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Review

Phage therapy: the *Escherichia coli* experience

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Phages have been proposed as natural antimicrobial agents to fight bacterial infections in humans, in animals or in crops of agricultural importance. Phages have also been discussed as hygiene measures in food production facilities and hospitals. These proposals have a long history, but are currently going through a kind of renaissance as documented by a spate of recent reviews. This review discusses the potential of phage therapy with a specific example, namely *Escherichia coli*.

Why *E. coli*?

The World Health Organization estimates that 5 million children die each year as a consequence of acute diarrhoea (Snyder & Merson, 1982). *Escherichia coli* is the cause of a third of cases of childhood diarrhoea in developing and threshold countries (Albert *et al.*, 1995) and is also the most prominent cause of diarrhoea in travellers to developing countries (Black, 1990). *E. coli* is also prominently associated with diarrhoea in pet and farm animals. Due to its malleable genetic character, *E. coli* has one of the widest spectra of disease of any bacterial species (Donnenberg, 2002). The recent emergence of *E. coli* O157 as a major food pathogen is a lively reminder of its dynamic character. Furthermore, *Shigella* species, the cause of dysentery, taxonomically constitute a subspecies of *E. coli*.

In addition, effective treatment and prevention measures are lacking for *E. coli* diarrhoea. The mainstay of treatment is oral rehydration (Bhan *et al.*, 1994). This simple and inexpensive measure has saved countless lives, but it does not influence the natural course of disease and has no intrinsic antibacterial activity. Antibiotic use is of doubtful value since resistance is widespread in *E. coli*, and vaccines are still in the early development phase (Savarino *et al.*, 2002). Water and sanitation programmes could improve the quality of drinking water, but are prohibitively expensive for many developing countries.

Furthermore, there are good historical reasons to single out *E. coli* diarrhoea and *Shigella* dysentery for phage therapy. As early as 1919, Félix d'Hérelle, the co-discoverer of phages, advocated their use for the treatment of bacterial diarrhoea (Summers, 1999, 2001). American pharmaceutical companies sold phage-based therapy in the 1930s (Duckworth, 1999). During World War II the German and Soviet armies used phages against dysentery and the US army conducted classified research on it (Häusler, 2003). After the war the Eliava Phage Institute in Tbilisi, Georgia, conducted a well-designed field study in the 1960s that came close to the standards of a placebo-controlled clinical trial (Babalova *et al.*, 1968). More recently, British scientists reported on the successful veterinary application of *E. coli* phages in the 1980s (Smith & Huggins, 1982, 1983; Smith *et al.*, 1987a, b) and phage therapy is now back in the headlines (Merril *et al.*, 2003).

Where to get the tools?

E. coli phages are commonly isolated from sewage, hospital waste water, polluted rivers and faecal samples of humans or animals. A surprising morphological diversity of coliphages is isolated from such samples (Ackermann & Nguyen, 1983). Stool samples from healthy subjects yielded mainly lambda-like Siphoviridae (Dhillon *et al.*, 1976; Furuse *et al.*, 1983), while stools of diarrhoea patients gave predominantly T4-like Myoviridae (phages with contractile tails) (Furuse, 1987). However, different phages were also isolated from the same stool samples when using different indicator cells (Chibani-Chennoufi *et al.*, 2004d). The mammalian gut seems to be the natural habitat of T4-like coliphages. Large T4 collections have been compiled (Ackermann & Krisch, 1997) and were investigated for genome evolution by Repoila *et al.* (1994). Important insights into the mechanism of host cell recognition were gained from the genetic analysis of the tail fibres that recognize outer-membrane proteins such as OmpC as well as inner parts of the lipopolysaccharide as cellular receptors. The comparison of the phage adhesins (gp37 in T4, gp38 in T2 phage) revealed a patchwork of shared and unique protein segments. Recombination between short regions of homology led to chimeric fibres and the acquisition of new host-range determinants (Tétart *et al.*, 1998). Genetic engineering might thus offer the possibility to extend the host range of a single master T4-like phage. In fact, some coliphages use this strategy naturally: phage Mu inverts the orientation of the receptor-interacting gene by a phage-encoded recombinase (Kamp *et al.*, 1978), and Scholl *et al.* (2001) described a coliphage possessing two different tail fibre proteins that showed the combined host range of phages containing one or the other protein. About 30 different O serotypes must be lysed to cover the majority of EPEC and ETEC strains worldwide

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(Robins-Browne, 1987; Goodridge *et al.*, 2003). Cocktails of several phages will be necessary to get sufficient breadth of host range and to reduce the probability of resistance developing.

