



Phage Interaction with the Mammalian Immune System

Jonas D. Van Belleghem, Krystyna Dąbrowska, Mario Vaneechoutte, and Jeremy J. Barr

1 Background: Human Immune Response

Vertebrates are constantly threatened by the invasion of microorganisms and have evolved systems of immune defense to eliminate infective pathogens in the body. The mammalian immune system can be divided in two branches: the innate and acquired or adaptive immunity (Table 1) (Akira et al. 2006). The innate immune response is the first line of host defenses against pathogens and is mediated by phagocytes including macrophages and dendritic cells (DCs). Acquired or adaptive immunity is involved in elimination of pathogens in the late phase of infection as well as the generation of immunological memory. The main cells involved in the acquired or adaptive immune response are T and B cells (Medzhitov and Janeway 2000).

The innate immune system is believed to have predated the adaptive immune response on several grounds. First, innate host defenses are found in all multicellular organisms, whereas adaptive immunity is found only in vertebrates. Second, innate immune recognition distinguishes self from nonself perfectly. Third, the innate immune system uses receptors that are ancient in their lineage, whereas adaptive

J. D. Van Belleghem (✉)

Laboratory Bacteriology Research, Department of Clinical Chemistry, Microbiology and Immunology, University Ghent, Ghent, Belgium

Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

K. Dąbrowska

Bacteriophage Laboratory, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

M. Vaneechoutte

Laboratory Bacteriology Research, Department of Clinical Chemistry, Microbiology and Immunology, University Ghent, Ghent, Belgium

J. J. Barr

School of Biological Sciences, Monash University, Melbourne, VIC, Australia

Table 1 Innate and adaptive immunity. Adapted from Janeway and Medzhitov (2002)

Property	Innate immune system	Adaptive immune system
Receptors	Fixed in genome Rearrangement is not necessary	Encoded in gene segments Rearrangement is necessary
Distribution	Non-clonal All cells of a class identical	Clonal All cells of a class distinct
Recognition	Conserved molecular patterns	Details of molecular structure
Self-nonsel discrimination	Perfect: selected over evolutionary time	Imperfect: selected in individual somatic cells
Action time	Immediate activation of effectors	Delayed activation of effectors
Response	Co-stimulatory molecules Cytokines Chemokines	Clonal expansion or anergy Cytokines

immunity appears to use the same effector mechanisms guided by clonally specific antibodies and T-cell receptors (TCR) encoded in rearranging genes of the Ig gene superfamily (Medzhitov and Janeway 1997). The virtues of having an innate immune system of pathogen recognition lies not only in the delaying tactics of inflammation upon infection, but also in the activation of the adaptive immune system only when the body is under attack by a specific pathogen (Janeway and Medzhitov 2002).

1.1 Innate Immunity

Innate immunity covers many areas of the host defense against pathogenic microbes and viruses, including the recognition of pathogen-associated molecular patterns (PAMPs) (Janeway 1989). In vertebrates, which are the only phylum that can mount an adaptive immune response, there are also mechanisms to inhibit the activation of innate immunity (Janeway and Medzhitov 2002). Innate immunity is an evolutionary ancient part of the host defense mechanisms, the same molecular modules are found in plants and animals, meaning it arose before the split into these two kingdoms (Hoffmann et al. 1999). Innate immunity lies behind most inflammatory responses; these are triggered in first instance by macrophages, polymorphonuclear leukocytes, and mast cells through their innate immune receptors (Janeway and Medzhitov 2002).

The innate immune system is the primary, or early, barrier to infectious agents and acts immediately upon recognition of a pathogen. It mounts an effective defense against infectious agents through the initiation of adaptive immunity, which is long-lasting and has immunological memory (Kumar et al. 2011). Invasion of a host by a pathogenic infectious agent triggers a battery of immune responses through interactions between a diverse array of pathogen-born virulence factors and the immune surveillance mechanisms of the host. Host-pathogen interactions are generally initiated via host recognition of conserved molecular structures known as pathogen-associated

molecular patterns (PAMPs) that are essential for the life cycle of the pathogen (Janeway and Medzhitov 2002; Kumar et al. 2011). However, these PAMPs are either absent or compartmentalized inside the host cell and are sensed by the host's germline-encoded pattern recognition receptors (PRRs), which are expressed on innate immune cells such as dendritic cells, macrophages, and neutrophils (Takeuchi and Akira 2010; Kawai and Akira 2010; Medzhitov 2007; Blasius and Beutler 2010).

Signaling receptors recognize PAMPs and activate signaling-transduction pathways that induce the expression of a variety of immune-response genes, including inflammatory cytokines (Takeuchi and Akira 2010; Kawai and Akira 2010; Ozinsky et al. 2000; Kumar et al. 2009). Toll-like receptors (TLRs) are the most widely studied PRRs and are considered to be the primary sensors of pathogens (Kumar et al. 2011). Based on their primary sequence, TLRs can be further divided into several subfamilies, each of which recognizes related PAMPs: the subfamilies of TLR1, TLR2, and TLR6 recognize lipids, whereas the highly related TLR7, TLR8, and TLR9 recognize nucleic acids (Table 2).

1.2 Adaptive Immunity

Adaptive immunity is a relative newcomer on the evolutionary landscape. Because the mechanism of generating receptors in the adaptive immune system involves great variability and rearrangement of receptor gene segments, the adaptive immune system can provide specific recognition of foreign antigens, immunological memory of infection, and pathogen-specific adaptor proteins. However, the adaptive immune response is also responsible for allergy, autoimmunity, and the rejection of tissue grafts (Janeway and Medzhitov 2002). The adaptive immunity adds specific recognition of proteins, carbohydrates, lipids, nucleic acids, and pathogens to the underlying innate immune system, using the same activated, but not antigen-specific, effector cells generated by the innate immune recognition. The two systems, i.e., the innate and adaptive immune system, are linked in the use of the same effector cells (e.g., dendritic cells or macrophages) (Janeway 1989).

Activation of the adaptive immune system occurs only upon pathogen recognition by dendritic cells, where they play a pivotal role at the interface of innate and adaptive immunity (Pulendran et al. 2001). Pathogen recognition is mediated by innate receptors such as RLRs and NLRs (Kumar et al. 2009). Immature dendritic cells reside in the peripheral tissues, where they actively sample their environment by endocytosis and micropinocytosis (Orsini et al. 2003).

