Phage therapy in allergic disorders?

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Impact statement

Allergic disorders pose a growing challenge to medicine and our society, so new approaches to prevention and therapy are urgently needed. Our article summarizes progress that has been recently made and presents a shift in our understanding of the immunobiological significance of bacterial viruses (phages). Currently, phages may be considered not only as mere "bacteria eaters" but also as regulators of immunity. The new understanding of phages as important factors in maintenance of immune homeostasis opens completely new perspectives for their use in controlling aberrant immune responses. It is likely that this new knowledge could be translated into novel means of immunotherapy of allergic disorders.

Abstract

Allergic disorders pose a growing challenge to medicine and our society. Therefore, novel approaches to prevention and therapy are needed. Recent progress in studies on bacterial viruses (phages) has provided new data indicating that they have significant immunomodulating activities. We show how those activities could be translated into beneficial effects in allergic disorders and present initial clinical data that support this hope.

Keywords: Allergy, immunomodulation, inflammation, phage

***Experimental Biology and Medicine* 2018; 243: 534–537. DOI: 10.1177/1535370218755658**

Introduction

The allergy epidemic has become a great challenge to medicine and society. While currently available therapies provide some relief and benefit, all those treatments have significant drawbacks, and therefore novel approaches are urgently needed.[1] Bacterial viruses (phages) have recently gained greatly increased attention in view of their ability to kill bacteria, including antibiotic-resistant strains. Consequently, phage therapy (PT) has remained of interest as a potential weapon to combat the microbial resistance believed today to be a grave challenge to medicine and civilization. While available data indicate high safety and strongly suggest efficacy of PT, it is expected that ongoing clinical trials will provide awaited proof of efficacy in accordance with the requirements of evidence-based medicine.[2]

Phages as regulators of immune and inflammatory responses

The growing interest in PT is paralleled by better understanding of the actual significance and role of phages, especially as potential regulators of immunity. Initially considered as mere "bacteria killers," today phages are recognized as an important part of the mammalian immune system. Phages present in mammalian organisms (endogenous phages, e.g. in the intestines) may exert immunomodulating action similar to probiotics[2,3] and, by their ability to translocate from the gut to other tissues, they can mediate such activities, locally contributing to maintenance of immune homeostasis.[4,5] Interestingly, phages have been shown to cause strong anti-inflammatory effects reducing levels of C-reactive protein and other indices of inflammation in patients receiving PT even though the infection has

not been eliminated, thus suggesting that some phage effects are at least partly independent from their direct antibacterial action.[6] The possible mechanisms of immunomodulating and anti-inflammatory activities of phages have recently been discussed in detail.[7] Those observations have been confirmed and extended by other authors.[8,9] Of particular interest are the recent data of van Belleghem *et al.*,[10] who studied the effect of purified phages on immune responses of human peripheral blood mononuclear cells and showed that their prevailing effect is anti-inflammatory. Thus, phages were shown to induce the anti-inflammatory IL-1 receptor antagonist (IL-1RA) and strong upregulation of IL-10. This cytokine has been recognized as having anti-inflammatory properties blocking the expression of pro-inflammatory cytokines and inhibiting the activities of Th1 cells, NK cells and macrophages. Similar data were obtained by Sun and Feng,[11] who showed that phage films downregulate the inflammatory response and induce high IL-10 expression. Van Belleghem's group also showed a marked reduction of TLR4 expression on human mononuclears; TLR4 is known to induce pro-inflammatory cytokines and chemokines.[10] Also of interest are data indicating that phages do not induce degranulation of human granulocytes and markedly decrease inflammation caused by the autoimmune reaction.[12,13]

How phages may counteract allergen-induced immunopathology

Evidence has accumulated that IL-10, a cytokine which is upregulated by phages, is a strong inhibitor of allergen-induced inflammation and airways hyper-responsiveness.

Administration of IL-10 reduces the number of eosinophils and mast cells alleviating nasal inflammation, thus showing potential as an inhibitor of allergic rhinitis.[14] Moreover, IL-10 was shown to stabilize mast cells, protecting against degranulation.[15] CD5 + B cells suppress IgE- and antigen-mediated activation of mast cells *in vitro* and allergic responses in mice in an IL-10-dependent manner.[16] Also, IL-10 production by T cells coincided with inhibition of eosinophilic airways inflammation and epithelial mucus plugging.[17] What is more, specific immunotherapy causes increased IL-10 production and resulting anergy of T cells and switching of specific IgE towards normal IgG4-related immunity.[18] Similar allergy-attenuating effects have been described for IL-1RA. Thus, an adenovirus expressing IL-1RA was observed to attenuate allergic airways inflammation in a mouse model of asthma.[19] The ability of IL-1RA to reduce allergen-induced airway inflammation and mucus secretion in mice has also been reported by Gurusamy *et al.*[20] IL-1RA has also been shown to prevent experimentally induced allergic eye disease in mice by downregulation of the recruitment of eosinophils and other inflammatory cells.[21]

