

Bacteriophage - A Common Divergent Therapeutic Approach for Alzheimer's Disease and Type II Diabetes Mellitus

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Abstract: Alzheimer's disease, the most important neurodegenerative disorder, is an irreversible, age-dependent disease of the brain characterized by problems in progressive impairments in memory, language, reasoning, behavior and visuospatial skills. It is characterized by the deposition of amyloid beta peptide, forming compact fibrillar plaques and neurofibrillary *tau* tangles. Another major and much more prevalent cause of morbidity and mortality in world is diabetes especially type 2 diabetes mellitus. It is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Chronic wounds caused by antibiotic resistant bacterial infections that fail to heal are a common complication of diabetes mellitus and the most frequent reason for nontraumatic lower limb amputation. Holistically, these two diseases are linked at molecular level but the exact mechanism is a topic of debate. Bacteriophages are viruses infecting bacteria and lack ability to infect mammalian cells. They are neither causative agent for Alzheimer's disease or type 2 diabetes mellitus nor involved in their pathogenicity but promises for a novel divergent therapeutic approach. The great versatility of the phage system has led to the development of improved phage delivery vectors, as well as immunomodulation of anti-amyloid beta peptide response. Phages could also constitute valuable prophylaxis against bacterial infections, especially in immunocompromised patients like in the case of diabetes. Patients having diabetes have a high risk of developing foot ulcers which are difficult to be treated by antibiotics alone due to ever increasing antibiotic resistance strains. Combination therapy based on multiple phage and broad spectrum antibiotics holds great promise. The potential therapeutic phage therapy arises from its lack of natural tropism for mammalian cells, resulting in no adverse effects.

Keywords: Type 2 diabetes mellitus, Alzheimer's disease, bacteriophage therapy, immunomodulator, vatches.

INTRODUCTION

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disorder and leading cause of dementia in elderly and affects more than 36 million people at present and with about 115 million people expected to have the disease by 2050 worldwide [1-3]. It is one of the most important health care, social and economic challenges of the 21st century. Currently available treatments for AD have very minimal effect on the course of the disease [4, 5]. The important characteristic symptoms of AD, includes progressive cognitive impairment, loss of memory and behavioral deficits, closely related to pathologic changes in the brain associated with the excessive deposition of amyloid β (A β) peptide plaques and neurofibrillary tangles [6, 7]. It is characterized in part by the deposition of A β , a peptide cleavage product of amyloid precursor protein (APP), in compact fibrillar plaques [8]. These structures can induce an innate immune response in the brain, which triggers

progressive inflammation, neuronal loss, and further acceleration of plaque formation [9]. AD is divided into familial and sporadic forms. While etiological events leading to disease remain unresolved, a small percentage has been shown to be genetic in origin and is termed Familial AD. The genes responsible for early onset (<60 yrs) are Presenilin2, Presenilin1, ApoE and APP. Familial pattern of inheritance is in which the patient had at least two first degree relatives with a history of AD, whereas sporadic refers to AD cases when no other cases have been seen in close family members. Approximately 25% of AD is familial, while the rest sporadic.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Chronic diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms, cerebrovascular problems and sexual dysfunction [10, 11]. Several studies have reported association between the two health burdens, AD and type 2 diabetes mellitus (T2DM) [3].

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Bacteriophages are simplest form of "life"; bacteria attacking viruses in possession of DNA/RNA and a proteinaceous structure, and proposed as the origin of higher cellular organisms. They were observed by Twort and d'Herelle in 1915 and 1917, respectively. Bacteriophages are the most numerous life forms on earth and found almost everywhere, from the ocean depths to hot springs, and can be isolated from soil, water as well as human or animal body (e.g., saliva, feces, and skin). Bacteriophages are known to be very common in the gastrointestinal tract together with their bacterial hosts [12-14]. Mammalian organisms are very frequently exposed to interactions with bacteriophages and these natural contacts are not incidental, but rather constant and intensive. However, they do not cause AD or T2DM and not involved in disease mechanism but has potential to be used as a therapy for both.