Unlike the innate mechanisms of host defense, the adaptive immune system manifests exquisite specificity for its target antigens. Adaptive responses are based primarily on the antigen-specific receptors expressed on the surface of T and B lymphocytes (Chaplin 2010; Bonilla and Oettgen 2010; Schroeder and Cavacini 2010). The adaptive immunity is mediated by immunoglobulins and T-cell receptors (TCRs) (Tonegawa 1983). A major challenge faced by the immune system is to identify host cells that have been infected by microbes that subsequently use the cell to multiply within the host. A major role of the T-cell arm of the immune response is

Table 2 TLR recognition of bacterial or viral components. Adapted from Akira et al. (2006)

Microbial components	Species	TLR usage	References
Bacteria			
LPS	Gram-negative bacteria	TLR4	Poltorak et al. (1998); Shimazu et al. (1999)
Diacyl lipopeptides	Mycoplasma	TLR6/ TLR2	Thoma-Uszynski (2001)
Triacyl lipopeptides	Bacteria and mycobacteria	TLR1/ TLR2	Thoma-Uszynski (2001)
Lipoteichoic acid (LTA)	Group B Streptococcus	TLR2	Alexopoulou et al. (2002); Ozinsky et al. (2000); Takeuchi et al. (2000, 2001)
Peptidoglycan (PG)	Gram-positive bacteria	TLR2	Alexopoulou et al. (2002); Ozinsky et al. (2000); Takeuchi et al. (2000, 2001)
Porins	Neisseria	TLR2	
Lipoarabinomannan	Mycobacteria	TLR2	Gilleron et al. (2003)
Flagelin	Flagellated bacteria	TLR5	Hayashi et al. (2001)
CpG-DNA	Bacteria and mycobacteria	TLR9	Hemmi et al. (2002)
ND	Uropathogenic bacteria	TLR11	Zhang et al. (2004)
Viruses			
DNA	Viruses	TLR9	Hochrein et al. (2004); Krug et al. (2004a, b); Lund et al. (2003); Tabeta et al. (2004)
dsRNA	Viruses	TLR3	Alexopoulou et al. (2001)
ssRNA	RNA viruses	TLR7 and TLR8	Diebold (2004); Heil (2004); Hemmi et al. (2002)
Envelope proteins	RSV, MMTV	TLR	Kurt-Jones et al. (2000)
Hemagglutinin protein	Measles virus	TLR2	Bieback et al. (2002); Compton et al. (2003)
ND	HCMV, HSV1	TLR2	Kurt-Jones et al. (2004)

to identify and destroy infected cells. T cells can also recognize peptide fragments of antigens that have been taken up by antigen presenting cells (APCs) through the process of phagocytosis or pinocytosis. The immune system permits T cells to recognize infected host cells by the recognition of both self-component and a microbial structure. This is mediated by the major histocompatibility (MHC) molecules. MHC molecules (also called human leukocyte antigen (HLA) antigens) are cell surface glycoproteins that bind peptide fragments of proteins that either have been synthesized within the cell (class I MHC molecules) or have been ingested by the cell proteolytically processed (class II MHC molecules) (Chaplin 2010; Menéndez-Benito and Neefjes 2007; Davis and Bjorkman 1988; Watts 2004).

1.3 Immune Cell Communication: The Language of Cytokines and Chemokines

Cells of the immune system require communication networks that can, as required, act locally or at a distance, specifically or globally, and transiently or in a sustained manner. This immune cell communication is conducted mainly by cytokines and chemokines. The term *cytokine* defines a large group of nonenzymatic protein hormones whose actions are both diverse and overlapping and which affect diverse and overlapping target cell populations (Kelso 1998; Opal et al. 2000). Chemokines on the other hand are essential for the trafficking of immune effector cells to sites of infection. Moreover, their function is necessary to translate an innate immune response into an acquired response. Innate immune stimuli, through activation of TLRs, set in motion a genetic program that induces the expression of a subset of chemokines from resident tissue macrophages and dendritic cells and modulates the expression of chemokine receptors on dendritic cells (Luster 2002; Nomiyama et al. 2010).

1.3.1 Cytokines

Cytokines are local mediators produced by cells of the lymphoid and macrophage lineage as well as by epithelial and mesenchymal cells. Cytokines are involved in a variety of biological processes, including cell activation, growth, and differentiation, and they are central to the development of inflammation and immunity (Sartor 1994; Elson 1996). Cells of the innate immune system, such as macrophages and monocytes, are able to mount a rapid response to a danger signal, e.g., an infectious agent, by secreting several pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-12, and tumor necrosis factor (TNF)- α . The cytokine milieu subsequently directs the development of adaptive immunity mediated by T and B lymphocytes (Papadakis and Targan 2000). Some cytokines clearly promote inflammation and are called pro-inflammatory cytokines, whereas other cytokines suppress the activity of pro-inflammatory cytokines and are called anti-inflammatory cytokines (Dinarello 2000).

The concept that some cytokines function primarily to induce inflammation while others suppress inflammation is fundamental to cytokine biology and also to clinical medicine (Dinarello 2000; Opal et al. 2000). A dynamic and ever-shifting balance exists between pro-inflammatory cytokines and anti-inflammatory components of the human immune system. The regulation of inflammation by these cytokines and cytokine inhibitors is complicated by the fact that the immune system has redundant pathways with multiple elements having similar physiologic effects (Kasai et al. 1997; Munoz et al. 1991). The net effect of any cytokine is dependent on the timing of cytokine release, the local milieu in which it acts, the presence of competing or synergistic elements, cytokine receptor density, and tissue responsiveness to each cytokine (Dinarello 1998; Cannon 2000). Different immunogens induce the synthesis of different cytokines which in turn activate different immune effector mechanisms. Although every nucleated cell type can produce cytokines, most lineages express only a subset of cytokine genes (Kelso 1998; Cannon 2000).

Here we will discuss some (i.e., IL-1, IL-6, and IL-10) but not all interleukins. The IL-1 cytokine family comprises four main members IL-1 α , IL-1 β , IL-1 receptor antagonist (IL-1ra/IL-1RN), and IL-18 (Girm et al. 2007). The IL-1 family is primarily considered to be pro-inflammatory, as it can upregulate host defenses and act as an immunoadjuvant (Dinarello 1997b). IL-1 β plays a significant role in inflammation; it has been implicated in enhancing expression of cell adhesion molecules on the endothelial surface and has consequently been deemed to be pro-atherogenic (Dinarello 1999). The only member of this family with paradoxical properties is IL-1RN, a naturally occurring cytokine antagonist, which plays an anti-inflammatory role in regulating IL-1 function (Dinarello and Thompson 1991; Perrier et al. 2006). IL-1RN blocks the action of IL-1 α and IL-1 β functional ligands by competitive inhibition at the IL-1 receptor level. IL-1RN is produced by monocytes and macrophages and is released into the systemic circulation in >100-fold excess than either IL-1 α or IL-1 β after lipopolysaccharide (LPS) stimulation (Dinarello 1998). The anti-inflammatory cytokines IL-4, IL-6, IL-10, and IL-13 inhibit the synthesis of IL-1 β and stimulate the synthesis of IL-1RN (Dinarello 1997a).

IL-6 has long been regarded as a pro-inflammatory cytokine induced by LPS along with TNF- α and IL-1. It is often used as a marker for systemic activation of pro-inflammatory cytokines (Barton and Medzhitov 2002). Like many other cytokines, IL-6 has both pro- and anti-inflammatory properties. Although IL-6 is a potent inducer of the acute-phase protein response, it has anti-inflammatory properties as well (Barton et al. 1996). IL-6 attenuates the synthesis of the pro-inflammatory cytokines while having little effect on the synthesis of anti-inflammatory cytokines such as IL-10 and transforming growth factor- β (TGF- β). IL-6 induces the synthesis of glucocorticoids and promotes the synthesis of IL-1RN and soluble TNF receptor release in human volunteers (Ruzek et al. 1997; Tilg et al. 1994). At the same time, IL-6 inhibits the production of pro-inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN- γ) and MIP-2 (Barton 1997).