Phage virulence factors

A number of important human bacterial pathogens owe their virulence factors to prophages integrated into the bacterial genome (for recent reviews see Brüssow *et al.*, 2004; Wagner & Waldor, 2002). This is also true for coliphages: in the sequenced *E. coli* O157 strains prophages encode the major virulence factor, the Shiga-like toxin. Even the 'academic' phage lambda carries *bor*, which confers serum resistance to the lysogen (Barondess & Beckwith, 1990). A survey of phage and prophage genomes has revealed that mainly phages of the lambda-like genus of Siphoviridae harbour proven or potential virulence factors (Boyd & Brüssow, 2002; Canchaya *et al.*, 2003). Many other genera of coliphages can establish lysogeny, but only few have actually been shown to contain established virulence genes. Nevertheless, to be on the safe side, temperate phages should not be selected for phage therapy. A priori, candidates for phage therapy should come from the group of 'professional virulent' phages. The term 'virulent' is in this context misleading since for phage biologists it means obligate lytic phages as opposed to temperate phages. T4-like phages belong to this class and no sequenced member carries known virulence genes (http://phage.bioc.tulane.edu/). These phages do not have integrases; they degrade and recycle the bacterial host genome for their own DNA synthesis and thus lack the molecular basis for coexistence with the host (Karam, 1994). This property also reduces the likelihood of *in situ* DNA transformation resulting from phage lysis. Sequencing of the phages enables exclusion of undesired genes or known virulence factors. However, even in the case of T4, many genes lack known functions and database matches (Miller *et al.*, 2003). This genetic 'terra incognita' is even greater in T4-like coliphages (http://phage.bioc.tulane.edu/).

Safety issues

Several safety issues have been raised over the years with respect to the therapeutic use of phages. Extensive recombination clearly goes on among T4-like phages, as one can see by examining the relationships of various genes that have been sequenced (Repoila *et al.*, 1994). However, one does not see the same sort of reshuffling of modules (i.e. groups of functionally related genes) as described for lambdoid coliphages or dairy phages (Chibani-Chennoufi *et al.*, 2004b). Since no toxin genes have been found in the T4-like phages, the issue of recombinants among T4-like phages is not particularly relevant for phage therapy.

Another issue concerns phage gene activity in mammalian cells. For example, a galactose transferase gene incorporated into the phage lambda genome could be expressed as mRNA and translated as protein in human fibroblast cells exposed to the viable phage or phage DNA (Geier & Merril, 1972; Merril *et al.*, 1971). However, there is no indication that the complex series of processes involved in reproducing phage T4 could be carried out in a eukaryotic cell, where the whole machinery and regulatory mechanisms are very different from those in bacteria. There are in general even substantial limitations as to the range of bacteria in which infection with a given phage can occur.

Furthermore, geneticists have reported that minute amounts of orally fed phage M13 DNA were taken up by the gut and could even be integrated into the mouse chromosome (Schubbert *et al.*, 1997). This observation was not specific for phage DNA since plasmid DNA had the same fate (Schubbert *et al.*, 1998). It is difficult to comment on these intriguing data since the experiments have not been repeated by any other group. With all the phages and bacteria constantly present in the human gut, one would think it would have been reported more often if this were a common occurrence. Phage lambda or M13 is normally found in 1–4 % of stool samples from humans (Schluederberg *et al.*, 1980). About every third stool sample from diarrhoea patients yielded a coliphage even though only two indicator strains were being used in this particular study (Chibani-Chennoufi *et al.*, 2004d). This percentage was 70 % with diarrhoea patients and 34 % with healthy controls when 10 indicator strains were used (Furuse, 1987).