BACTERIOPHAGE

Bacteriophages are being utilized extensively in scientific research and medical therapeutics as vehicles for protein and DNA vaccines delivery, gene therapy, alternatives to antibiotics, in diagnostic tools to detect pathogenic bacteria, screening libraries of proteins, peptides or antibodies [15]. The structure and genetic information of filamentous phages, such as M13, f1 or fd have been reported and they contain circular single-stranded DNA molecule, encapsulated in a protein envelope forming a rod-shaped cylindrical structure [16]. Two coat proteins, (major and minor) encoded by gene VIII and gene III exposed on N-terminal domains that tolerate foreign peptide inserts (Fig. 1). Filamentous bacteriophages are excellent vehicles for the expression and presentation of foreign peptides in a variety of biological systems [17, 18]. Large repertoires of peptides and antibody fragments are displayed on their surface by cloning random oligonucleotides at the 5'-end of the genes coding for the phage coat proteins, minor protein pIII and major coat protein gene VIII. The approach for recombinant filamentous phage to obtain specific peptide antigens has a greater advantage over chemical synthesis. *In vivo* administration of filamentous phages induces a strong immunological response to the phage proteins pIII and pVIII in all animals tested without any evidence of toxic effects. The display of short immunogenic determinants fused to the phage surface provides the basis for the development of novel peptide vaccines [19-22]. Peptides can be chemically conjugated to the phage, or displayed as recombinant fusions to the coat proteins [23]. The immunogenicity of short peptide epitopes is enhanced when they are displayed on the phage capsid and chemical stability is increased [24]. Phage-based products have been recently approved in food safety by the US Food and Drug Administration [25, 26]. Different kinds of

bacteriophages (filamentous phage [27], lambda phage [28], T4 [29] and T7 [30]) can be utilized in phage-display vaccination and DNA vaccination [31].

Filamentous bacteriophages (Ff, M13, f1, fd, Ike, N1, Q β etc) are excellent carriers for the expression and presentation of foreign peptides in a variety of biological systems. Since the first description of the phage display technique 28 years ago [18], filamentous bacteriophages have been largely employed for the generation of peptide libraries, based on phage virions displaying peptides encoded by degenerate oligonucleotide sequences cloned into a gene coding for one of the viral coat proteins [32-36].

BACTERIOPHAGE FOR AD THERAPY

AD is characterized by two hallmark pathologies: plaques of A β peptide, and neurofibrillary *tau* tangles [3-4, 6]. A β peptide is a vital therapeutic biomarker in AD with numerous pathological, biochemical, and genetic evidence supporting its involvement in the ailment. Multiple symptomatic treatment options are being used presently, but no specific disease-modifying therapies are available. A β has central role in AD pathogenesis and various anti-A β strategies are being pursued, with active and passive immunization being among the most advanced approaches [4, 37-39]. The great versatility of the phage system has led to the development of novel and improved phage delivery vectors, as well as immunomodulation of anti-A β peptide response. The potential therapeutic phage therapy stems from its lack of natural tropism for mammalian cells, resulting in no adverse effects. Major applications of Bacteriophages are: (a) Immunomodulators Against A β Peptide, (b) Delivery vector of antibodies imaging/treatment and (c) Anti-aggregating agent of A β .

Bacteriophage as Immunomodulators Against A β Peptide

A series of immunomodulatory antigens have been developed displaying the 4 amino acid "EFRH" epitope on filamentous phages with varied number of EFRH copies [40]. Epitope EFRH, A β ₍₃₋₆₎ peptide, acts as a regulatory site controlling the conformational changes of whole A β peptide. Locking of the epitope by antibodies affects the dynamics, prevents self-aggregation and enables resolubilization of already formed aggregates [41]. Intranasal administration of phage-antibodies or antigens provides a simple and rapid delivery route of therapeutic agents into the CNS due to the unique connection between the nose and brain. Substances delivered by intranasal administration exert their effect on the brain rather than on peripheral tissues. EFRH

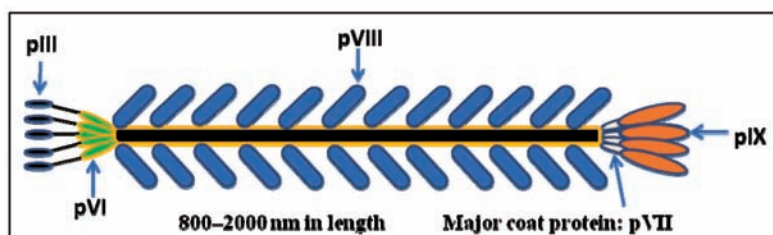


Fig. (1). Schematic illustration of filamentous phage.

immunization of human APP transgenic (Tg) mice has been done [40]; by preparing antigens containing between 10-300 copies of different numbers of EFRH displayed on the phage. Treatment of Tg Mice Model of AD with Phage Single Chain Variable Fragment (ScFv) molecules was done. Intranasal administration of 10 μ l containing 1011 phages per mouse was done every 3 weeks for a total period of 6 months. Thioflavin-S staining of mice-brain sections showed that plaque load in the treated mice was an average of 50% of that in the control tg mice. No toxic effects were observed following EFRH phage immunizations in the challenged animal sections, including brain, liver, kidney, spleen and lung.

