at Pliva, PLD118 does not appear to be metabolized via the cytochrome P450 (CYP450) enzyme system and, therefore, unlike the azole antifungals, could carry a low risk of interactions with other drugs. This could be an important advantage in the treatment of patients taking multiple drugs.

An oral formulation of PLD118 is now undergoing Phase I clinical evaluation. Scientists at Pliva believe that patients with *Candida* infections, including non-albicans strains and azole-resistant strains, could benefit from treatment with PLD118, and these patients will be studied in ongoing and future clinical trials.

Other new antifungal compounds

A variety of antifungal agents are currently under investigation as treatments for candidiasis. Some are variations on the triazole theme, such as voriconazole, posaconazole and ravuconazole, but others, such as PLD118, offer completely new approaches to treating the infection.

The triazole antifungal drugs inhibit fungal CYP450-dependent lanosterol 14-α-demethylase, which is essential for the conversion of lanosterol to ergosterol in fungal cell membranes. Inhibition of this enzyme causes an accumulation of toxic ergosterol precursors in membranes and thus inhibits cell growth. The newer triazole drugs have a broader spectrum of activity than the older drugs that includes *Aspergillus* sp. and possibly other fungi as well as *Candida* sp. [6].

New classes of antifungal drugs – echinocandins, pneumocandins, sordarin derivatives, and the nikkomycins – take different approaches [6]. The echinocandins (caspofungin, anidulafungin, mycofungin) kill fungal cells by destabilizing their cell walls. This is achieved by inhibiting the 1,3-β-D-glucan synthase complex that is responsible for incorporating glucan fibrils into cell walls. Because glucan fibrils are not present in human cells, this is a promising fungi-specific drug target. So far, the echinocandins show promise against a variety of fungi and triazole-resistant *Candida* sp. [6].

The sordarin derivatives show most promise against *Candida* infections. These drugs block fungal protein synthesis by inhibiting elongation factor-2, which promotes the movement of the ribosome along mRNA. Finally, the nikkomycins are another new class of antifungal agents that competitively inhibits chitin synthase and blocks the formation of chitin, a component of the fungal cell wall. Although the nikkomycins have shown activity against some fungi, they have not, to date, shown significant activity against yeasts such as *Candida* sp. [6].

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Viral Trojan horse for combating tuberculosis

Nicole Johnston, freelance writer

The emergence of pathogenic bacteria resistant to one or more antibiotics has outpaced the development of new drugs. Using bacteriophage, Raul Barletta (Dept of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE, USA) and colleagues at the California Pacific Medical Center (San Francisco, CA, USA) have devised a promising new approach to killing the intracellular pathogens *Mycobacterium avium*, which commonly afflicts AIDS patients, and *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Their findings were presented at the *41st Interscience Conference on Antimicrobial Agents and Chemotherapy* hosted by the American Society for Microbiology in Chicago, IL, USA [1].

Why bacteriophage?

Bacteriophage (phage) are viruses that infect their specific bacterial host, but do not infect other bacterial species or

eukaryotic cells. Phage have been studied for use as anti-infectives for nearly a century but waned in popularity in western society following the advent of the antibiotic era [2]. However, the threat of a post-antibiotic era is renewing interest in phage research.

Lytic bacteriophage are lethal to their bacterial hosts by virtue of their replication. Phage surface-ligands target bacterial cell-surface receptors, enabling the phage to inject their nucleic acid and take over bacterial metabolism to produce progeny phage. At the end of the lytic cycle, new phage exit the cell by bursting through the bacterial cell-wall peptidoglycan, which consists of cross-linked peptides and sugars. To do this, the phage produce a protein called holin, which permeabilizes the cell membrane. An enzyme called lysin then enters through the cell membrane and rapidly cleaves the bonds that maintain peptidoglycan structure, destroying the cell wall and enabling phage particles to escape [3].