There is ample evidence that allergic disorders such as asthma, rhinitis and atopic dermatitis may be mediated by oxidative stress.[22] Endogenous and exogenous reactive oxygen species (ROS) have been shown to be responsible for the airway inflammation in allergic asthma. In animal models, excessive ROS production may cause airway inflammation and hyper-responsiveness, tissue injury, and remodeling.[23] In this regard, it is noteworthy that phages – in contrast to pathogenic viruses and bacteria – do not induce ROS[24] and inhibit ROS production by phagocytes.[25,26]

TLR4 antagonist has been shown to reduce asthma features provoked by an allergen.[27] Therefore, phage ability to downregulate its expression might cause similar effects. Recent data suggest that the microbiomes of the lung and gut contribute to the pathogenesis of asthma and allergy.[28] Allergic children harbor higher counts of coliforms and *Staphylococcus aureus*.[29] It is also well known that local allergic reactions can be induced and aggravated by microorganisms.[30] As phages usually have very narrow spectra, in contrast to antibiotics (whose use is believed to be associated with the rising prevalence of allergies), phage application could thus selectively eliminate those bacterial pathogens and perhaps alleviate or even prevent symptoms of allergy. Table 1 briefly summarizes what is known about phage activities *in vitro* and *in vivo* and how those findings can be translated into beneficial effects in allergic disorders.

Table 1. Phage activities *in vitro* and *in vivo* which may be beneficial in allergic disorders.

In vitro	*In vivo*
Reactive oxygen species ↓(26)	Circulating eosinophils – (31)
IL-10 ↑ (10,11)	C-reactive protein↓ (6)
IL-1 receptor antagonist ↑ (10)	Erythrocyte sedimentation rate ↓ (6)
TLR4↓(10)	Leukocytosis↓ (31)
Degranulation of granulocytes – (12)	Autoimmune reaction ↓ (13)Inflammatory infiltration of skin(32) and lung(33) ↓
	Local reactions to phage administered subcutaneously – (34)

Note: Relevant references are given in parentheses.
↓ downregulation, ↑ upregulation, – no effect.

PT in allergic patients

Interestingly, in >150 patients who received PT significant side-effects including some signs of allergic reactions occurred in only 1.4% of cases. What is more, eosinophil counts remained within a normal range in all of them.[31] A search of the non-English literature from Eastern Europe has revealed publications reporting lack of local reactions to phage preparations in patients.[34] Intravenous phage phi X174 has been used to study immunocompetence in patients with the hyper-IgE syndrome and in children with steroid-dependent asthma.[34,36] The above data suggest that phage administration in humans rarely induces allergic reactions. Moreover, there are some data claiming efficacy of PT in allergic patients. Sakandelidze *et al.*, reported success in "infectious allergoses."[37] Similarly, good results were reported in patients with allergy to antibiotics.[38] American physicians as long ago as in the 1950s and 1960s suggested that PT may be helpful in

controlling allergy and asthma.[39,40] Recently, successful PT of a boy with Netherton syndrome with atopic diathesis was reported. By the seventh day of the therapy, a significant improvement including a marked reduction of skin involvement was noted. No allergic reactions to the phage were observed.[41]

Conclusions

Phages exert anti-inflammatory action *in vitro* and *in vivo* and can downregulate aberrant immune reactions. Initial observations in patients receiving PT suggest that allergic reactions to phage administration are rare; furthermore, PT may be useful in specific cases of allergic disorders. Further studies and clinical trials of phage efficacy in those disorders are warranted.

Authors' Contributions: AG drafted the main part of the manuscript. EJ-M, M**Ł**-S, RM, BW-D, and JB contributed parts of the manuscript. All authors revised the manuscript.

DECLARATION OF CONFLICTING INTERESTS

A Górski, R Międzybrodzki, B Weber-Dąbrowska, and J Borysowski are co-inventors of patents owned by the Institute of Immunology and Experimental Therapy and covering phage preparations.

FUNDING

This work was supported by grant DEC-2013/11/B/NZ1/02107 from the National Science Center (NCN). The publication was also supported by Wroclaw Centre of Biotechnology, the Leading National Research Center (KNOW) program for the years 2014–2018.

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