Therapeutic potential of phages in autoimmune liver diseases

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Summary

Autoimmune liver disease (ALD) poses a difficult medical challenge, as there is a significant number of patients in whom current therapy offers questionable or no benefit, yet its side effects may be serious, including the development of malignancy. Bacterial viruses (phages) have been recognized increasingly as immunomodulators contributing to immune homeostasis and curbing inflammation. Accumulating data suggest that phages may be useful in immunotherapy of ALD. Phages have been shown to down-regulate the expression and/or production and activity of factors associated with hepatic injury [reactive oxygen species, Toll-like receptor (TLR)-4 activation, nuclear factor kappa B (NF-κB) activation, proinflammatory and procoagulant activities of platelets] and up-regulate the expression and/or production of factors demonstrated as playing a protective role [interleukin (IL)-10, IL-1 receptor antagonist].

Keywords: hepatitis, interleukin-10, Kupffer cells, phage, reactive oxygen species

Introduction

Autoimmune liver disease (ALD) includes three main clinical entities: autoimmune hepatitis (AH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). While being considered as autoimmune, the aetiology of each of those disorders remains obscure [1]. Even though immunosuppressive therapy may be beneficial in some patients, there is a significant proportion of patients with absent or unsatisfactory response to the treatment [2]. This applies particularly to PBC and PSC, where a shortfall of available therapies exists [3]. Patients usually require immunosuppressive treatment (steroids and azathioprine) to induce and maintain remission of inflammation and prevent fibrosis progression. In patients intolerant to azathioprine, 6-mercaptopurine or mycophenolate mofetil may also be used. Most patients require life-long treatment. Patients not responding adequately may be treated with calcineurin inhibitors (cyclosporin, tacrolimus). However, those drugs have a narrow therapeutic window and are associated with significant side effects. There are also attempts to use biological therapy such as anti-tumour necrosis factor (TNF) or anti-CD20 monoclonal antibodies, which may control the progress of disease but infections are a serious complication [2]. Ursodeoxycholic acid (UDCA) and colchicine are used with some success in

patients with PBC, but the treatment remains a difficult dilemma. Treatment of PSC poses a similar challenge, and there is a shortfall of available therapies for the majority of patients [3]. In addition, side effects of immunosuppressive therapy including increased risk of malignancy suggest strongly that other targeted treatment options are needed urgently [4]. An increased risk of malignancy is a well-established complication of long-term immunosuppressive therapy and a major source of morbidity and mortality. Virus infections, immunodeficiency and drug-induced promotion of carcinogenesis (for example, through production of cytokines regulating tumour growth, metastasis and angiogenesis) are most probably inducers of malignancy in those patients [5]. In this context it should be noted that phage therapy is safe and may be applied in immunodeficient cancer patients [6]. Furthermore, there are data suggesting that it may have anti-viral action and induce tumour and metastasis regression in experimental mice [7–9].

The need for more targeted therapy of ALD coincides with the marked progress in research on immunobiology of bacterial viruses (phages). In the face of increasing drama of anti-microbial resistance, phages offer a broad medical promise as a weapon against antibiotics-resistant bacteria [10]. However, phages should no longer be

considered as mere bacterial viruses but also as potential modulators of immunity [11–16], a finding which may have an important application in therapy including auto-immune diseases [12].

Phages as immunomodulators

Accumulating data indicate that phages may modulate the immune response, contributing to maintenance of immune homeostasis in the gastrointestinal tract and possibly at other sites [11,17–19]. The recent review discusses in detail those immunomodulating activities of phages [11]. In brief, phages can diminish T cell activation, alloantigen-induced immunoglobulin production *in vitro* and extend the skin allograft survival in naive and sensitized mice [13,14]. In addition, phages may reduce autoimmune reaction when a mouse model of autoimmunity was used (collagen-induced arthritis) [12]. Skin and organ inflammatory infiltration induced by alloantigens and endotoxin can also be reduced markedly by phage or a phage protein administration [13,20].