Without knowing we constantly consume phages with our fermented food in yogurt (Brüssow *et al.*, 1994), sauerkraut (Lu *et al.*, 2003b), pickles (Lu *et al.*, 2003a) or salami (Chibani-Chennoufi *et al.*, 2004c). From a clinical standpoint, phages appear to be innocuous. Oral coliphages were given in controlled clinical tests to many thousands of children and adults in the former Soviet Union – apparently without major adverse effects (see below). We conducted a small safety trial with adult volunteers who received a total of 10^8 p.f.u. T4 phage orally. The volunteers did not report adverse events and the levels of two enzymes diagnostic for liver cell damage remained in the normal range (Bruttin & Brüssow, 2005).

Industrial phage production

Problems of large-scale production of dysentery phages were addressed in a series of papers during the 1940s (Schade & Caroline, 1943, 1944a, b). Lyophilized phages were shown to be superior to liquid preparations. The advantages included greater stability, decrease in bulk size and simpler means of oral administration as pills. Repeated cycles of freezing and thawing were not linked to activity loss, while acidity below pH 3·5 decreased the phage activity substantially. Out of a large number of substances only egg yolk had some protective properties on the phage preparation. Under dry conditions the phage preparation resisted temperatures at least up to 55 °C; at room temperature the lyophilized phages were stable for at least 14 months. Even early crude preparations containing cellular debris of the lysed *E. coli* did not cause much adverse reaction when

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applied orally, reflecting the relatively low sensitivity of the human gut to endotoxin. T4-like phages grown on a sequenced strain of K-12 devoid of inducible prophages, virulence genes and the O side chain of LPS (Chibani-Chennoufi *et al.*, 2004d, e) maintained their broad host range against pathogenic *E. coli*, so therapeutic phage production could be carried out without biohazard containment conditions and in cheap media, providing an affordable technology even where the burden of *E. coli* infection is the highest.

Pharmacokinetics of oral phage: can oral phage reach its target cell?

When given orally to adult mice and without an antacid, doses as low as 10^3 p.f.u. T4 per ml drinking water led to detection of phage in the faeces. Increasing the oral phage concentration resulted in dose-dependent increases of faecal phages. When given at a dose of 10^4 p.f.u. T4 per ml or higher, phage appeared and disappeared from the faeces with a time lag of 1 and 2 days, respectively (Chibani-Chennoufi *et al.*, 2004e).

A series of elegant mouse experiments conducted in the 1940s revealed other remarkable pharmacological aspects of *Shigella* phages (Dubos *et al.*, 1943). These experiments showed that phages can be carried to wherever they are needed – even across the blood–brain barrier – and multiply there (in the presence of an appropriate bacterial host), at levels that are far higher than those in blood. When 10^5 phage were applied intraperitoneally about 10^2 phage arrived in the brain of control mice. When the experiments were conducted with mice that were intra-cerebrally inoculated with *Shigella*, a massive increase of phage was observed in the brain to 10^9 phage after 8 h, indicating amplification of phage *in vivo* in a tissue that is protected by a tight barrier. When 10^9 phage were injected intraperitonally, phage appeared with titres up to 10^7 in the blood, but began to fall hours after injection. Decades later it was shown that phages circulating in the blood were sequestered in the spleen, but mutant phages with prolonged circulation times could be easily obtained by serial passages in mice (Merril *et al.*, 1996). If the mice received a *Shigella* strain that was not susceptible to the phage *in vitro*, the *in vivo* phage amplification was not observed (Morton & Perez-Otero, 1945). In further experiments, Morton & Engley (1945) demonstrated that the protective effect of the phage could be diluted; limiting efficiency was reached at a 10 : 1 bacterium : phage injection dose. The treatment could be delayed for 3 h after bacterial challenge, and phage treatment could precede the bacterial challenge by 4 days and still prevent death. Heat-inactivated phage had no effect on survival of mice under these conditions. The authors described broad reactivity of the phages against *Shigella* strains; their general observation was a close correspondence between *in vitro* and *in vivo* lytic activity.