IL-10 is the most important anti-inflammatory cytokine found in the human immune response (Opal et al. 2000). It is a potent inhibitor of T_H1 cytokines, including both IL-2 and IFN- γ , but also of IL-1, IL-6, and TNF- α (Hagenbaugh et al. 1997; Opal et al. 1998; Howard and O'Garra 1992; Lalani et al. 1997). IL-10 is a pleiotropic cytokine produced by a variety of cells, including T and B lymphocytes, thymocytes, macrophages, mast cells, keratinocytes, and intestinal epithelial cells. IL-10 is also a potent deactivator of monocyte/macrophage pro-inflammatory cytokine synthesis (Clarke et al. 1998; Brandtzaeg et al. 1996). It also inhibits cell surface expression of MHC class II molecules and the LPS recognition and signaling molecule CD14 (Opal et al. 1998).

The cytokine induced immune responses can be further regulated by suppressors of cytokine signaling (SOCS) and cytokine-inducible SH2 protein (CIS) family of intracellular proteins (Yasukawa et al. 2000; Larsen and Röpke 2002; Greenhalgh et al. 2002). In total, there are eight SOCS proteins (i.e., SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7, and CIS) (Illson et al. 1998). The most well-characterized

SOCS family members, SOCS1, SOCS2, SOCS3, and CIS (Table 3), seem to act in a classical negative-feedback loop to inhibit cytokine signal transduction (Larsen and Röpke 2002). SOCS1 has an important regulatory function in macrophages and dendritic cells. The inhibitory activity of SOCS2 is not as strong as that of CIS (Metcalf et al. 2000). Both SOCS1 and SOCS3 can inhibit JAK tyrosine kinase activity.

1.3.2 Chemokines

Chemokines are small heparin-binding proteins that form a family of chemotactic cytokines that regulate migration and tissue localization of various kinds of cells in the body (Moser et al. 2004; Zlotnik and Yoshie 2000; Charo and Ransohoff 2006). In particular, they participate in inflammatory leukocyte recruitment, in lymphocyte recirculation and homing, and even in cancer metastasis (Gerard and Rollins 2001; Ben-Baruch 2008). Chemokines are known to have well-conserved four cysteines and are grouped into five subfamilies, CXC, CC, XC, CX₃C, and CX, based on the arrangement of the two N-terminal cysteine residues (Table 4) (Nomiyama et al. 2010). A single chemokine can bind to several chemokine receptors, whereas a single chemokine receptor can have multiple chemokine ligands (Zlotnik and Yoshie 2012). The recognition of chemokine-encoded messages is mediated by specific cell surface G-protein-coupled receptors (GPCRs) with seven transmembrane domains (Murphy 2002).

Infectious microorganisms can directly stimulate chemokine production by tissue dendritic cells (DCs) and macrophages as well as by many parenchymal and stromal cells. Conserved microbial PAMPs induce chemokines through PRR, such as TLRs or NOD1 and NOD2 (Girardin et al. 2003; Janeway and Medzhitov 2002). Classically the major inflammatory and immunomodulatory cytokines such as IL-1, TNF- α , IFN γ , IL-4, IL-5, IL-6, IL-13, and IL-17, induced in injury or infection, stimulate through their respective receptors the production of many different chemokines (Luster 1998; Rollins 1997; Baggiolini et al. 1997).

2 The Human-Phage Story: More than We Thought

2.1 Phage-Mammalian Host Interactions

The human body is colonized by commensal microorganisms; most of these microorganisms reside at body surfaces that are in direct contact with the environment, including the intestine, skin, and respiratory tract. Research efforts focused primarily on the bacterial component of the human microbiota and its associated genes have yielded a wealth of insight about the composition of human-associated bacterial communities (Duerkop and Hooper 2013). It has clarified how these resident bacteria interact with the immune system and how bacteria-immune system interactions are altered in disease (Hooper et al. 2012; Lozupone et al. 2012). Recently, it has become apparent that the microbiota of healthy humans also include viruses (White et al. 2012). Metagenomic studies have revealed that the human microbiome includes many viral genes (the virome) (Minot et al. 2011; Reyes et al.

Table 3 Cytokine induction and activation of SOCS proteins. Adapted from Alexander (2002) and Alexander and Hilton (2004)

SOCS protein	Induced by	Inhibits	References
CIS	IL-2, IL-3, IL-6, IL-9, IL-10, GM-CSF, GH, PRL, TSLP, EGF, CNTF, leptin, EPO, TPO	IL-2, IL-3, GH, IGF1, leptin, EPO	Adams et al. (1998); Aman et al. (1999); Bjørnbæk et al. (1999); Emilsson et al. (1999); Isaksen et al. (1999); Lejeune et al. (2001); Okabe et al. (1999); Pezet et al. (1999); Ram and Waxman (1999); Sadowski et al. (2001); Shen et al. (2000); Starr et al. (1997); Yoshimura et al. (1995); Zong et al. (2000)
SOCS1	IL-2, IL-4, IL-6, IL-9, IL-10, G-CSF, GH, PRL, TSH, SCF, insulin, LIF, CT1, CNTF, EPO, IFN- α/β , IFN- γ , TNF	IL-2, IL-3, IL-4, IL-6, IL-7, M-CSF, GH, IGF1, PRL, TSLP, SCF, Flk ligand, insulin, LIF, OSM, CT1, EPO, TPO	Adams et al. (1998); Bjørnbæk et al. (1999); Bourette et al. (2001); De Sepulveda et al. (1999); Endo et al. (1997); Hamanaka et al. (2001); Isaksen et al. (1999); Kawazoe et al. (2001); Lejeune et al. (2001); Losman et al. (1999); Morita et al. (2000); Naka et al. (1997); Park et al. (2000); Pezet et al. (1999); Sadowski et al. (2001); Shen et al. (2000); Song and Shuai (1998); Sporri et al. (2001); Starr et al. (1997); Trop (2001); Wang et al. (2000a, b); Zong et al. (2000)
SOCS2	IL-2, IL-6, GH, PRL, insulin, CNTF	GH, IGF1, LIF	Zong et al. (2000); Ram and Waxman (1999); Minamoto et al. (1997); Starr et al. (1997); Adams et al. (1998); Pezet et al. (1999); Sadowski et al. (2001); Bjørnbæk et al. (1999)
SOCS3	IL-1, IL-2, IL-3, IL-6, IL-9, IL-10, IL-11, IL-22, GH, PRL, TSH, EGF, insulin, PDGF, BFGF, LIF, OSM, CT1, CNTF, leptin, EPO, TPO, TNF	IL-1, IL-2, IL-3, IL-4, IL-6, IL-9, IL-11, GH, IGF1, PRL, insulin, LIF, OSM, CT1, CNTF, leptin, EPO, INF- α/β , INF- γ	Adams et al. (1998); Auernhammer et al. (1998); Auernhammer and Melmed (1999); Bjørnbæk et al. (1998, 1999); Boisclair et al.