Bacteriophage as Vector for Drug and Antigen Delivery to the Brain

The filamentous bacteriophages are being utilized as vector for delivering antigens and small peptides and drugs. The induction of an effective immune response requires antigen uptake and processing by antigen-presenting cells, T cell priming, and activation of B and T cells. The antigen delivery system can confer immunogenicity to short peptides that are not by themselves immunogenic, and can overcome the limitations inherent to synthetic peptides in terms of stability and toxicity. The linear structure of the filamentous phage enables penetration to the brain *via* intranasal administration through the olfactory tract, conferring to the phage properties as a delivery vector of drugs [42]. Direct correlation exists between the number of applications and the amount of phage detected in the brain. The filamentous phage maintains the biological activity of displayed foreign molecule of anti-A β P ScFv, and efficiently penetrates biological membranes, strongly indicating that the olfactory route may target the plaques into specific regions. Filamentous bacteriophage exhibits penetration properties to the CNS, and the ability to carry foreign molecules to target brain regions [36]. Genetically engineered filamentous bacteriophage proved to be an efficient and non-toxic viral delivery vector to the brain exhibiting penetration properties to the CNS, offering an obvious advantage over other mammalian vectors. The bacteriophage lacks the ability to infect mammalian cells unless designed to do so. Due to its structure, the filamentous phage is highly permeable to different kinds of membranes and, following the olfactory tract, it may directly target affected sites in the brain [42, 43].

Bacteriophage as Anti-Aggregating A β Agent

Misfolded protein, especially in β -sheet conformation is further stabilized through aggregation or oligomerization. Preventing the folding of nascent A β monomer into self-aggregating toxic conformers or oligomers would have therapeutic benefit [44]. The phage's long thin filamentous shape may be the basis underlying its anti-aggregating function. Modifying the phage's linear structure and rendering it spherical abolishes its disaggregating activity [45]. This strategy for the production and targeting of anti-aggregating antibodies against A β plaques to disease affected regions in the CNS has clinical potential for treatment of AD.

Effect of Phage Display on Antigen Uptake and Immune Response

Antigens can easily enter in lymph vessels but the antigen presenting cells takes these antigens very inefficiently but particulate antigen is up taken more efficiently [46]. Peptides derived from the bacteriophages reach and loaded on both the major histocompatibility complex class I and II [47]. The stimulation of CD4 and CD8 T cells by MHC explain the ability of bacteriophages displaying foreign T-cell epitopes to prime strong T-helper-dependent cytotoxic T cell responses [48, 49]. The particulate nature of phage-displayed antigens and the size of phages most probably underlie the remarkable effects of phage-display on antigen uptake and processing [46, 50].

Response of A β Epitopes Antibodies

In the development of effective and safe immunotherapy for AD, the most important challenges are low immunogenicity of the A β peptide and detrimental autoimmune responses. When the whole A β peptide was used for immunization in human, then adverse reactions was observed mediated by T cells [22, 37, 39, 41, 51-56]. The good antibody response was observed to short fragments of A β by delivering through "amyloid displayed" filamentous bacteriophages (fdAD). A phage-based anti-A β vaccine was developed with EFRH epitope of A β by the group of Beka Solomon [43]. Solomon and collaborators improved the system by generating phages that only display the EFRH epitope. It was observed that phages expressing 300 copies of the peptides were more immunogenic than phages expressing 150 copies of the peptide which suggest that epitope density was the main limiting factor for immunization. EFRH phages induced an immune response against A β resulting in the reduction of the amyloid load and improving cognition [40, 45, 53, 57, 58]. In particular, epitope 2-6 (alanine EFRH), that is nearly identical to the EFRH epitope analyzed by Solomon at a maximum expression level of 300 copies per phage particle [40], was expressed at 810 copies per phage particle [43]. A marked difference in the immunogenicity of the A β epitopes displayed by the 4 fdAD phages was observed. In other instances it has been shown that immunogenicity is enhanced by increasing the length of A β epitope [59]. Significant reduction was seen in the number of A β plaques in the hippocampus and cortex of 8-month-old AD model mice immunized monthly with fdAD (2-6) from age 2 months, suggesting that the treatment effectively delays the onset of plaque pathology [60]. AD model mice treated with phage-EFRH showed a considerable improvement in their cognitive behavior in the Morris Water Maze test [40]. Overall, data from anti-A β immunization experiments show that phage-based antigens represent a good strategy to focus the immune response to a defined B cell epitope, and obtain antibody levels that afford a therapeutic effect. In contrast, marked differences were observed in the immunogenicity of different A β epitopes displayed by the phages, despite incorporation of recombinant pVIII proteins in the phage capsid [60]. The genetically engineered filamentous bacteriophage proves to be an efficient and nontoxic viral delivery vector to the brain. This was the first demonstration that filamentous bacteriophage exhibits penetration properties to the central nervous system while preserving