Phages do not impair granulocyte and monocyte ability to kill bacteria; conversely, this activity may be normalized in patients on phage therapy who had abnormally low values prior to the therapy (a finding with prognostic significance for the outcome of the therapy) [14]. Capturing of lipopolysaccharide (LPS) does not appear to be responsible for this effect, as it is observed using phages against both Gram$^-$ and Gram$^+$ bacteria. Reducing the bacterial burden by phages may enhance phagocytic functions, in accord with the concept of 'immunophage synergy' – synergistic action of phages and phagocytes [21]. Also, degranulation of human granulocytes is not affected by phages [22]. Some of those original observations have been confirmed recently by other authors, including the data on phage translocation from the gut to other tissues [15,17].

Phages as anti-inflammatory agents

It has been noted that clinical phage therapy may cause a decrease of inflammatory markers [C-reactive protein (CRP), sedimentation rate, leucocytosis], even though eradication of infections has not been achieved [23]. Further studies have shown that phages may down-regulate the inflammatory process by reducing the production of reactive oxygen species (ROS) induced by bacteria and by endotoxin [24]. Of particular interest here are data showing that a phage protein diminishes organ leucocytic infiltration markedly (including the liver) in mice challenged with endotoxin [20]. Recently, Van Belleghem *et al.* [25] have shown that human immune responses to phages *in vitro* are predominantly anti-inflammatory and – interestingly – moderate amounts of endotoxin can up-regulate those effects further. The authors have shown that adding an intermediate level of endotoxin up-regulates

Table 1. Phage-mediated changes in immune parameters which may be beneficial in the treatment of autoimmune liver disease

Interleukin (IL)-10 production	↑
Reactive oxygen species production	↓
Activation of nuclear factor-kappa B	↓
Bacteria translocation	↓
IL-1 receptor antagonist	↑
Toll-like receptor-4 expression	↓

interleukin-1 receptor antagonist and reduces strongly the expression of proinflammatory cytokines chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL5. This fully confirms and extends our data derived from studies using phage preparations containing endotoxin showing that such preparations may reduce the level of inflammation markers (CRP, leucocytosis and sedimentation rate) [23]. Table 1 summarizes phage-mediated changes in immune parameters which may be beneficial in the treatment of auto-immune liver disease.

Phages, Kupffer cells and immune homeostasis

Liver macrophages (Kupffer cells: KC) are of paramount importance for maintenance of liver and systemic homeostasis; a recent comprehensive review depicts in detail their role in modulating inflammation and inducing immunological tolerance [26]. KC mediate immunosuppression by production of IL-10, by expression of the negative co-stimulator for T cell activation programmed cell death ligand-1 (PD-L1) [27] and by preventing cytotoxic lymphocyte responses [28]. Those activities translate into induction of KC-dependent protective immune tolerance in liver [29]. Down-regulating KC-dependent signalling by IL-10 reduces inflammation and fibrosis [30]. Deletion of KC in experimentally induced hepatitis suppresses liver damage as well as collagen-induced autoimmune arthritis in mice [31]. Interestingly, we noted similar effects of phages using the latter model of autoimmune disorder [12]. KC may also be involved in mediating the phenomenon of liver allograft tolerance, which occurs in different species so that transplant survival may be accomplished without concurrent immunosuppression – a long-term dream in transplantation [32]. Moreover, when liver and kidney are transplanted simultaneously the liver is immunoprotective for the kidney [33]. KC production of IL-10 may be up-regulated by encapsulated platelets; therefore, when KC are first activated by liver injury such administered platelets cause increased secretion of IL-10 and down-regulation of ROS [34]. Interestingly, increased phagocytosis by KC leads to attenuation of hepatitis through IL-10-mediated suppression of ROS and inflammatory cytokines [35]. In conclusion, as pointed out by Krenkel and Tacke [26], available data from experiments in animals and early clinical trials suggest strongly that

targeting pathogenic Kupffer cells may be a novel promising approach in acute and chronic liver diseases. Interestingly, 70–90% of phages administered intravenously in mice are taken up by liver (the value for the liver is approximately 12 times higher that that for the spleen) [36]. Other authors have confirmed that liver and spleen are the primary organs responsible for the uptake of phages [37]. Of note, liver Kupffer cells that are primarily responsible for this uptake are unable to prime lymphocytes for antibody responses against phage; therefore, no intrahepatic phage-neutralizing antibody response should be expected. In contrast, almost the entire humoral response to phage is attributable to spleen [38]. Interestingly, unstimulated human peripheral blood granulocytes do not inactivate phages, a finding that highlights further the significance of Kupffer cell interactions with phages [39].