(continued)

Table 3 (continued)

SOCS protein	Induced by	Inhibits	References
			(2000); Cacalano et al. (2001); Cohnney et al. (1999); Emanuelli et al. (2000); Hamanaka et al. (2001); Hong et al. (2001); Karlsen et al. (2001); Kotenko et al. (2001); Lejeune et al. (2001); Losman et al. (1999); Magrangeas et al. (2001a, b); Minamoto et al. (1997); Nicholson et al. (1999); Park et al. (2000); Pezet et al. (1999); Sadowski et al. (2001); Sasaki et al. (2000); Shen et al. (2000); Song and Shuai (1998); Starr et al. (1997); Terstegent et al. (2000); Wang et al. (2000a, b); Zong et al. (2000)

BFGF basic fibroblast growth factor, *CIS* cytokine-induced SRC-homology-2 protein, *CNTF* ciliary neurotrophic factor, *CTI* cardiotrophin-1, *EGF* epidermal growth factor, *EPO* erythropoietin, *G-CSF* granulocyte colony-stimulating factor, *GH* growth hormone, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IFN* interferon, *IGF1* insulin-like growth factor-1, *IL* interleukin, *LIF* leukaemia inhibitory factor, *M-CSF* macrophage colony-stimulating factor, *OSM* oncostatin M, *PDGF* platelet-derived growth factor, *PRL* prolactin, *SCF* stem-cell factor, *SOCS* suppressor of cytokine signaling, *TNF* tumor necrosis factor, *TPO* thrombopoietin, *TSH* thyrotropin, *TSLP* thymic stromal lymphopoietin

2010; Handley et al. 2012). Additionally, there are viruses associated with the intestine and the skin that replicate either in eukaryotic cells or in bacteria (Zhang et al. 2006; Minot et al. 2011; Reyes et al. 2010).

Bacteria that inhabit the intestine and skin are generally regarded as stable residents that confer metabolic and/or immune benefits to their hosts (Turnbaugh et al. 2009). The question can be raised whether a stable association between human healthy tissues and viruses can exist. It is interesting to consider whether phage predation of intestinal bacteria could alter community composition in ways that impact function of the immune system and influence the spread of pathogenic viruses (Duerkop and Hooper 2013; Ivanov et al. 2008; Mazmanian et al. 2005). Limiting pathogen colonization through niche occupation and resource use is part of how the microbiota impact host immunity. These indirect protective effects could extend to the viral members of the microbiota, of which there are an estimated 10⁹ viruses per gram of feces. Some of these viruses target mammalian cells, but phages make up the majority of this viral community (Cadwell 2015).

Table 4 CC, CXC, CX₃C, and XC families of chemokines and chemokine receptors

Receptor	Chemokine ligands	Cell types	References
CCR1	CCL3, CCL5, CCL7, CCL14	T cells, monocytes, eosinophils, basophils	Mahad et al. (2004); Proudfoot et al. (2003); Trebst et al. (2001)
CCR2	CCL2, CCL8, CCL7, CCL13, CCL16	Monocytes, dendritic cells, (immature) memory T cells	Charo (2004); Charo and Peters (2003); Gu et al. (2000); Proudfoot et al. (2003)
CCR3	CCL11, CCL13, CCL7, CCL5, CCL8, CCL13	Eosinophils, basophils, mast cells, T _H 2 cells, platelets	Daly and Rollins (2003)
CCR4	CCL17, CCL22	T cells (T _H 2), dendritic cells (mature), basophils, macrophages, platelets	Calzascia et al. (2005); Flier et al. (2001)
CCR5	CCL3, CCL4, CCL5, CCL11, CCL14, CCL16	T cells, monocytes	Mahad et al. (2004); Proudfoot et al. (2003); Trebst et al. (2001)
CCR6	CCL20	T cells (T regulatory and memory), B cells, dendritic cells	Schutysen et al. (2003)
CCR7	CCL19, CCL21	T cells, dendritic cells (mature)	Cyster (2003, 1999); Mantovani (1999); Sozzani et al. (2000)
CCR8	CCL1	T cells (T _H 2), monocytes, dendritic cells	Qu et al. (2004)
CCR9	CCL25	T cells, IgA ⁺ plasma cells	Calzascia et al. (2005)
CCR10	CCL27, CCL28	T cells	Homey et al. (2002); Wang et al. (2000a, b)
CXCR1	CXCL8 (IL-8), CXCL6	Neutrophils, monocytes	Gerszten et al. (1999); Liehn et al. (2013); Tachibana et al. (1998)
CXCR2	CXCL8, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6	Neutrophils, monocytes, microvascular endothelial cells	Gerszten et al. (1999); Liehn et al. (2013); Tachibana et al. (1998)
CXCR3-A	CXCL9, CXCL10, CXCL11	Type 1 helper cells, mast cells, mesangial cells	Flier et al. (2001); Sørensen et al. (1999)
CXCR3-B	CXCL4, CXCL9, CXCL10, CXCL11	Microvascular endothelial cells, neoplastic cells	Flier et al. (2001); Sørensen et al. (1999)
CXCR4	CXCL12	Widely expressed	Ma et al. (1998); Zou et al. (1998)
CXCR5	CXCL13	B cells, follicular helper T cells	Cyster et al. (1999); Müller et al. (2003)
CXCR6	CXCL16	CD8 ⁺ T cells, natural killer cells, memory CD4 ⁺ T cells	Matloubian et al. (2000); Shimaoka et al. (2000)
CX ₃ CR1	CX3CL1	Macrophages, endothelial cells, smooth-muscle cells	Bazan et al. (1997); Pan et al. (1997)
XCR1	XCL1, XCL2	T cells, natural killer cells	Kelner et al. (1994)

Although humans are routinely exposed to phages on a daily basis, concerns persist over their immunogenicity and overall safety, presenting an additional stumbling block for the adoption of phage therapy (Cooper et al. 2016). It becomes clearer that phages can do more than exercise antibacterial properties, they can become a part of the mucus layers (Barr et al. 2013, 2015) and even migrate through cell layers (Nguyen et al. 2017) or form an additional virulence factor elevating the bacterial fitness (Penner et al. 2016; Secor et al. 2015). When these phages enter the blood, they can interact with immune cells and induce innate and adaptive immune responses (Van Belleghem et al. 2017; Majewska et al. 2015; Miernikiewicz et al. 2013; Hodyra-Stefaniak et al. 2015).