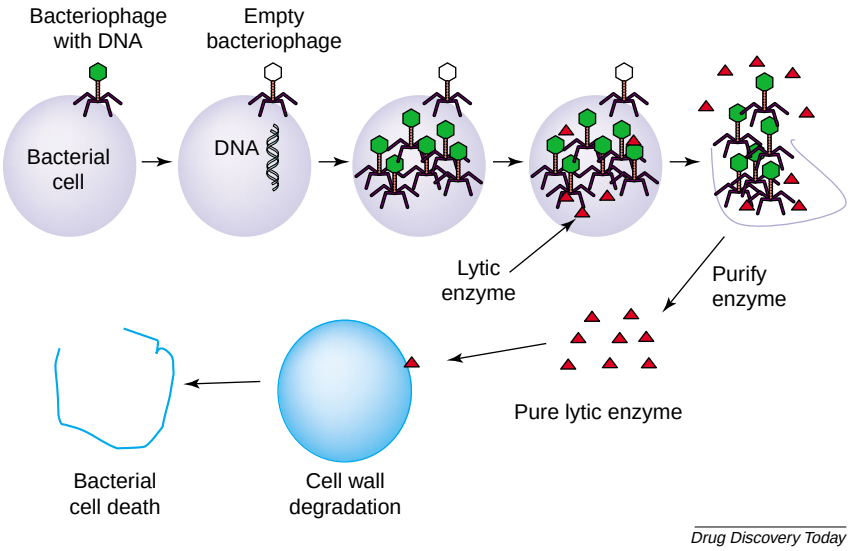

Figure 1. The lytic cycle and purification of lytic enzymes. Specific ligands on the bacteriophage (phage) tail target bacterial cell-surface receptors, enabling the phage to inject their nucleic acid and produce progeny phage. The progeny phage escape from the bacteria by producing lysin, an enzyme that degrades the peptidoglycan structure of the bacterial cell wall, causing bacterial cell death and allowing the phage to propagate. By purifying the lytic enzymes of phage, scientists have harnessed the antibacterial properties of the phage while eliminating the difficulty of having to deliver whole phage to bacterial cells found on mammalian mucous membranes.

Trojan horse

Until now, targeting intracellular pathogens with phage was believed to be limited in scope because bacteriophage cannot reach bacterial hosts residing inside eukaryotic cells [2]. To overcome this problem, Barletta and colleagues devised a 'Trojan horse' approach by using the non-pathogenic mycobacterium, *Mycobacterium smegatis*, to ferry lytic phage TM4 into macrophages. Once inside, the lytic phage then escape from *M. smegatis* and begin infecting and killing the pathogens.

After establishing macrophage monolayers infected with *Mycobacterium avium* or *Mycobacterium tuberculosis in vitro*, the cells were then incubated with either TM4-phage-infected *M. smegmatis* or non-infected *M. smegmatis*. 'Each species is initially contained within individual vacuoles, which subsequently fuse, thereby bringing *M. smegatis* together with either pathogen within the same vacuole,' explains Barletta. 'Pathogenic bacteria are known to modify vacuoles in some way to allow this fusion to take place, but the precise modification is not known,' he says. 'When in the same vacuole, the viruses start lysing the non-virulent *M. smegmatis* and start infecting the pathogenic bacteria.' The team found that this resulted in a tenfold and 100-fold reduction in the amount of *M. avium* and *M. tuberculosis* present, respectively. Subsequent studies, Barletta says, will involve carrying out this study in an animal model, as well as devising other ways of introducing phage into the interior of vacuoles, such as using liposome-mediated delivery.

Harnessing lysins

Using another new approach, researchers at The Rockefeller University (New York, NY, USA) have developed a highly potent means of killing pathogenic bacteria that does not require delivery of the actual phage. Instead, the group led by Vincent A. Fischetti (Co-Head, laboratory of Bacterial Pathogenesis and Immunology at The Rockefeller University) has isolated and purified species-specific lytic enzymes, or lysins, from double-stranded DNA phage, and are using these enzymes directly to eliminate the presence of pathogenic streptococci (Fig. 1) [4,5]. So far, they have shown that a lysin specific for group A streptococci, which causes 'strep throat', rheumatic fever and necrotizing fasciitis, can kill 10^7 streptococci in culture with as little as 10 ng of purified lysin [4].

