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REVIEW

The type I interferon response during viral infections: a "SWOT" analysis

Giel R. Gaajetaan, Cathrien A. Bruggeman and Frank R. Stassen*
Department of Medical Microbiology, Maastricht University Medical Center, The Netherlands

SUMMARY

The type I interferon (IFN) response is a strong and crucial moderator for the control of viral infections. The **strength** of this system is illustrated by the fact that, despite some temporary discomfort like a common cold or diarrhea, most viral infections will not cause major harm to the healthy immunocompetent host. To achieve this, the immune system is equipped with a wide array of pattern recognition receptors and the subsequent coordinated type I IFN response orchestrated by plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs). The production of type I IFN subtypes by dendritic cells (DCs), but also other cells is crucial for the execution of many antiviral processes. Despite this coordinated response, morbidity and mortality are still common in viral disease due to the ability of viruses to exploit the **weaknesses** of the immune system. Viruses successfully evade immunity and infection can result in aberrant immune responses. However, these weaknesses also open **opportunities** for improvement via clinical interventions as can be seen in current vaccination and antiviral treatment programs. The application of IFNs, Toll-like receptor ligands, DCs, and antiviral proteins is now being investigated to further limit viral infections. Unfortunately, a common **threat** during stimulation of immunity is the possible initiation or aggravation of autoimmunity. Also the translation from animal models to the human situation remains difficult. With a **Strengths–Weaknesses–Opportunities–Threats** ("**SWOT**") analysis, we discuss the interaction between host and virus as well as (future) therapeutic options, related to the type I IFN system. Copyright © 2011 John Wiley & Sons, Ltd.

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INTRODUCTION

For centuries, infectious diseases have been the most common cause of morbidity and mortality worldwide. Due to achievements like vaccination and antimicrobial drugs, many infectious diseases can now be prevented or controlled. Most striking in this respect is the development of a vaccine against smallpox, a lethal virus that globally claimed millions of lives. Although the vaccination procedure was already developed in the 18th century, it lasted until the end of the 20th century before the world was declared smallpox-free. Based on this success, there was great confidence that viral infections could be conquered definitely, either by vaccination or by antiviral drug treatment. Inspired by these successes, the US Surgeon General William Stewart stated in 1967 that "The time has come to close the book on infectious diseases". Unfortunately, the future has shown otherwise.

In 1983, the HIV was discovered as the AIDS causing agent. Despite massive efforts, HIV is still a major problem worldwide [1,2]. In addition, the rise of new (variants of) viruses like influenza A strains [3,4] and severe acute respiratory syndrome corona virus (SARS CoV) and their potential pandemic threat is a general and realistic concern [5]. Furthermore,

*Correspondence author: Dr. F. R. Stassen, Department of Medical Microbiology, Maastricht University Medical Center, P.Debyelaan 25, 6202 AZ, Maastricht, The Netherlands. E-mail: f.stassen@maastrichtuniversity.nl

Abbreviations

APOBEC3G, apolipoprotein B-mRNA-editing enzyme-catalytic polypeptide-like 3G; BST-2, bone marrow stromal antigen-2; cDC, conventional dendritic cell; CLR, C-type lectin receptor; COPD, chronic obstructive pulmonary disease; CpG ODN, CpG oligodeoxynucleotides; DAI, DNA-dependent activator of IFN-regulatory factors; DC, dendritic cell; dsRNA, double stranded RNA; HPV, human papilloma virus; IFNAR, interferon α/β receptor; IRF, interferon response factor; ISG, interferon-stimulated genes; ISG15, IFN-stimulated protein of 15 kDa; LPS, lipopolysaccharide; MDA5, melanoma differentiation-associated gene 5; miRNA, microRNA; Mx1, myxovirus resistance 1; NF-κB, nuclear factor κB; NK, natural killer; NS1, non-structural 1; OAS1, oligoadenylate synthetase 1; PAMP, pathogen-associated molecular patterns; pDC, plasmacytoid dendritic cell; PKR, protein kinase R; Poly(I:C), polyinosinic:polycytidylic acid; PRR, pattern recognition receptor; RIG-I, retinoic acid inducible gene I; RNAseL, ribonuclease L; SARS CoV, severe acute respiratory syndrome corona virus; SLE, systemic lupus erythematosus; ssRNA, single stranded RNA; SWOT, strengths-Weaknesses-Opportunities-Threats; TLR, Toll-like receptor; TRIM5α, tripartite motif 5α.

seasonal respiratory viral infections and various other viruses can cause major inconvenience in healthy people and can be life-threatening in the immunocompromised [5,6]. Thus, despite vaccines and antiviral drugs, viral disease is still common and requires development of additional therapeutics.