2.2 Phages in the Mucus: Non-host-Derived Immunity

A critical immunological barrier protecting all animals against invading bacterial pathogens but also supporting large communities of commensal microorganisms are the mucosal surfaces (e.g., human gut and respiratory tract) (Linden et al. 2008; Johansson et al. 2008). The mucus is predominantly composed of mucin glycoproteins that are secreted by the underlying epithelium. The amino acid backbone of these proteins incorporates tandem repeats of exposed hydrophobic regions alternating with blocks bearing extensive O-linked glycosylation (Cone 2009). By offering both structure and nutrients, mucus layers commonly support higher bacterial concentrations than the surrounding environments (Martens et al. 2008; Poulsen et al. 1994). When invaded by pathogens, the epithelium may respond by increasing the production of antimicrobial agents, hypersecretion of mucin, or alteration of mucin glycosylation patterns to subvert microbial attachment (Gill et al. 2013; Jentoft 1990; Schulz et al. 2007). Besides bacteria, phages are also present in these mucus layers. Moreover, phage concentrations in mucus are elevated relative to the surrounding environment (Barr et al. 2013).

Phages in the human gut encode a population of hypervariable proteins (Minot et al. 2012). Approximately half of these encoded proteins possessed the C-type lectin fold previously found in the major tropism-determinant protein at the tip of the *Bordetella* phage BPP-1 tail fibers; six others contained Ig-like domains (Medhekar and Miller 2007). These Ig-like proteins, similar to antibodies and T-cell receptors, can accommodate large sequence variation (Halaby and Mornon 1998). Ig-like domains also are displayed in the structural proteins of many phages (Fraser et al. 2006, 2007). That most of these displayed Ig-like domains are dispensable for phage growth in the laboratory led to the hypothesis that they aid adsorption to their bacterial prey under environmental conditions (McMahon et al. 2005; Fraser et al. 2007). The increased concentration of phage on mucosal surfaces is mediated by weak binding interactions between the variable Ig-like domains on the T4 phage capsid and mucin-displayed glycans (Fig. 1). These Ig-like domains are present in approximately one quarter of the sequenced genomes of tailed DNA phages, i.e., the *Caudovirales*, and are only found in the virion structural proteins and are typically displayed on the virion surface (Fraser et al. 2006). Most of these structurally displayed Ig-like domains are dispensable for phage growth in the laboratory,

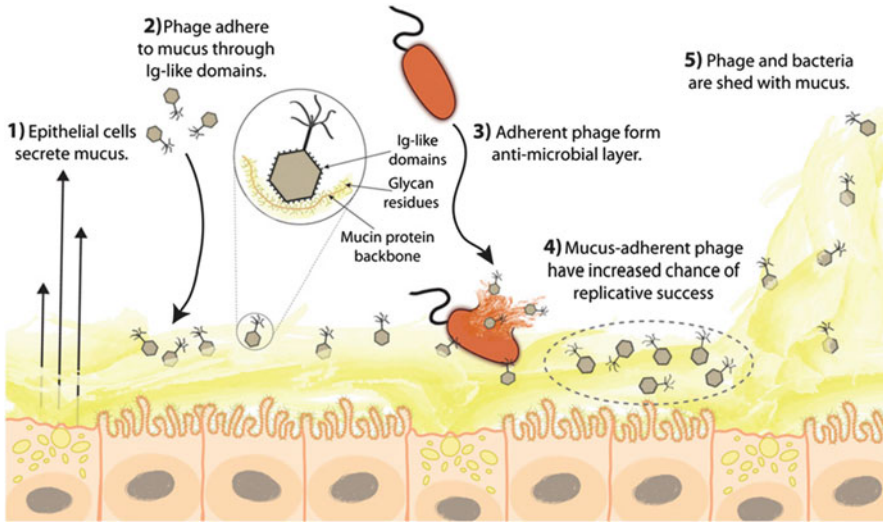


Fig. 1 The bacteriophage adherence to mucus (BAM model). (1) Mucus is produced and secreted by the underlying epithelium. (2) Phage bind variable glycan residues displayed on mucin glycoproteins via variable capsid proteins (e.g., Ig-like domains). (3) Phage adherence creates an antimicrobial layer that reduces bacterial attachment to and colonization of the mucus, which in turn lessens epithelial cell death. (4) Mucus-adherent phages are more likely to encounter bacterial hosts and thus are under positive selection for capsid proteins that enable them to remain in the mucus layer. (5) Continual sloughing of the outer mucus provides a dynamic mucosal environment. (Figure adapted from Barr et al. (2013))

which led to the hypothesis that they aid the phage in the adsorption to their bacterial host under environmental conditions (McMahon et al. 2005; Fraser et al. 2007). This concept was further extended showing that phages use the variable Ig-like protein to adhere to the ever-changing patterns of mucin glycosylation.

Furthermore, the presence of an Ig-like protein displayed on the capsid of T4 phage (highly antigenic outer capsid protein, Hoc) significantly slowed the diffusion of the phage on mucin solutions. Although phage particles, being inanimate and small, act as colloidal particles, they use subdiffusive motions instead of a Brownian motion. This was shown in experiments using phage T4, where the subdiffusive motions of phage T4 in mucus increase the frequency of host encounters. Thus, phage Ig-like domains that bind effectively to the mucus layer would be under a positive selection. These findings lead to the development of the bacteriophage adherence to mucus (BAM) model (Fig. 1), which provides a non-host-derived antibacterial defense (Barr et al. 2013, 2015).

2.3 Phage Transcytosis

The cellular epithelium forms another physical barrier, besides the mucosal surface, that separates the heavily colonized mucosa from the normally sterile regions of the

body. Due to their ubiquity within the epithelial mucus layer, phages are in constant and continual contact with the epithelial layers. The passage of commensal bacteria colonizing the intestine through the mucosa to local lymph nodes and internal organs is termed bacterial translocation and is a critical step in the pathology of various disorders (Guarner and Malagelada 2003; Wiest and Garcia-Tsao 2005). While bacterial translocation is a well-described phenomenon, little is known about the translocation of bacterial viruses.

Low internalization of bacteriophages by enterocytes and other endothelial cells was demonstrated for M13 phages (empty vectors used as a control in phage display) in vivo by Costantini et al. (2009) and in vitro by Ivanenkov et al. (1999). Clathrin-dependent endocytosis was proposed as the pathway, since chloroquine blocked then in vitro uptake (Ivanenkov et al. 1999). Since this type of endocytosis is strictly receptor-mediated, i.e., external objects must be bound to a membrane receptor to be dragged into pits, there is a reason to think that such phage uptake can be a consequence of specific phage-to-epithelium interactions.

Effective (Keller and Engley 1958; Wolochow et al. 1966; Reynaud et al. 1992; Jaiswal et al. 2014; Jun et al. 2014) or ineffective (Duerr et al. 2004; Bruttin and Brüßow 2005; Denou et al. 2009; Oliveira et al. 2009; Letarova et al. 2012; McCallin et al. 2013) systemic dissemination after oral administration has been demonstrated in vivo using nonengineered phages. This suggests that the translocation of natural phage from the gut to circulation is possible. It also shows it is dependent on specific conditions, probably comprising both physiological status of a host (Górski et al. 2006; Majewska et al. 2015) and the characteristics of the phage. Physical parameters of phage particles like their size and shape, can in some extent, influence the phage's ability to penetrate mammalian bodies. The most important factor seems to be the dose, which correlates strongly with the probability that an orally applied phage can be found in circulation or in tissues. This is in line with the fact that phages may differ in their ability to propagate on gut bacteria and this ability may further limit their systemic dissemination after application per os (Oliveira et al. 2009; Weiss et al. 2009).