both the inert properties of the vector and the ability to carry foreign molecules. The linear structure of the phage is suggested to confer penetration properties *via* various membranes. No evidence was shown of spread of filamentous phage to other brain sections, strongly emphasizing the olfactory tract as the most probable path in this case. Additional studies are needed toward early diagnosis of the disease and therapeutic intervention before cognitive decline occurs [42]. It has been reported that the filamentous bacteriophages breakdown the amyloid plaques of AD. The intranasal administration of wild type M13 bacteriophage may serve as a safe alternative to immunotherapy to disaggregate existing amyloid plaques and prevent formation of new ones, countering the neurodegeneration associated with AD. Phages are eliminated from the brain and secreted from the body *via* urine and feces without adverse effects. Intranasal administration of wild type M13 bacteriophages overcame drawbacks of immunotherapy, paving the way for therapeutic application to AD [43].

Active Immunization

The active A β immunotherapy could reduce A β load *in vivo* and was originally reported by Schenk *et al.* in 1999 [61]. The anti-A β antibodies generated following immunization prevented plaque deposition and leads to clearance of pre-established plaques in the brains of the animals. Vaccination also attenuated gliosis and neuritic dystrophy. Diverse reports have confirmed and extended these findings in various transgenic AD mouse models, by using various routes of administration, adjuvants and immunogens [56, 61, 62]. The first active immunization clinical trials were stopped for AN1792 which used full length aggregated A β_{1-42} as an immunogen, because 6% of vaccinated patients developed meningoencephalitis. This complication happened due to excessive inflammation mediated by T-helper-1, whereas the amyloid-reducing effects are mainly linked to humoral T-helper-2-related immunity. In a second-generation A β CAD106 vaccine, a small A β fragment (A β_{1-6}) was used coupled to an adjuvant carrier formed by multiple copies of the coat protein of bacteriophage Q β , an icosahedral virus with a diameter of 25 nm. Its host is *Escherichia coli*. A β immunogen avoids the Th2-related T-cell epitopes present in full length A β_{1-42} , reducing the possibility of encephalitis as a complication [4-5, 37-39, 63]. In Tg2576 mice (a plaque-bearing transgenic mouse model of AD), however, although A β_{1-42} vaccination in young, pre-plaque animals prevented plaque deposition, the same vaccine was much less effective in clearing pre-established plaques in aged mice [64, 65]. In 18 month-old 3xTg-AD mice-transgenic animals that exhibit both amyloid plaques and neurofibrillary tangles- active vaccination with fibrillar A β_{1-42} did not reduce plaque number, or the levels of insoluble A β or insoluble *tau*. Immunization did, however, lead to an improvement in behavioral performance and a reduction in soluble A β levels in these animals, suggesting that soluble A β species might be directly linked to the behavioral impairment observed in the 3xTg-AD model [66]. A peptide that specifically binds A β_{1-10} modulates A β aggregation and A β -induced neuronal damages, opening possibilities for the development of a novel therapeutics for the AD treatment [67].