In this review, we apply a Strengths–Weaknesses–Opportunities–Threats (SWOT) analysis to discuss virus-immune interactions and speculate on (im) possibilities how to use these interactions in view of new treatment options.

STRENGTHS

Once the virus has been able to cross first barriers like the skin or mucosa, the **strength** of the host's natural defense system will determine the outcome of the infection. In the succeeding text, we will briefly discuss some of the initial key steps involved in the antiviral response (see also Figure 1).

Recognition: pattern recognition receptors

Before an appropriate immune response can be generated, the virus needs to be recognized. For this, immune cells are equipped with different groups of receptors, which are able to sense microbial intruders including viruses. These pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs), which are fundamentally different from host structures. One of the first discovered and best characterized PRRs are the Toll-like receptors (TLRs) [7–10], which are mostly present on antigen-presenting cells like macrophages and dendritic cells (DCs) [8,9], but also on non-immune cells like fibroblasts and epithelial cells [10]. These transmembrane receptors are located on the cell surface or at the endosome [7,9–11]. The cell surface-located TLRs recognize mainly lipids and proteins from bacteria and yeasts [10]. Viruses, on the other hand, are intracellular parasites, which

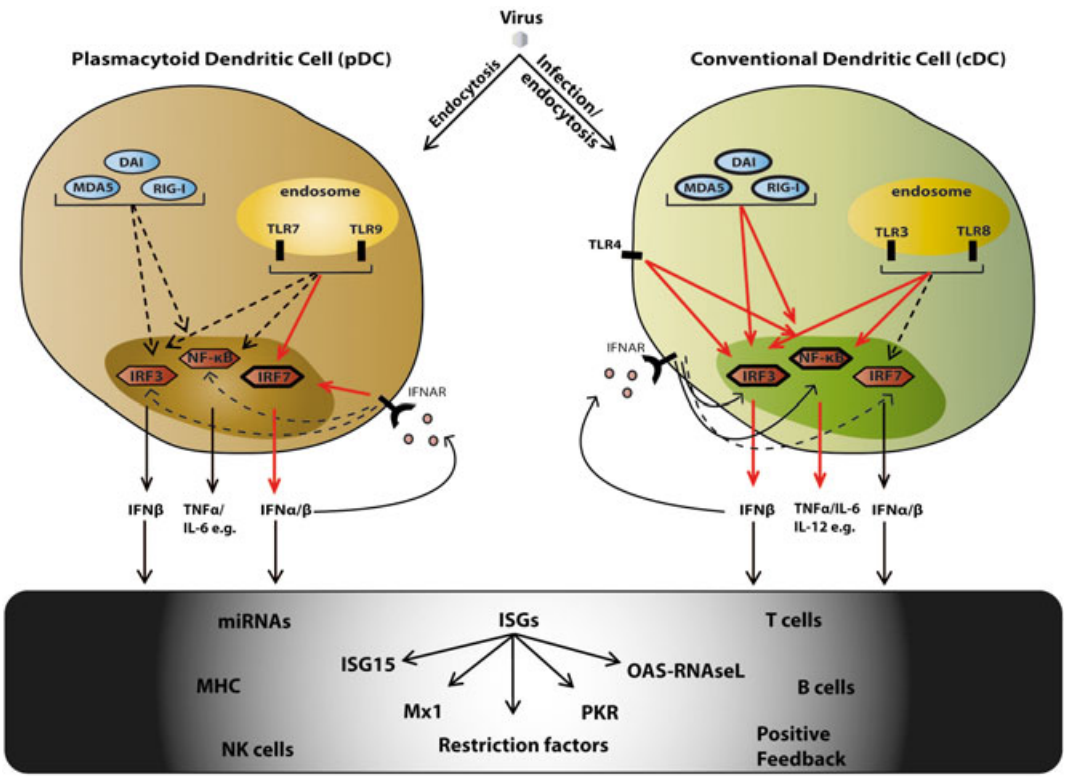

Figure 1. Schematic overview of different signal transduction pathways that are activated in plasmacytoid dendritic cells (pDCs) and conventional dendritic cell (cDCs) following viral encounters. In general, pDCs endocytose the virus and subsequently Toll-like receptor 7 (TLR7) and/or TLR9 is stimulated. Interferon response factor 7 (IRF7) is activated and induces transcription of IFNα/β. Besides execution of many antiviral functions, autocrine signaling via the interferon α/β receptor (IFNAR) also induces more type I IFN production. In contrast, infection of or endocytosis by cDCs results in activation of the cytoplasmic pattern recognition receptors, TLR3, and TLR8. Accordingly, IRF3 and nuclear factor-κB (NF-κB) facilitate transcription of IFNβ and proinflammatory cytokines. Via IFNAR, IRF7 is activated and induces production of type IFNα/β. Red indicates major routes, dotted arrows indicate minor routes

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may explain the endosomal localization of the viral nucleic acid-recognizing TLR3, TLR7, TLR8, and TLR9 (Figure 1) [11–19]. Also, this endosomal location of the TLRs probably serves to ensure tolerance for "self" molecules and to promote ligand accessibility [10,14]. Interestingly, in addition to the well-known lipopolysaccharides from Gram-negative bacteria, cell surface TLRs have also been associated with viral recognition. TLR4 has been shown to recognize the fusion protein of RSV [10,20]. Likewise, next to the recognition of Gram-positive bacteria, TLR2 is involved in detection of various DNA viruses like HSV1 and 2, measles virus, vaccinia virus, and CMV [21–23]. Interstingly, this TLR2-dependent detection seems to be regulated especially by monocytes [21,22,24].