It is important to consider whether phages can cross the mucosal barrier at sufficient numbers to bypass and interact with the cellular epithelium. It has been demonstrated, in vitro using cell lines, that phages can enter and cross epithelial cell layers by a nonspecific transcytosis mechanism, in an apical-to-basal direction (Nguyen et al. 2017). This transcytosis occurs across different types of epithelial cell layers (e.g., gut, lung, liver, kidney, and brain cells) and for diverse phage types and morphologies (e.g., *Myoviridae*, *Siphoviridae*, and *Podoviridae*). Roughly, 10% of epithelial cells endocytosed phage particles, which appeared to be localized within membrane-bound vesicles, as shown through microscopy analyses. The few cells that did endocytose phage particles appeared to contain large numbers of such vesicles. These endocytosed phage particles traffic via the Golgi apparatus before being functionally exocytosed at the basal cell layer. The transcytosis of phages across epithelial cell layers provides a mechanistic explanation for the systemic occurrence of phages within the human body in the absence of disease (Nguyen et al. 2017).

2.4 Intracellular Interaction of Phages with Mammalian Cells

The direct contact of phages with eukaryotic cells is accomplished through the penetration of phages in higher organisms. It is thus important to know whether these phages can interact or infect eukaryotic cells. Genuine infection seems unlikely, as elements of the phage tail structure only binds to specific receptors on the surfaces of their target bacteria. Intracellular replication is unlikely due to the major differences between eukaryotes and prokaryotes in regard to key intracellular machinery that are essential for translation and replication (Sharp 2001), nevertheless phage gene transcription and translation might be a possibility. Di Giovine et al. (2001) demonstrated that re-engineering of filamentous phage M13 enables it to infect mammalian cells. Although subsequent binding and internalization of the engineered phage was observed, no multiplication of the phage was detected (Di Giovine et al. 2001). Infection aside, as this is outside the scope of this chapter, it is feasible that phages can directly interact with the eukaryotic cell, either extra- or intracellularly. Phages have been attributed as being anti-tumorigenic. Genetic modification of phage M13 (designated WDC-2) led to the production of a tumorigenic phage that was able to bind 93% of the tested tumor cells (Eriksson et al. 2009). Moreover, the administration of the tumor-specific phage initiated the infiltration of neutrophilic granulocytes with subsequent regression of established B16 tumors in mice (Eriksson et al. 2007, 2009). The mechanisms of this phage-induced tumor regression are TLR dependent as no signs of tumor destruction or neutrophil infiltration were observed in tumors of MyD88^{-/-} mice, where TLR signaling was abolished.

Cellular fractionation of epithelial cells, incubated with phage, has been performed by Nguyen et al. (2017) and showed complete perfusion of the eukaryotic cell, with phage particles seen within all endomembrane compartments. Phage particles are likely degraded, shuffled, and transported throughout the cell, providing ample opportunities to interact with eukaryotic cellular components. The question rises whether these interactions occur with the whole phage particle or with specific components of the phage such as the genetic material of the phage (e.g., dsDNA or ssDNA). The specific mechanisms here remain largely uninvestigated but could conceivably include recognition or binding with phage structural proteins or recognition, binding, transcription, or translation of phage nucleic acids (Lengeling et al. 2013). *E. coli* phage PK1A2 can actively bind and penetrate eukaryotic neuroblastoma cells in vitro, through an interaction and binding of cell surface polysialic acid. This cell surface polysialic acid shares structural similarity with the bacterial phage receptor (Lehti et al. 2017). These phage particles were able to be present in these cells for up to 24 h without affecting cell viability. Uptake of these phage particles may also lead to the activation of intracellular immunity, potentially priming the eukaryotic cell into an antimicrobial state or enhancing barrier function (Tam and Jacques 2014). Further research is needed within this area to elucidate intracellular phage-eukaryote interactions.

2.5 Phage-Mammalian Immune Response

Phages clearly interact with nontarget tissues to some extent. For example, at least some phages are taken up from the gastrointestinal tract into the blood and there is reason to think that such uptake can be a consequence of specific phage-to-epithelium interactions, as also appears to be the case given phage interaction with the reticuloendothelial system (Merril 2008; Górski et al. 2006; Duerr et al. 2004). Surprisingly, the early phage workers did not seem to be concerned about the immunological responses to phage therapy (Summers 2001).

2.5.1 Direct Phage-Mammalian Interaction

The fact that phages can directly interact with mammalian cells was first shown in 1940 by Bloch. He observed an accumulation of phages in cancer tissue and inhibition of tumor growth (Bloch 1940). Later on, it was demonstrated that phages can bind cancer cells *in vitro* and *in vivo* and attach to the plasma membrane of lymphocytes (Northrop 1958; Kantoch and Mordarski 1958; Wenger et al. 1978; Dąbrowska et al. 2004).

A hypothesis concerning the molecular basis of such interaction was coined by Gorski et al. (2003). He suggested that this interaction occurs through the presence of a Lys-Gly-Asp (KGD) tripeptide motif present in the phage T4 capsid protein gp24. This peptide motif acts as a ligand for the $\beta 3$ integrins on cells. A genetic modification of phage M13 (designated WDC-2, containing a TRTKLPRLHLQS peptide motif) was reported to lead to the production of a tumor-specific phage that was able to bind 93% of tested tumor cells (Eriksson et al. 2009). Moreover, administration of this tumor-specific phage initiated the infiltration of neutrophilic granulocytes with subsequent regression of established B16 tumors in mice (Eriksson et al. 2007, 2009). The authors observed that the mechanisms of this phage-induced tumor regression were TLR-dependent as no signs of tumor destruction or neutrophil infiltration were observed in tumors of MyD88^{-/-} mice, that lack TLR signaling.

2.5.2 The Cellular Immune Response Against Phages

Phage Can Induce Phagocytosis of Bacteria

It has been postulated that purified phages have anti-inflammatory effects via the suppression of reactive oxygen species (ROS) production and inhibition of NF- κ B activity (Górski et al. 2012) and even affecting the cytokine production (Van Belleghem et al. 2017). Neutrophils and monocytes play an important role in host defenses against microbial pathogens, and ROS constitutes to their antimicrobial arsenal. Phagocyte-derived ROS may overwhelm the body's endogenous antioxidant defense mechanism when produced in excess, leading to oxidative stress and causes tissue damage. This forms a major contributing factor to the high mortality rates associated with sepsis and endotoxic shock (El-Benna et al. 2005; Riedemann et al. 2003; Pawlak et al. 1998; Sikora 2002). Hyperresponsiveness and immune cell apoptosis can be induced by ROS, while antioxidants can alleviate this effect (Betten et al. 2004; Malmberg 2004). Not much is not known about the effects

of bacteriophages on the ROS production, whereas the effect of bacteria and eukaryotic viruses on ROS activity have been described. A preliminary study performed suggested that phage T4 influences the phagocyte system (Przerwa et al. 2006), showing that phages could inhibit a ROS production in response to pathogenic bacteria (i.e., *E. coli*). This phenomenon appears to depend on specific phage-bacteria interactions, as phage F-8 (infecting *P. aeruginosa*) did not affect the ROS production induced by *E. coli* on the phagocytic cells. It could be argued that this reduction might be caused by a reduction of bacteria infected and lysed by the phage. This might also explain why phage T4 had an effect and not phage F-8 on the reduction of ROS induced by *E. coli*.