Passive Immunization

Passive immunization with antibodies devoid of fragment crystallizable region may prevent over-activation of microglia and thus, attenuates auto-antibody triggered neuroinflammation. This prevents the plaque deposition and leads to reduction in the levels of soluble and insoluble A β , as well as an increase in the levels of A β -anti-A β antibody complexes in the blood with an anti-A β monoclonal antibody in young PDGF-driven human A β precursor protein mice which recognized an epitope in the middle region of the peptide (A β_{13-28}). This finding supports a role for passive A β immunotherapy in the prevention of AD [68]. In older plaque-bearing mice, only passive immunization with monoclonal antibodies that recognized the amino-terminal epitopes of A β led to a reduction in pre-established plaques [52]. Generally, studies in aged transgenic mice have reported that passive A β immunotherapy, when initiated after the onset of pathology, has lowered the plaque burden and cerebral A β_{1-42} levels, reduced neuritic dystrophy, and reversed behavioral deficits (in some studies behavioral improvements occurred in the absence of changes in brain A β levels) [69, 70]. Systemic injections of some anti-A β antibodies, lowers A β -plaque burden, resulting an increase in the occurrence of microhemorrhages in cerebral amyloid angiopathy area. In a study, reduction in plaque burden and an improvement in behavioral deficits were observed despite an increase in microhemorrhage passive immunization with anti-A β monoclonal antibodies directed against the carboxyl terminus of the peptide (A β_{28-40}) [71, 72]. In another study, reduction of plaque burden and rate of early aggregation of phosphorylated *tau* was seen by using direct intra hippocampal injection of anti-A β monoclonal antibodies in 12 month-old 3xTg-AD mice [62].

The A β plaques can be dissolved *in vivo* by anti-A β ScFv-phage with repeated intranasal administration to human APP transgenic mice. The cognitive average of the treated animals was found to be close to that of the non-transgenic animals, indicating a healthy pattern of learning and memorizing of the new information [45, 73]. Intranasal treatment for six months with phage anti-A β ScFv of transgenic mice over expressing hAPP resulted in reduction of plaque load and considerable reduction of brain inflammation. Repeated applications of phage-ScFv demonstrate the ability of single-chain antibodies to dissolve A β aggregates and to clear their deposits from the brain, indicating that alternative mechanisms beside fragment crystallizable-mediated phagocytosis by microglia are involved in this clearance [74]. ScFv antibodies delivered by intranasal application are more likely to reach the brain and exert their effect there, rather than reaching and affecting peripheral tissues. Intranasal administration was chosen as a direct delivery route to the CNS *via* the olfactory system [75-77]. The feasibility of these novel strategies, using filamentous phages for the production and targeting of anti-aggregating antibodies against A β plaques to disease affected regions in the CNS, may have clinical potential for treatment of AD (Fig. 2).

The involvement of A β in the pathogenesis of AD is unequivocally proved. The view that A β deposition drives the pathogenesis of AD has received support from a wide range of molecular, genetic and animal studies [78-80]. A β