In addition to the well-described TLRs, other PRRs also play an important role in viral recognition. The cytoplasmic PRRs, such as retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and DNA-dependent activator of IFN-regulatory factors (DAI), recognize viral nucleic acids [25,26] and are, in contrast to TLRs, expressed in all cells. RNA viruses are differentially recognized by RIG-I and MDA5, but activate similar pathways (Figure 1) [26–28]. Although RIG-I can respond to both positive and negative strand RNA viruses, MDA5 senses mainly picornaviruses like rhinovirus and poliovirus [29,30]. Earlier data suggested that MDA5 preferentially binds long dsRNA (picornaviruses), whereas shorter fragments of dsRNA and other specific nucleotide sequences are sensed by RIG-I [30,31]. However, some viruses can be detected by both receptors [29]. Also, the recently discovered receptor DAI is important for intracellular detection of viral DNA [32,33].

C-type lectin receptors (CLRs) and NOD-like receptors (NLRs) also belong to the large family of PRRs. CLRs are present on DCs and recognize carbohydrate structures present on pathogens [34,35] and are especially important for induction of antigen presentation to T cells, but also in modulating TLR responses [36]. NLRs, a group of cytoplasmic proteins formerly thought to detect only bacterial PAMPs, also sense RNA [37–39] and DNA viruses [33,40,41]. This induces the production of the proinflammatory cytokines IL-1β and IL-18 via the inflammasome, a complex composed of NLRs, and leads to the recruitment of immune cells to the site of infection [42,43].

Taken together, the innate immune system is equipped with a large variety of PRRs and this extended array is essential to sense the various microbial components and to prevent or limit viral spread as much as possible [7,44,45].

Implementation of antiviral immunity: conventional and plasmacytoid dendritic cells

After recognition of a virus, a cell-dependent signaling cascade will be initiated. Infection of non-immune cells usually results in detection of viral DNA/RNA or their intermediates by the cytoplasmic PRRs and the production of IFNβ, which is required to limit the infection. This antiviral cytokine also primes cells to produce other type I IFNs, which comprise all IFNα subtypes, IFNβ, and various other IFN types, essential to initiate production of antiviral proteins [46].

Dendritic cells are better equipped than non-immune cells for the initiation of an antiviral response. Conventional dendritic cells (cDCs) recognize viral invaders with both extracellular (TLR 4 and CLRs) and intracellular PRR (TLR3, 8, RIG-I, MDA5), which are highly expressed on cDCs (Figure 1) [11,12,47,48]. As in infection of non-immune cells, viral nucleic acids need to be detected before IFNβ and other type I IFNs can be produced.