A more comprehensive follow-up study was conducted by stimulating polymorphonuclear leukocytes (PMN) with one of three different R-type *E. coli* strains (i.e., *E. coli* B and *E. coli* J5, both susceptible for T4, and *E. coli* R4, resistant to T4) and LPS derived from these three strains (Miedzybrodzki et al. 2008). The R-type strains were used as their LPS was able to activate the peripheral blood PMN ROS production (Kapp et al. 1987). Through this setup, a reduction in ROS production could be observed in the presence of phage T4 when PMNs were stimulated with either the live bacteria or their LPS. Moreover, this reduction was seen not only when T4 was able to infect the *E. coli* strains but also for the T4 resistant *E. coli* strain. Although the T4 resistant *E. coli* strain induced a less strong ROS production compared to the T4 susceptible strains. These results indicate that phage can directly interact with mammalian cells and could even have anti-inflammatory properties (Miedzybrodzki et al. 2008). Furthermore, the reduction of ROS by phage could be due to the T4 phage tail adhesion gp12, which specifically binds bacterial LPS. This could subsequently lead to a decrease in the availability of LPS and reduce its potential to induce an inflammatory response (Miernikiewicz et al. 2016).

When phages were administered together with the host bacteria, some studies showed that phages were able to stimulate bacterial phagocytosis, and this is attributed to certain opsonization of bacterial cells by phages. In addition, phages can remain active and infective when adsorbed onto the bacteria on intake by granulocytes (Kaur et al. 2014). Therefore, some authors have suggested that during phagocytosis, phages continue lysing the phagocytosed bacteria, helping the activity of phagocytic cells (Górski et al. 2012; Jończyk-Matysiak et al. 2015). Phages might also inhibit the adhesion of platelets and, to some extent, T cells to fibrinogen, a protein which plays an important role in transplant rejection, angiogenesis, and metastasis (Kurzepa et al. 2009).

Phage Innate Immune Response

The innate immune system, particularly the components of the mononuclear phagocyte system (MPS), could form a mechanism for removing phages that are circulating in the human body (Navarro and Muniesa 2017; Górski et al. 2012). Among the mechanisms responsible for the recognition of microbial and viral structures are the TLR (Kawai and Akira 2011). Viral nucleic acids act as PAMPs and are recognized by multiple TLRs. It could thus be postulated that phage DNA might be recognized by TLR9, which is responsible for the recognition of DNA

(Janeway and Medzhitov 2002), after phagocytosis of the phage. The intracellular phages are subsequently uncoated in the cytoplasm and the nucleic acid released. The MPS was credited for the rapid removal of administered wild-type phage λ from the circulatory system in humans (Merril et al. 1973). Moreover Merrill et al. (1996) were able to identify certain phage λ mutants that was capable of circumventing the MPS immune response, these mutants prevailed for longer periods in the blood stream than the wild-type phage (Merril et al. 1996).

Immunological studies on the cellular immune response induced against phages have been conducted in recent year, in vitro as well as in vivo. However, it should be noted that many experiments concerning immune responses induced by phages have been carried out using phage lysates. This means that bacterial fragments, proteins, or LPS could still be present in these preparations, making it often difficult to determine whether the observed response can be attributed to the phage.

Mice treated intraperitoneally for 5.5 h with four T4 capsid proteins (i.e., gp23*, gp24*, Hoc, and Soc) showed that no cytokines were induced (Miernikiewicz et al. 2013). This lack of cytokine production might be explained by the early time point by which the mice were tested for the presence of cytokines or through the rapid removal of the phages from circulation. Another immunological study evaluated the cytokine production in mice induced by phage T7, after the mice were fed for 10 days with phage T7. A single dose was fed every 24 h, although an exact concentration was not provided by the authors (Park et al. 2014). Although this study had its limitations, the authors were able to demonstrate that phage T7 induced a very minor increase of inflammatory cytokine production in mice, but no histological changes were observed in the tissues of the gastrointestinal organs. As no caution was taken to the presence of endotoxins, the immune responses that were observed could be, partially, due to endotoxin contamination of the used phage stock.

2.5.3 Cytokine Response Against the Phage

Phages have the potential to induce cytokine responses, as indicated by several studies, often these studies make use of phage preparations that where not fully purified from bacterial endotoxins or proteins. The effect of phages on the production of tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) in human serum has also been studied, as well as the ability of blood cells to produce these cytokines in culture. The authors of this study used blood derived from 51 patients with long-term suppurative infections of various tissues and organs caused by drug-resistant strains of bacteria (Weber-Dąbrowska et al. 2000). These patients were treated with phages and blood samples were collected and tested for the presence of TNF- α and IL-6. The authors were able to observe a reduction in the production of these cytokines after long-term treatment (i.e., 21 days). Unfortunately, the authors were not able to show whether the observed immune response was due to the presence of the phage or due to the reduction of the bacterial count through their lysis by the phage. On the other hand, analysis of the cytokine production of mice treated intraperitoneally for 5.5 h with either a highly purified phage T4 or four phage T4 capsid proteins (i.e., gp23*, gp24*, Hoc, and Soc) showed that no inflammatory mediating cytokines were detected (Miernikiewicz et al. 2013).

The maturation of dendritic cells can be induced by *Cronobacter sakazakii* phage ES2 through the induction of the expression of IL12p40 via NF- κ B signaling (An et al. 2014). This maturation presumably happens after the phagocytosis of the phage by the dendritic cells. The maturation of these dendritic cells play an important role in generating a cell-mediated immune response and subsequently in the production of phage specific antibodies. It has even been shown that phages have conserved anti-inflammatory properties. Using highly purified phages targeting two different pathogens, *P. aeruginosa* and *S. aureus*, it was shown that these phages induced very comparable immune responses (Van Belleghem et al. 2017). Especially the upregulation of the anti-inflammatory markers suppressor of cytokine signaling 3 (SOSC3), IL-1 receptor antagonist (IL1RN), and IL-6 was very similar between the different phages. These anti-inflammatory phage properties are also in line with some previous observations suggesting an immunosuppressive effect of phages in murine in vivo models of xenografts (Gorski et al. 2016). It should however be emphasized, that potential anti-inflammatory or immunosuppressive action of bacteriophages cannot be considered as comparable to physiological effects exerted by well-known anti-inflammatory or immunosuppressive drugs. Phage activity seems to be much weaker and it rather impacts the ecological balance in microbiota inside bodies than the physiological status of the organisms.