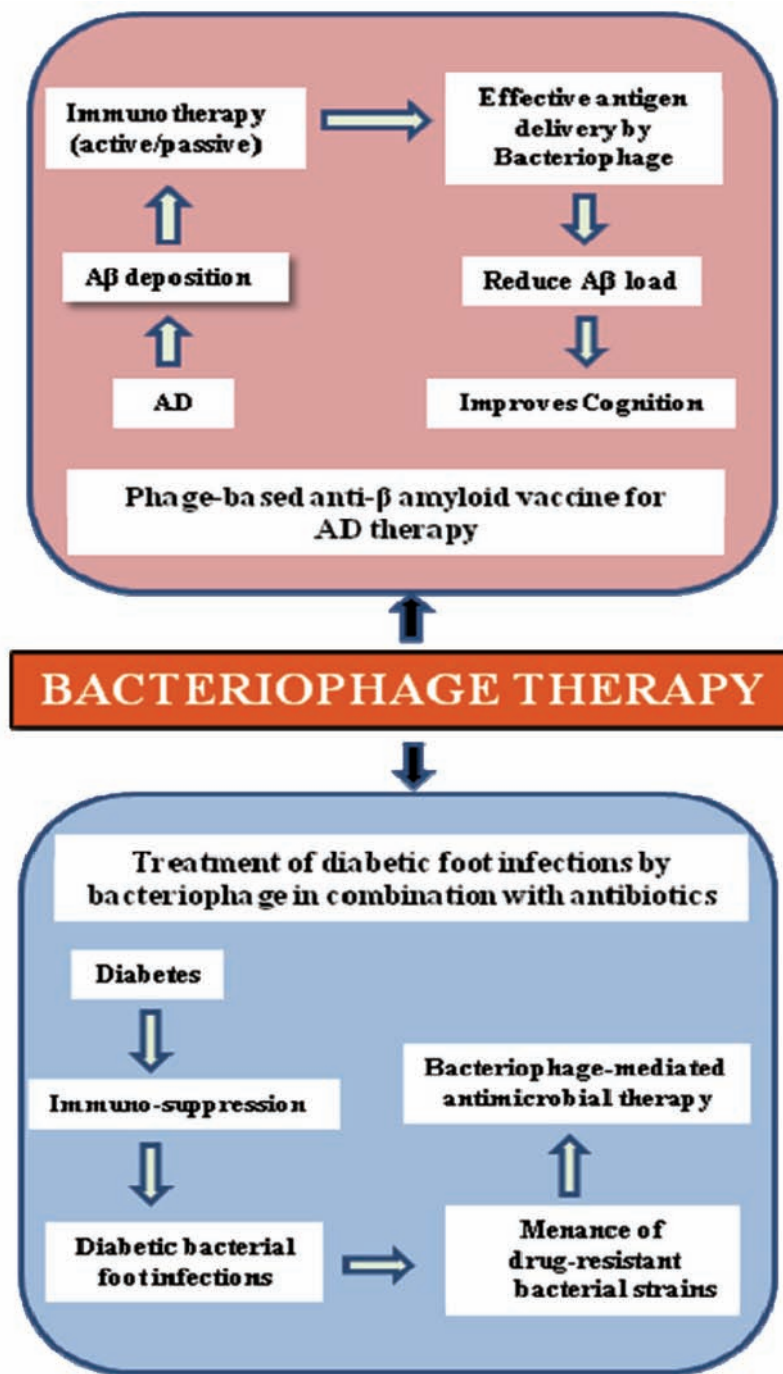


Fig. (2). Graphical depiction of divergent therapeutic approaches for AD and T2DM using bacteriophages.

peptides are not easily amenable for any kind of biophysical, structural and functional studies. However, numerous independent attempts to intervene in the Aβ production (inhibitors of beta- and gamma-secretases) or to neutralize the molecule (immuno approach) have been unsuccessful. Recently a therapeutic approach was developed that uses anti-Aβ AN 1792 vaccine to generate antibodies which target Aβ in order to facilitate its removal from the brain. Such vaccines were successfully tested in mouse models of AD [61, 81, 82] and they showed no adverse side effects in Phase I clinical trials. However, Phase IIa clinical trials had to be abandoned after some patients who had received the AN 1792 vaccine developed meningoencephalitis. Despite

good tolerability and marked reduction in Aβ plaques, several patients had developed inflammation of the CNS and worsened dementia at the time of death. These outcomes were not anticipated by experiments on transgenic mice because compared to humans, these mice have less genetic variability, and their plaques have a different chemical composition, making them far more soluble and easier to remove. The active immunization approach has led to significant side effects such as meningoencephalitis [83-86]. The most important lesson to be learned from the AN 1792 trials is that new strategies for treating AD should not be tested on humans until they have been extensively tested on non-murine species.

BACTERIOPHAGES FOR T2DM THERAPY

Diabetes mellitus, insulin-dependent, 2 is a multifactorial disorder of glucose homeostasis that is characterized by susceptibility to ketoacidosis in the absence of insulin therapy. Insulin, a secretory protein decreases blood glucose concentration and increases cell permeability to monosaccharides, amino acids and fatty acids. It accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in liver. Clinical features are polydipsia, polyphagia and polyuria which result from hyperglycemia-induced osmotic diuresis and secondary thirst. These derangements result in long-term complications like retinopathy, nephropathy, neuropathy, and cardio-vascular problems.

Chronic wounds that fail to heal are a common complication of diabetes mellitus and the most common precipitating reason for nontraumatic lower limb amputation [87]. The bacterial species that cause these infections are increasingly becoming antibiotic resistance making treatment difficult. Mendes and his co-workers investigated the antimicrobial activity and wound-healing capability of topically delivered bacteriophage solutions against wounds with chronic *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* infections in two animal models of diabetes mellitus (rat and porcine). Microbiological (colony counts), planimetric (ulcer size), and histological parameters (epithelial and dermal gap) were compared in debrided infected wounds with or without topical bacteriophage treatment in a previously optimized rodent wound infection model in chemically induced diabetic Wistar rats, and in a modified pig wound infection model in animals with chemically induced diabetes.

