252
30. Ronda C, López R, García E (1981) Isolation and characterization of a new bacteriophage, Cp-1, infecting *Streptococcus pneumoniae*. J Virol 40:551–559
31. Ronda C, López R, Gómez A, García E (1983) Protease-sensitive transfection of *Streptococcus pneumoniae* with bacteriophage Cp-1 DNA. J Virol 48:721–730
32. Sánchez-Puelles JM, Ronda C, García JL, García P, López R, García E (1986) Searching for autolysin functions. Characterization of a pneumococcal mutant deleted in the *lytA* gene. Eur J Biochem 158:289-293
33. Salas M, Viñuela E (1980) Proteins covalently linked to viral nucleic acid. Trends Biochem Sci 5:191–193.
34. Sanz J, López R, García JL (1988) Structural requirement of choline derivatives for convertion of pneumococcal amidase. FEBS Lett 232:308-312
35. Schuch R, Nelson D, Fischetti VA (2002) A bacteriolytic agent that detects and kills *Bacillus anthracis* (2002) Nature 418:884–889
36. Sheehan M, García JL, López R, García P (1997) The lytic enzyme of the pneumococcal phage Dp-1: a chimeric lysin of intergenic origin. Mol Microbiol 25:717–725
37. Tiraby JG, Tiraby E, Fox MS (1975) Pneumococcal bacteriophages. Virology 68:566–569
38. Wagner PL, Waldor MK (2002) Bacteriophage control of bacterial virulence. Infect Immun 70:3985–3993

Streptococcus pneumoniae y sus bacteriófagos: una prolongada controversia

Resumen. Las enfermedades infecciosas matan anualmente a unos 15 millones de personas y la OMS estima que 1,6 millones de esas muertes se deben a infecciones neumocócicas. *Streptococcus pneumoniae* (neumococo), una bacteria con una notable contribución histórica a la biología, es el mejor ejemplo que ilustra el rápido desarrollo de la resistencia a los antibióticos, lo que puede originar un grave problema sanitario. Esta revisión analiza las bases moleculares de los dos factores principales de virulencia en el neumococo, la cápsula y las hidrolasas de la pared celular y describe nuevos enfoques para el desarrollo de nuevas herramientas médicas para prevenir las infecciones neumocócicas. También se analizan el conocimiento actual de la posible contribución de los fagos de neumococo a la virulencia de esta bacteria y el uso como arma terapéutica de las enzimas líticas codificadas por estos fagos. [**Int Microbiol** 2004; 7(3):163–171]

Palabras clave: *Streptococcus pneumoniae* · neumococo · cápsula · bacteriófagos de neumococo · enzimas líticas

Streptococcus pneumoniae e seus bacteriófagos: uma prolongada controvérsia

Resumo. As enfermidades infecciosas matam anualmente cêrca de 15 milhões de pessoas. A OMS estima que 1,6 milhões dessas mortes são decorrentes de infecções pneumocócicas. *Streptococcus pneumoniae* (pneumococo), uma bactéria com uma notável contribuição histórica para a biologia, é o melhor exemplo que ilustra o rápido desenvolvimento da resistência aos antibióticos, o que pode originar um grave problema sanitário. Esta revisão analisa as bases moleculares dos fatores principais de virulência no pneumococo, a cápsula e as hidrolases da parede celular e descreve novos enfoques para o desenvolvimento de ferramentas médicas inovadoras para prevenir as infecções pneumocócicas. Também foi analisado o atual conhecimento da possível contribuição dos fagos de pneumococo para a virulência desta bactéria e o uso como arma terapéutica das enzimas líticas codificadas por estes fagos. [**Int Microbiol** 2004; 7(3):163–171]

Palavras chave: *Streptococcus pneumoniae* · pneumococo · cápsula · bacteriófagos de pneumococo · enzimas líticas