2.5.4 Anti-phage Antibody Production

It is very easy to generate phage antisera by immunization of humans or animals with phage lysates (Górski et al. 2012; Bacon et al. 1986; Puig et al. 2001) and forms one of the major consequences of using phage as therapeutics (Kamme 1973; Smith et al. 1987; Górski et al. 2012). Soon after the discovery of phages, antibodies against the phage could be observed in humans and animals (Jerne 1952, 1956). Natural occurring bacteriophages are able to induce a humoral immunity, i.e., phage-neutralizing antibodies that were not stimulated by phage treatment were detected in the sera of different species (e.g., human) (Dabrowska et al. 2005). In fact, immunization with bacteriophage ϕ X174 has been used extensively to diagnose and monitor primary and secondary immunodeficiencies since the 1970s without reported adverse events, even in patients in whom prolonged circulation of the phage in the bloodstream was observed (Ochs et al. 1971; Rubinstein et al. 2000; Shearer et al. 2001; Fogelman et al. 2000). Besides, the humoral response does not follow a simple schema of induction. It depends on the route of administration and on individual features of the phage. Moreover, it depends on the dose and application schedule and possibly on other, not yet specified, features (Górski et al. 2006, 2012; Dąbrowska et al. 2014; Łusiak-Szelachowska et al. 2014). The humoral response induced against phages can be devastating (Huff et al. 2010), but it has also been reported that the anti-phage activity of serum does not exclude a favorable result of phage therapy in humans (Łusiak-Szelachowska et al. 2014).

Initial safety studies of phage T4 performed on humans revealed no antibody induction in phage-related volunteers at all (Bruttin and Brüssow 2005). Evaluation of serum derived from 50 healthy volunteers who had never been subjected to phage therapy or were involved in phage work showed that 82% significantly decreased

phage T4 activity (Dąbrowska et al. 2014). In these positive sera, natural IgG antibodies specific to the phage proteins gp23*, gp24*, Hoc, and Soc were identified. It is not the highly antigenic outer capsid (Hoc) protein that induced the most of the humoral responses, but the antibodies specific to the major capsid protein gp23* (Dąbrowska et al. 2014).

The production of IgG, IgA, and IgM in human patients undergoing phage therapy was conducted where 20 patients were treated with the MS-1 phage cocktail (containing three lytic *S. aureus* phages, 676/Z, A5/80, and P4/6409) either orally or locally (Żaczek et al. 2016). Few patients produced elevated levels of IgG or IgM. Nevertheless, the presence of anti-phage antibodies did not translate into an unsatisfactory clinical result of the phage therapy. The small time-scale by which the patients were treated could explain the low antibody production against the phage cocktail. On the other hand, the elevated antibody production in a few patients could be due to a previous encounter of one of the phages used in the cocktail and the presence of an immunological memory.

Studies concerning the anti-phage antibody production in humans are rare, nevertheless an extensive study on the antibody production in mice are more common. The antibody production against a single phage (i.e., *E. coli* phage T4) in mice has been studied over a time period of 240 days (Majewska et al. 2015). The long-term oral treatment of mice with phage T4 demonstrated a humoral response, in contrast to previous human trials where no such responses were detected (Bruttin and Brüssow 2005). This response emerged by the secretion of IgA in the gut lumen but also as an IgG production in the blood (Majewska et al. 2015). The intensity of this response and the time necessary for its induction depend on the exposure to phage antigens, which is related to the phage dose. The specific IgA production seemed to be the limiting factor of phage activity in the. This was shown by the presence of phages in the feces when the secretory levels of IgA were low. When the IgA level, around day 80, increased, there were no active phages present in the feces.

The induction of serum IgG in these mice suggested that phages can translocate from the gut lumen to the circulation. This is further strengthened by the ability of phage to transcytose epithelial layers (Nguyen et al. 2017). It was even possible to isolate phages from murine blood after application of high phage doses (4×10^9 pfu/ml of drinking water).

Additionally, it is interesting to evaluate the immune responses induced to individual phage proteins, besides the whole phage particle. Majewska et al. demonstrated that phage T4 Hoc protein and gp12 strongly stimulated the IgG and IgA antibody production in the blood and gut, respectively, while gp23*, gp24*, and Soc induced low responses.

2.6 Relevance of Phage Host Immune Responses

It is becoming more evident that phages can interact with a mammalian or human host in diverse ways. The adherence of phages to mucosal surfaces provides an additional antimicrobial defense (Barr et al. 2013, 2015). The inclusion of symbiotic

phages within the mucosal surface provides the eukaryotic host with additional potential benefits, whereby the phages offer a selective antimicrobial defense. This operates at a much finer spectrum than some other broad-spectrum host secretions, such as the antibacterial lectin RegIII-Y (Vaishnava et al. 2011). The phages obtain a higher probability to contact epithelial cells and transcytose through them when they bind to the mucosal layers. The potential of phages to be internalized by eukaryotic cells raises the question whether they can induce intracellular immune responses. Might it additionally be possible that after internalization these phages can interact or even infect mitochondria? Although the presence of phages in mammalian cells has been observed (Nguyen et al. 2017; Di Giovine et al. 2001), replication of these viruses in these cell types has not yet been observed.

Important implications for the use of phage in therapeutic settings are the observations that they can induce certain (anti-inflammatory) cytokines (Van Belleghem et al. 2017). The effect of phage anti-inflammatory properties on the outcome of a bacterial infection has been highlighted in *in silico* models. These phage immune responses could have a much broader effect; they could not only lead to a rapid clearing of a bacterial infection but could also lead to a higher persistence of the bacterial infection. Additionally, phages could even be used as nanocarriers for targeted drug delivery or display selected antigens (Majewska et al. 2015; Eriksson et al. 2007, 2009).

Phages can have a direct impact on sepsis, where the lytic activity of the phage can reduce the bacterial burden. The immunomodulating properties of the phage could lead to a, partial, dampening of the inflammatory response induced by the bacteria. The immune response could be further altered by using phage or phage-derived proteins that interact with bacterial components, e.g., endotoxins (Miernikiewicz et al. 2016). These phage anti-inflammatory properties could be exploited in the future to develop phage protein-based anti-inflammatory agents, leading to a possible new type of anti-inflammatory drugs with a new mode of action. These phage or phage-derived proteins could potentially possess less side effects compared to the classic nonsteroid anti-inflammatory drugs (NSAIDs).

3 Conclusion

It is becoming evident that phages can directly interact with mammalian cells and interact with the immune system. Current data indicates that high phage concentrations induce immune responses whereas low phage concentrations have less to none observable immune responses. If the anti-inflammatory property of phages is widespread between the different types of phages, this can have a profound effect on understanding different bacterial pathologies (e.g., *P. aeruginosa* infection of cystic fibrosis patients) but also further add to our current understanding of phage therapy. The study of phage-mammalian cell interaction may alter our view of the function of phages in the microbiota, showing the potential of phage anti-inflammatory properties to more rapidly remove a bacterial infection or lead to a

more severe infection. It is thus becoming clear that the study of phage-mammalian interactions leads to many new, exciting study opportunities.

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