Phages represent a quickly diluted medicine in case of absence of the target bacterium and an amplifiable medicine in the presence of the target pathogen. Phage therapy is thus a unique medicine, which challenges current pharmacokinetic concepts. Two types of phage therapy have been distinguished: passive (where the initial phage dose removes the pathogen) and active (where the effect is due to the *in vivo* replication of the phage on the pathogen). In the latter case the phage behaves as a self-amplifying drug, which leads to unfamiliar kinetic phenomena like treatment failure when combined with antibiotic therapy or when given too early and at too low a phage dose (Payne & Jansen, 2003). Some controversy concerns threshold phenomena. *In vitro* experiments using T4 phage suggested that phage amplification did not occur below a critical threshold of 10^4 susceptible host cells per ml (Wiggins & Alexander, 1985). The existence of such a threshold was recently disputed (Kasman *et al.*, 2002). The field is currently dominated by the mathematical modelling of phage infections in test tubes, which do not seem to reflect the *in vivo* situation of phage transit in mice (Chibani-Chennoufi *et al.*, 2004a, e) and rats (Weld *et al.*, 2004).

Human volunteers showed a very similar faecal phage excretion pattern to mice (Bruttin & Brüssow, 2005). More than 10 % of the orally applied phage was recovered from the faeces. When the volunteers were put back on phage-free drinking water, faecal phage titres quickly dropped below the threshold of detection when no infective *E. coli* strain was present in the gut.

Host specificity and collateral damage to the commensal biota?

Antibiotics kill bacteria rather unspecifically and can therefore lead to numerous side effects. In contrast, species specificity is the rule for phages and is commonly quoted as one of the major assets of phage therapy. A polyvalent phage refers to a virus that infects many strains within a bacterial species. Many coliphages have been reported to also infect other enterobacteria than *E. coli*. This has frequently been seen in the phages used therapeutically in Eastern Europe and reported in many laboratories over the years.

In practical terms, the host specificity of coliphages is a major limitation for phage therapy, necessitating the use of phage cocktails potentially causing problems for the commensal *E. coli* gut biota, which might suffer from oral T4 phage exposure. However, mice exposed to an oral four-phage cocktail did not experience a decline of their commensal *E. coli* biota (Chibani-Chennoufi *et al.*, 2004e). Likewise human volunteers orally exposed to phage T4 maintained their commensal *E. coli* population (Bruttin & Brüssow, 2005).

Physiological aspects: starving *E. coli* are not a target

Most studies of T4 development have been conducted under typical laboratory conditions (Abedon *et al.*, 2003; Hadas *et al.*, 1997). The ribosome number was the most important

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factor determining the growth of the coliphage, followed by limitations of the transcription and then the DNA synthesis apparatus (You *et al.*, 2002). However, it was noted as long as 10 years ago that laboratory growth conditions do not correspond to those prevailing in the mammalian gut, the major habitat of T4 (Kutter *et al.*, 1994). In the colon, *E. coli* has to grow under anaerobic conditions and it lacks the enzymes to ferment the polysaccharides available there (Chang *et al.*, 2004). *E. coli* is nutritionally a bystander of anaerobes like *Bacteroides* that dominate the human intestinal biota. The relatively low concentration of commensal *E. coli* in the intestine indicates that the competition for nutrients is high. In fact, *E. coli* from the intestinal lumen is starving and not dividing (Poulsen *et al.*, 1995). An actively growing commensal *E. coli* population with coccoid morphology was found in mucosal microcolonies overlying the epithelial cells of the large intestine (Krogfelt *et al.*, 1993). It is not clear how these factors affect the ability of T4 to infect *E. coli in vivo*. Notably, T4 grows similarly under aerobic and anaerobic laboratory conditions (Kutter *et al.*, 1994).

Resistance development: a practical or an academic issue?

A classical paper by the founders of phage biology started with the sentences: 'When a pure bacterial culture is attacked by a bacterial virus, the culture will clear after a few hours. However, after further incubation for a few hours, or sometimes days, growth of a bacterial variant [is observed] which is resistant to the action of the virus' (Luria & Delbrück, 1943). Was this already the death of phage therapy in the eggshell? In fact, the interaction of bacteria and their bacteriophages became a cornerstone in community ecology and evolution. It was extensively investigated with *E. coli* strain B and the phages of the T series (for a recent review see Bohannan & Lenski, 2000). In the T4–*E. coli* B system oscillations were observed in the chemostat community that were induced by the competition between phage-sensitive and phage-resistant bacteria, while evolution of T4 was not observed. In contrast, phage T7 starts a co-evolutionary arms race. However, the race is asymmetrical and favours the bacteria. Also phage T2 can respond with extended host range mutants. Resistance to phage T5 apparently comes without a metabolic cost to *E. coli* B; consequently, T5 quickly becomes extinct, raising the question how this phage could survive in nature.