If enhanced phagocytosis by Kupffer cells may translate into attenuation of hepatitis (see above), then it may be expected that phage uptake by Kuppfer cells may also mediate similar effects. In addition, if the effect of phages on granulocyte and peripheral blood monocyte ROS production is comparable to their effect on Kupffer cells then phage-induced decrease of ROS and enhancement of IL-10 production by these cells may also contribute significantly to achieving immune homeostasis.

Phages in potential immunotherapy of ALD

Recent findings indicate that phages induce IL-10 production by human mononuclear cells [25]. This cytokine, known for its anti-inflammatory action, has been recognized as playing a protective role against hepatic injury. Administration of IL-10 in mice reduces apoptosis of hepatocytes and hepatic neutrophil infiltration dramatically and delays hepatic necrosis. In a model of liver injury IL-10 reduces markedly the level of transaminases and haemorrhagic liver damage; it also has anti-fibrotic properties [40]. IL-10-producing T cells prevent liver damage during chronic hepatitis C virus infection [41]. Phages cause a moderate inhibitory effect on the activation of nuclear factor kappa B (NF-κB) [13] – such action is known to inhibit liver inflammation and injury [42].

As already mentioned, phages reduce excessive ROS production [24]. Oxidative stress with concurrent excessive production of ROS has been recognized as an important factor in progression of liver injury, while Kupffer cells are potentially more exposed or sensitive to ROS. Antioxidative therapy aiming to prevent the progression of liver injury represents a reasonable means of treatment. Although experimental studies *in vitro* and in animals are encouraging, only few clinical trials have demonstrated the beneficial effects of anti-oxidants regarding the the prevention of progression of ALD [43,44].

Phages have been found to up-regulate the expression of IL-1 receptor antagonist (IL-1RN) [25]. It is noteworthy that the evolution to a more aggressive form of chronic active hepatitis is associated with an excess of IL-1 over IL-1RN at tissue level [45]. Furthermore, IL-1RN plasma concentrations are increased by interferon (IFN)-α treatment in patients with hepatitis C virus (HCV)-released chronic hepatitis, and it was suggested that this constitutes the key mechanism of the action of IFN-α in this disorder [46]. Thus, phage-mediated IL-1RN up-regulation may be of potential therapeutic benefit.

Toll like receptor-4 (TLR-4) is known to be present in the liver (Kupffer cells, hepatocytes, stellate cells, dendritic cells, endothelial cells) and – upon triggering – produce proinflammatory cytokines (IL-1, IL-6, etc.) as well as anti-inflammatory cytokine IL-10. Also, biliary epithelial cells bear TLR-4. There is increasing evidence that the receptor plays a key role in HCV infection and replication. TLR-4 signalling is controlled by a variety of regulators, while contributing inhibitorily to prevention of inflammation-induced damage. For example, its genetic deletion or mutation lowers liver infiltration and injury in the preclinical mouse model of chronic hepatitis [47]. TLR-4 has been identified as a factor associated with a high risk of developing cirrhosis in patients with chronic hepatitis C. Moreover, TLR-4 activation is associated with the progress of chronic liver diseases. including AH, PBC and PSC. Inhibitors of TLR-4 are being tested in the hope that they might prevent the progression of chronic hepatitis [48,49]. As purified phages may down-regulate TLR-4 [25], they are an obvious candidate for such trials.

While platelets have been recognized as a source of mediators enhancing liver regeneration, they can also contribute to the initiation of liver inflammation promoting leucocyte recruitment to this organ and amplifying hepatic injury with subsequent hepatocarcinogenesis [50]. Anti-platelet therapy prevents the development of hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B [51]. It is also known that fibrinogen deposits are observed in liver injury which form a matrix attracting and inducing proliferation of inflammatory cells [52]. Again, phages may reduce the extent of this pathology, as they inhibit platelet adhesion to fibrinogen and also, weakly, T cell adhesion [11].

Recently, another support for our concept was provided by the data showing that in a mouse model of autoimmune disorder phages can inhibit the development of autoimmune inflammatory responses with efficacy comparable to standard immunosuppression [12].

An expansion of individual microbes and increased microbial translocation have also been implicated in the pathogenesis of ALD [53]. In fact, oral vancomycin was reported to cause complete normalization of laboratory parameters in a patient with PSC [4]. Thus, phages may mediate beneficial effects in ALD by both their immunomodulating and anti-bacterial actions.