They found that bacteriophage treatment effectively decreased bacterial colony counts and improved wound healing, as indicated by smaller epithelial and dermal gaps, in *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections but was not as effective against *Acinetobacter baumannii*. Although the improvements were more significant in the rodent model than in the porcine model, results suggest that topically administered bacteriophage treatment may be effective in resolving chronic infections, especially when applied in conjunction with wound debridement.

This suggests that bacteriophage-containing topical antimicrobial therapy may be a viable treatment for diabetic foot infections by drug-resistant bacteria. Although additional studies are necessary, it may be an effective and novel therapeutic approach for addressing the serious problems associated with diabetic foot infections and other chronic skin and soft tissue infections [88].

In another independent study, protective effect of bacteriophage was assessed against experimental *Staphylococcus aureus* lethal bacteremia in streptozotocin induced-diabetic and non-diabetic mice. Intraperitoneal administrations of *S. aureus* of 2×10^8 colony forming unit caused fatal bacteremia in both diabetic and non-diabetic mice. A sole administration of a newly isolated lytic phage strain considerably protected diabetic and non-diabetic mice from lethal bacteremia (mice survival rate of 90% and 100% for diabetic and non-diabetic bacteremic groups versus 0% for saline-treated groups). These results imply that phages could

constitute valuable prophylaxis against *S. aureus* infections, especially in immunocompromised patients [89].

Diabetes leads to immunosuppression impairing the overall immune functioning of body [90] and to worsen the situation, wounds inflicted with drug resistant strains worsen the situation in diabetic patients. Methicillin resistant *S. aureus* accounts for approximately 42.86 % of the *S. aureus* isolates from diabetic foot infections [91]. A recent study focuses on the use of lytic bacteriophage in combination with linezolid, a synthetic antibiotic as an effective and safe treatment strategy against these resistant strain mediated foot infection in diabetic animals [92]. Combination therapy using both was more effectual in seizing the infection (bacterial load, lesion score, foot myeloperoxidase activity and histopathological analysis) and hastened tissue healing. This approach promises to reduce the rate of emergence of resistant strains, thereby, handling the menace of antibiotic resistance in immunosuppressed diabetic patients.

In phage therapy, the patient is given phages in combination with antibiotics, the bacterial pathogens are not likely to be able to become resistant to both, because the mechanisms of resistance are fundamentally different and thus resistance to one agent will not enable the bacterium to be resistant to the other simultaneously [93, 94]. An important benefit of phage therapy is derived from the observation that bacteriophages are much more specific than most antibiotics that are in clinical use. Theoretically, phage therapy is harmless to the eukaryotic host undergoing therapy, and it should not affect the beneficial normal flora of the host. Phage therapy also has few, if any, side effects, as opposed to drugs, and does not stress the liver. Since phages are self-replicating in their target bacterial cell, a single, small dose is theoretically efficacious. On the other hand, this specificity may also be disadvantageous because a specific phage will only kill a bacterium if it is a match to the specific subspecies. Thus, phage mixtures may be applied to improve the chances of success, or clinical samples can be taken and an appropriate phage identified and grown. Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics, especially successful where bacteria have constructed a biofilm composed of a polysaccharide matrix that antibiotics cannot penetrate (Fig. 2) [95].

Bacteriophage therapy is typically an 'active' treatment requiring multiplication in the bacterial host and therefore the factors that govern its success are different from those of conventional antibiotics. From the pharmacokinetic and pharmacodynamic points of view, time of treatment, dosage depending on the site of infection and the composition of the bacteriophage formulation (single vs multiple strains) need careful consideration when designing clinical trials. Scientific evidence regarding inflammatory effects, potential for gene transfer and phage resistance, need to be evaluated through such trials. However purity, stability and sterility of preparations for human use can be addressed through Good Manufacturing Practices to reduce many potential safety concerns [96].

Several studies have reported on the bacteriology of diabetic foot infections and therapy directed on the causative bacteria, with varied results. The antibiotic-resistant *Enterococci* problem in diabetic foot has not received

adequate attention from the medical fraternity and also the pharmaceutical pipeline for new antibiotics is drying up. Multidrug-resistant *Enterococci* are a real problem and continuous surveillance is necessary. Today, resistance has rendered most of the original antibiotics obsolete for many infections, mandating the development of alternative anti-infection modalities. One of such alternatives stemming up from an old idea is the bacteriophage therapy. A recent study demonstrated viable phages against multiple drug resistance *E. faecalis* [97]. They prospectively studied adult diabetic patients admitted for lower extremity infections and recovered 32 strains of *Enterococcus* spp. and screened the isolates for high-level aminoglycoside resistance. Multidrug resistance and concomitant resistance of these strains to other antibiotics were quite high.