From a population-dynamic perspective, the interactions between phages and bacteria are analogous to those of a predator and a prey. Quite detailed mathematical models have been developed for this interaction, and the population and evolutionary dynamics relevant for phage therapy was recently reviewed (Levin & Bull, 2004). Serial passages of *Pseudomonas fluorescens* and its phage in the laboratory showed that phages are not fundamentally constrained in their ability to co-evolve with bacteria. Long-term antagonistic co-evolution characterized by multiple cycles of defence (development of phage-resistant bacteria) and counter-defence (phage populations from two transfers in the future showed consistently greater infectivity to bacteria than contemporary phage) were observed (Buckling & Rainey, 2002). Comparable data have now also been obtained for *E. coli* and its phages. Over a 200 h experiment on the T2-like phage PP01 (Morita *et al.*, 2002) with *E. coli* O157 in continuous culture (Mizoguchi *et al.*, 2003), a series of bacterial mutants was sequentially observed. They differed in colony morphology, the nature of phage receptors OmpC and LPS, and phage susceptibility. Phage PP01 evolved by broadening its host range. The system reached a coexistence of phage and bacteria, both at high titre levels, and continued to evolve. The dynamics in these interacting systems were largely determined by the trade-offs between resistance to phage (which is normally metabolically costly) and competitiveness with the parental strain for limiting resources. Smith & Huggins (1982) arbitrarily targeted a virulence factor of *E. coli* like the K1 antigen with a K1-specific phage *in vivo*; a low number of phage-resistant *E. coli* strains were isolated from the calf intestine, but due to the loss of the K1 antigen the strain had concomitantly lost its pathological potential in mice. Indeed, it might well turn out that the *in vitro* experiments are more of academic than of practical interest since they do not account for the complexity of phage–host interaction in the natural environment. In the defined laboratory system, the only competitor for the phage-resistant cell is its phage-susceptible ancestor cell, which is counter-selected in the presence of a phage. In the wild, the phage-resistant clone must also compete with many other strains that do not feel this phage pressure. A microcosmos consisting of only two strains (*E. coli* and *Salmonella*) largely prevented the development of the phage resistance phenotype (Harcombe & Bull, 2005).

Food sanitation

At slaughter about 7 % of cattle harbour *E. coli* O157 in their faeces, which then become a source for meat contamination. Several groups have explored the use of O157-specific phage for food sanitation (Kudva *et al.*, 1999; O'Flynn *et al.*, 2004). When meat was experimentally contaminated with O157, high doses of phage were needed to decrease the cell count *in situ*. Similar high phage titres were needed to free chicken skin from *Salmonella* and *Campylobacter* contaminations (Goode *et al.*, 2003). The high phage titres might reflect the diffusion-limitation of phage–food pathogen encounters on a carcass surface, contaminated by only a low concentration of the pathogen. Laboratory and ecological experiments have identified threshold concentrations below which phage infection chains are frequently interrupted (Chibani-Chennoufi *et al.*, 2004a; Wommack & Colwell, 2000). *In vitro*, O157 developed phage resistance with a 100-fold higher frequency against Siphoviridae than against Myoviridae.

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Animal studies

Chickens. *E. coli* causes severe respiratory infections in broiler chickens. In one study, phages were applied by aerosol spraying, followed by injection of 10^4 c.f.u. *E. coli* directly into the thoracic air sac (Huff *et al.*, 2002). Aerosol containing 10^7 p.f.u. of two phages halved the mortality when the challenge was done on the day of phage spraying. When the dose of the phage was increased to 10^8 p.f.u. significant protection against infection was still observed up to 3 days after phage spraying. Another study documented efficacy of phage applied intramuscularly against lethal *E. coli* infections for chickens. When phage and bacteria were given in equal numbers, no morbidity was observed at all in chicken, but 100-fold lower phage titres also conferred significant protection, demonstrating the *in vivo* multiplication of the phage. Intramuscularly administered phage also protected against intracranial *E. coli* infection. Phage therapy was even effective when given at the onset of clinical symptoms (Barrow *et al.*, 1998).