Table 2. Different liver pathologies, therapies and potential phage application

Liver pathology	Origin	Treatment	Potential phage application
Autoimmune hepatitis	Unknown, immune-mediated chronic inflammatory disease	Immunosuppression to induce and maintain remission of inflammation and prevent fibrosis progression to cirrhosis; insufficient response in up to 20% of patients	To reduce inflammation and prevent progression to end-stage liver disease
Primary biliary cirrhosis	Autoimmune disorder marked by anti-mitochondrial antibodies leading to biliary destruction and liver cirrhosis	Ursodeoxycholic acid (UDCA) may improve survival; approx. 1/3 of patients do not respond; other agents (e.g. colchicine, fibrates) have uncertain therapeutic value	To reduce autoimmune reactions
Primary sclerosing cholangitis	Immune-mediated disease of intra-and extrahepatic bile ducts leading to end-stage liver disease	UDCA value uncertain; current immunosuppression largely disappointing. Prophylactic and long-term antibiotics may improve biochemical parameters and symptom profile. Only curative therapy liver transplantation. Unmet need for effective medical treatment [3]	To alleviate autoimmune reactions and combat bacteria-induced aggravation preventing progression to end-stage liver disease

Phages in viral hepatitis

As noted above, IL-10 production by T cells may prevent liver damage in chronic hepatitis C virus infection. Phages have been reported to inhibit duck hepatitis virus replication and their activity was higher than that of acyclovir [54]. This effect was due at least partly to induction of endogenous IFN by phage DNA. Furthermore, we showed that phage may interfere with absorption and replication of adenoviruses [55]. There are also more data to suggest that phages could also be used not only in ALD, but perhaps also in some forms of viral hepatitis [7]. As mentioned, phages and their nucleic acid may inhibit virus replication. They may compete with viruses for cellular receptors or evoke antibodies cross-reacting with pathogenic viruses. When administered orally, phage DNA can reach liver via the intestinal wall mucosa [56]; therefore, intrahepatic IFN induction by phage DNA could be helpful in eradication of viruses targeting the liver. Phages could also be used for the development of vaccine against HBV and production of nanomolecules displaying peptides that could interfere with attachment of pathogenic viruses and their entry into liver cells [57]. Those data are, of course, very preliminary and require further studies. Table 2 briefly summarizes basic data on the different liver pathologies, their therapies and potential phage application.

Safety of phage therapy

It should be emphasized that no clinical or laboratory signs of hepatic injury were noted in normal individuals and patients treated with phages [although some of them had some extent of liver dysfuntion (moderate elevation of aminotransferases)], so clinical phage therapy appears to be free of hepatotoxicity [58–60]. This confirms earlier data from experiments in mice [61] and in broilers in which dietary supplementation with phages increased liver weight [62].

Conclusions/perspective

The data available suggest that liver is an important target for phages administered *in vivo*. Phages accumulate within Kupffer cells believed to play a strategic role in maintenance of hepatic and systemic homeostasis. Recent data indicate that phages modulate the phenotype and function of immune cells towards an anti-inflammatory response which opens an interesting perspective for targeting pathogenic Kupffer cells by phage therapy to attenuate immune-mediated liver injury. This option appears to be especially attractive in view of the findings which suggest strongly that – in contrast to standard immunosuppression – phage therapy is safe and relatively free of side effects. ALD and rheumatic diseases co-exist in approximately 30% of cases, suggesting that they may share pathogenic mechanisms [63]. In this context, our recent data showing the efficacy of phage therapy in a mouse model of rheumatoid arthritis provide additional arguments to believe that relevant clinical trials should be on the horizon. We suggest PSC as a primary target for such a trial of phage therapy. The disease poses a great therapeutic challenge, while the available data suggest the involvement of both autoimmune and infectious factors. Portal venous bacteraemia and exposure to toxic bile acids produced by colonic bacteria can up-regulate immunoactivation pathways; in fact, antibiotics have been shown to bring some benefits in those patients [64]. Thus, in contrast to other drugs, phages could target

both factors contributing to pathology of the disease and therefore offer more chances for success. Furthermore, therapy-resistant patients with AH and PBC could also be candidates for phage therapy trials in ALD.

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Disclosure

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