VATCHES WITHIN A β AND THE MICROTUBULE PROTEIN TAU

The entire human genome is composed of viral DNA reflecting an evolutionary descent as suggested by J.B.S. Haldane and Felix D'Herelle over a century ago, and the insertion of multiple retroviral inserts over millions of years of co-existence, that are likely responsible for evolutionary jumps [98]. These sequences are short gapped segments that are identical in viral and human proteins, resulting in the protein translation of millions of contiguous amino acid stretches 5-12 amino acids long (Vatches = viral matches). These are homologous to proteins expressed by today's viruses and other pathogens. Viral infection will therefore interfere with host protein networks, and antibodies to the virus may also target the human homologous proteins leading to immune-related protein knockdown, and in some cases autoimmune destruction of the cells containing the human counterpart. This scenario is relevant to most, if not all, human diseases.

There are several million within the human proteome, derived from evolutionary descent and from the insertion of multiple viruses into the human genome over millions of years. This type of insertion is not restricted to retroviruses, as herpes viruses, hepatitis viruses, influenza and common cold virus, coronavirus, papillomavirus and lots of bacteriophages, among others, have all been inserted into different genomic regions or are homologous to the encoded protein products. This has occurred on several occasions during evolutionary time, and these reinsertions appear to be responsible for the creation of gene families (refer <http://www.polygenicpathways.co.uk/blasts.htm>), where over 2 million such alignments are available for multiple viral species. In effect, the entire human genome appears to be composed of viral DNA. For example, the coverage of human chromosome 10 is complete, with 119,867 human/viral DNA matches. Over millions of years, these DNA inserts have been extensively shuffled by recombination, but millions of consecutive sequences are retained that encode for the viral matching protein components [99, 100].

The general applicability of a peptide vaccine aimed at eliciting a T cell response to a specific CD4 or CD8 T cell epitope is limited by the HLA diversity in the human

population, as the presentation of the T cell epitope included in the vaccine can be restricted by a specific haplotype. In the case of phage-based vaccines, an alternative to the use of HLA-promiscuous epitopes would be the production of a mixture of different bacteriophages, each delivering a T cell epitope restricted by a different haplotype. On the other hand, non-linear epitopes can be mimicked by "mimotope" peptides, that can reproduce the epitope despite little or no amino acid sequence homology with the antigen, and are able to induce an antibody response that cross-reacts with the original antigen. Overall, filamentous bacteriophage represents a promising antigen delivery platform for the development of peptide vaccines, deserving further research.

CONCLUSION

Recent studies have linked the pathogenicity of AD, a neurological disorder with T2DM, a metabolic disorder emphasizing on genomic, epigenomic, proteomic, and viral links [101, 102]. Several preclinical studies support the notion that AD can be prevented by A β immunotherapies [103]. Chronic lesions like diabetic foot ulcers caused by multi-drug resistant bacterial strains that fail to heal are a common complication of T2DM. These ailments are inherent and have very limited treatment options available. Bacteriophage, the simplest life form on earth, has a key for novel therapeutic strategy. They have a powerful antigen delivery system that can be utilized to develop very stable, safe and inexpensive vaccines, because of their ability to display exogenous peptides on their surface as a fusion to phage proteins. Intranasal administration of genetically engineered filamentous phage offers a convenient way of treating AD as its linear structure confers effective penetration to the brain and disaggregates the A β plaque [104-106].

Phages constitute valuable prophylaxis against bacterial infections, especially in immunocompromised diabetic patients who have a high risk of developing foot ulcers difficult to be treated by antibiotics alone due to ever increasing antibiotic resistance. Combination therapy based on multiple phage and broad spectrum antibiotics holds great promise. The potential therapeutic phage therapy arises from its lack of natural tropism for mammalian cells, resulting in no adverse effects.

LIST OF ABBREVIATIONS

AD	= Alzheimer's disease
T2DM	= Type II diabetes mellitus
A β	= Amyloid beta or beta amyloid
APP	= Amyloid precursor protein
Tg	= Transgenic
CNS	= Central nervous system
EFRH	= Glutamic acid, phenylalanine, arginine, histidine
ScFv	= Single chain variable fragment

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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