Mice. In the 1980s Smith and Huggins conducted a careful series of phage therapy experiments in various animals, which resumed the tradition of the mouse experiments from the early 1940s. They started with a K1 *E. coli* meningitis mouse model (Smith & Huggins, 1982). Low doses of phage, given intramuscularly, protected mice against a massive dose of pathogen applied in the opposite muscle at the same time. The anti-K1 phage was *in vivo* more efficient than a large number of antibiotics. Multiplication of the phage occurred in the animal, and phage was disseminated from the site of inoculation into the blood and the spleen, where it was sequestered. However, phage treatment could not be delayed for more than 5 h after the pathogen challenge without loss of activity. Intramuscular phage also protected against intracerebral pathogen challenge. Only phages recognizing the K1 antigen were protective. Phages with high *in vitro* lytic activity were also the most effective in conferring protection *in vivo*. The results of Smith and Huggins were reproduced recently (Bull *et al.*, 2002).

Calves. Subsequently, Smith and colleagues infected calves with a natural bovine enteropathogenic *E. coli* strain causing high lethality. Convincing evidence for the efficacy of phage therapy was obtained in an extremely carefully documented series of experiments (Smith & Huggins, 1982, 1983; Smith *et al.*, 1987a, b). Diarrhoea could be prevented by phage given 1–8 h after infection. When phage application was delayed until the onset of symptoms, phage had no effect on diarrhoea, but still largely prevented death (Smith & Huggins, 1983). Phage titres increased in the faeces over time, with a concomitant decrease in the enteropathogen counts. In sacrificed animals this observation was confirmed at all anatomical levels of the gastrointestinal tract. Phage counts were 10-fold lower in mucosal scrapings than in the luminal content. Phage was not recovered from blood or spleen. Phage-resistant cells were observed in most of the calves, but their titres generally remained low. Upon reinoculation into new calves, the mutant cells were less competitive than the parental strain.

In a second series of experiments, a low dose of phage (10^5 p.f.u.) was given to calves at the onset of diarrhoea and animals were sacrificed in a time series. Phage counts as high as 10^{10} were observed during the first 12 h in the posterior parts of the small intestine, followed by a decline at 24 h and a disappearance of phage at 40 h when the pathogenic bacterium could no longer be detected. Phage-resistant *E. coli* appeared during the experiment, but they had lost the K1 antigen, their major virulence factor.

Control of the diarrhoea by a low dose of phage (10^5 p.f.u.) given 6 h or 10 min before infection of the calves with *E. coli* was only achieved with phages showing a high *in vitro* bacterial lytic activity (Smith *et al.*, 1987a). With an extremely low dose of phage (10^2 p.f.u.), prophylaxis was only possible when given 10 min, but not 6 h before bacterial challenge. Within 5 h the phage had multiplied up to 10^{11} p.f.u. *in vivo*, demonstrating an impressive phage multiplication *in vivo*. Very small doses of phage (down to 20 p.f.u.), given after the onset of diarrhoea, still resulted in an amelioration of the disease.

Calves held in a room previously occupied by phage-exposed calves could no longer be infected with the enteropathogen, coming close to d'Hérelles's initial idea of 'infectious protection' by phages. Also, spraying the litter of the calves in the room with a high or low dose of phage (10^{10} or 10^6 p.f.u.) prevented an infection of the calves with the pathogenic *E. coli* strain, applied either before or after transfer to the phage-inoculated room. When substantial pathogen counts were measured in the faeces, phage appeared with titres 10- to 100-fold higher than the bacterial counts. Phage survived in the room for up to a year and at least 100 days longer than the pathogenic bacteria, and was also more resistant to phenolic disinfectants than the enteropathogen (Smith *et al.*, 1987b).

Phage was sensitive to a low pH in the abomasum of the calves, but this problem could be solved by applying the phage with the milk feed or shortly after feeding. Barrow *et al.* (1998) confirmed that intramuscular phage injection in calves orally challenged with a $K1^+$ *E. coli* delayed the appearance of the bacterium in the faeces and the blood and lengthened the life span of the animals. Smith & Huggins (1983) extended the phage therapy experiments to piglets and lambs and confirmed their results in these animals.