In animal studies, a single treatment with the same quantity of lysin was sufficient to eliminate group A streptococci from the nasopharynx of heavily colonized mice. Most recently, Fischetti's group has demonstrated that a lysin specific for *Streptococcus pneumoniae*, which causes otitis media and pneumonia, can eradicate the organism in colonized mice and was even potent against penicillin-resistant *S. pneumoniae* [5].

Fischetti says that their approach will be most useful as a means of preventing

illness. 'The organisms you carry on your mucous membranes are the cause of infection 80% of the time, but people don't realize this,' he says. 'We have tolerated these organisms until they cause infection, and then we treat them. Finally we have something to eliminate this reservoir.' Resistance should not be a problem either; Fischetti says they have not seen any resistance at all despite repeated attempts to select for resistant bacteria. Moreover, unlike antibiotics, the lysins only target their specific host and don't affect neighbouring bacteria, thus avoiding disrupting the normal flora. These enzymes are highly stable and could be used in a liquid form as a nasal spray or lyophilized before use. The group is currently working on enzymes from other organisms, including staphylococci, enterococci, and *Bacillus anthracis*. Fischetti says they are currently in discussions with pharmaceutical companies and the food industry to begin Phase I clinical trials with the streptococcal enzymes.

'Both approaches are highly promising,' says Ry Young, an expert in phage biology at the Dept of Biochemistry and Biophysics, Texas A&M University (College Station, TX, USA). 'All bacterial control on earth is done by bacteriophage. There has been a war for billions of years where phage are preying on bacteria,' he says. 'It is clear that there will be many potential applications of phage biology. We are running out of antibiotics, so we have to look at the alternatives.'

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A 'C' change for hepatitis treatment

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A novel oral compound for the successful treatment of the hepatitis C virus (HCV) has been selected for development by Lilly (Indianapolis, IN, USA) and Vertex Pharmaceuticals (Cambridge, MA, USA). The compound, LY570310 (VX950), is an inhibitor of the hepatitis C protease, NS3-4A, which is considered to be essential for the replication of the virus. LY570310 is the first candidate of a novel class of antiviral drugs that are under investigation for the inhibition of HCV. It is currently in preclinical trials and is expected to enter Phase I clinical trials in early 2003.

The virus

HCV infects 3–4 million people in the USA (according to the Center for Disease Control and Prevention, Atlanta, GA, USA) and the worldwide figure is close to 170 million (according to the World Health Organization; http://www.who.int). Many victims are unaware of the infection, which can remain undetected for up to 20 years; this indicates that worldwide infection could be much higher than estimated. HCV causes inflammation of the liver, which can lead to fibrosis, cirrhosis, liver cancer and, ultimately, liver failure. Complications resulting from HCV infection claim 8000–10,000 lives, annually, in the USA (http://www.hepatitiscaware.org/).

HCV is a small (40–60 nm in diameter), enveloped single-stranded RNA virus of the family *Flaviviridae*. Detection of the disease is through elevated levels of the enzyme alanine aminotransferase (ALT); chronic HCV can result in an increase in ALT levels by up to 20-fold. The disease is initially characterized by flu-like symptoms: aching limbs, fever, headaches, appetite suppression and weight loss. Chronic infection occurs in up to 85% of cases and can be transmitted through the sharing of IV needles (in ~70% of cases) and blood transfusions (before routine testing became available in 1992).

Current treatments

At present, the only treatment for HCV that is approved by the Food and Drug Administration is interferon-α (IFN-α), taken in combination with the antiviral, nucleoside analogue ribavirin [1]. Ribavirin is only approved as a treatment of HCV when used in conjunction with IFN-α (Rebetron™, ICN Pharmaceuticals, Costa Mesa, CA, USA). IFN-α is known to bind to a membrane receptor, which elicits a signalling cascade resulting in the expression of target cell killing by lymphocytes.

Current treatments have only a 40–60% success rate, and can produce unpleasant side effects such as insomnia, depression, extreme fatigue, skin rashes, fevers, nausea and weight loss, which are similar to the symptoms of HCV.

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