Efficacy trial in humans

In 1963 a total of 30 769 children (6 months to 7 years old) were enrolled in Tbilisi, Georgia, in an oral phage prophylaxis trial against bacterial dysentery. About half of the children living on one side of the streets received, once every week, *Shigella* phages orally in a pressed tablet form, while children on the other side of the streets received a

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placebo. The children were followed for 109 days. Phage administration was associated with a 3·8-fold decrease in dysentery incidence (1·8 vs 6·7 episodes per 1000 children from treatment and placebo group, respectively). The culture-confirmed incidence was decreased 2·6-fold by phage application (0·7 vs 1·8 episodes, respectively). Phage exposure also decreased the incidence of any form of diarrhoea (15 vs 45 episodes per 1000 children 6 to 12 months old in treatment and placebo group, respectively). This latter observation suggests a protective effect of the anti-*Shigella* phage preparation against other serotypes of *E. coli*. Protective effects were most pronounced in children younger than 3 years. Note, however, that this apparently well-designed study was only documented in a 75-line publication written in Russian containing a single table (Babalova *et al.*, 1968).

The most detailed reports on phage therapy published in English are from Poland (Slopek *et al.*, 1987), dealing with cases of septicaemia (only some of these were caused by *E. coli*). The rate of success was greater than 90 %, including patients for whom antibiotic therapy was ineffective. Quite extensive double-blind phage prophylaxis and treatment trials were conducted with soldiers of the Red Army in four different geographical areas of the Soviet Union during 1982–1983. The authors reported that the incidence of dysentery was 10-fold less in the phage treatment as compared to the control group. These studies were insufficiently documented in the published Russian literature for a rigorous evaluation of these trials to be done (for a review of the Soviet literature see Alisky *et al.*, 1998; Sulakvelidze *et al.*, 2001; Sulakvelidze & Kutter, 2005).

Outlook

Until quite recently academic phage researchers remained rather sceptical about the medical or agricultural use of phages. The present literature survey does not paint a negative picture for the prospect of phage therapy against *E. coli* infections. However, one should refrain from over-interpreting the available clinical evidence and hail phages as a panacea against bacterial infections in general. Too many of the clinical studies with phages do not correspond to current standards of clinical and microbiological research and need to be repeated. Undue scepticism and unfounded optimism are both misplaced as regards the rediscovery of phage therapy. The technology holds at least the prospect of practical solutions to some urgent public health problems, and not just those caused by *E. coli*. The development costs of phage therapy are much lower than for a new antibiotic. In view of the emergence of new infectious diseases at an unpredicted pace and the escape of well-known bacterial diseases from antibiotic control, we are probably well advised to develop the necessary phage technology sooner rather than later. The chances of developing a successful phage approach to *E. coli* diarrhoea control are reasonably good since it can be based on decades of research with the bacterium and its phages. Research on *E. coli* and its phages played a major part in the molecular biology revolution. Why should *E. coli* not also lead us into the future? We could take the best of the reductionist approach (working with the simplest systems) and transfer it deliberately into the complexity of the gut of humans or animals living in their natural ecological context. This would ideally fit into the current trend towards systems biology.

Much of the future of phage therapy will be determined by the attitudes of the health authorities, which have to license the use of phages. Currently, we still have here a clash of cultures. The West, perhaps represented by the US Food and Drug Administration, seems to favour the use of a single well-defined phage, while the secrets of the apparent success in the East with phage therapy lay in phage cocktails. Even individualized treatments for each patient were used in surgical settings based on large phage collections and laboratory tests of phage sensitivity for the patient's specific pathogen. However, 'Intestiphage' was a fixed mix of a group of phages against *E. coli* and other enterobacteria (Sukalvelidze & Kutter, 2005). It was made in large quantity: 80 % of the 2 tonnes of phage-therapy products made in the 1980s were shipped off to the Soviet army, where most was used without any particular pretesting in the individual patients; much of it was prophylactic. It is not yet clear how the best of both worlds can be combined to the benefit of patients.

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