For the successful eradication or control of the virus, the intervention of plasmacytoid dendritic cells (pDCs) is indispensable. The pDC is one of the few cells that express both TLR7 and TLR9 (Figure 1), allowing detection of an extended repertoire of viruses. To initiate the antiviral response, viruses or virus-infected cells are first internalized by endocytosis or phagocytosis, respectively, and subsequently recruited to the endolysosomes of the pDC [49]. The acidic environment disassembles the virus, and viral nucleic acids are subsequently recognized by TLR7 or TLR9 [50,51]. Ultimately, massive amounts of type I IFN are produced. In contrast to cDCs and non-immune cells, in pDCs the TLRs contribute significantly more to viral recognition than the cytoplasmic PRRs RIG-I and MDA5 [26,52,53]. Consequently, pDCs are less dependent on steps in the viral life cycle for recognition, which significantly accelerates the response to an infection in these DCs.

The difference in response time between pDC and cDC is also because of marked differences in

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intracellular signaling cascades that are activated following PRR stimulation. In cDCs, viral components stimulate the TLRs (apart from the cytoplasmic PRRs) resulting in phosphorylation of interferon-regulatory factor 3 (IRF3). IRF3 is essential for the production of proinflammatory cytokines and IFNβ (the first wave IFN) and is constitutively expressed, not only in cDCs but in most cell types [10,54]. Next, because of autocrine or paracrine signaling through the interferon-α/β receptor (IFNAR), IRF7 is activated, leading to the production of all type I IFNs including the various IFNα subtypes (the second wave IFN) [55,56]. Alternatively, in pDCs IRF7 is constitutively expressed and activated immediately after stimulation of TLR7 or TLR9, and thus no prior phosphorylation of IRF3 or autocrine/paracrine signaling is required (Figure 1) [48,52,57–60]. Accordingly, a robust antiviral response is initiated that, in contrast to the response seen in cDCs, is rapid and characterized by the production of high amounts of type I IFNs [61,62].

Consequently, the pDC is clearly the major antiviral cell type due to its rapid and abundant IFNα production. Yet, the cDC is indispensable for clearance of a viral infection. This can be illustrated by the function of TLR8 expressed by cDCs. This receptor is similar to TLR7 in pDCs and also recognizes viral ssRNA. Interestingly, stimulation of TLR8 on cDCs and TLR7 on pDCs results in entirely different responses [63]. Although the pDC produces mainly IFNα, the cDC induces a pro-inflammatory profile in which nuclear factor-κB (NF-κB) is activated for the production of TNF-α and IL-6 [64]. More importantly, IL-12 is produced (Figure 1). This cytokine augments the cytolytic activity of natural killer (NK) cells and also induces the production of the immunoregulatory cytokine IFNγ by T and NK cells [65]. Thus, although both DC subsets use different antiviral pathways, they are certainly not mutually exclusive in their response to viral infection. Because of their different cytokine patterns, pDCs and cDCs respond collaboratively to viral infection and connect innate and adaptive immunity [66]. Communication and cooperation between these two DC subsets are vital to induce appropriate immune responses towards invading pathogens.

Effector: Type I interferon

The type I IFNs are key effector molecules of the innate immune system and are essential for the antiviral response towards a plethora of viruses. In humans, the type I IFN family comprises 13 IFNα subtypes, IFNβ, IFNκ, IFNε, IFNo, IFNτ, and IFNδ, and all these molecules engage the ubiquitously expressed IFNAR. Binding to IFNAR then stimulates more than 300 interferon-stimulated genes (ISGs) [67,68], which subsequently induce an antiviral state. The antiviral state is a collective term for limitation of viral replication, viral resistance of neighboring cells, and apoptosis of virally infected cells.

Although IFNAR signaling induces the transcription of more than 300 ISGs, surprisingly, few of these genes encode proteins with direct antiviral effects [69]. Those proteins target viruses in many different ways (Figure 1). For example, the protein ISG15 (IFN-stimulated protein of Mr 15 000) has been reported to prevent virus-mediated degradation of IRF3 [70], to enhance NF-κB signaling [71], and to modulate the immune response [72]. Myxovirus resistance 1 (Mx1) proteins target viral nucleocapsid-like structures [73] and mediate vesicle trafficking in the ER to effectively trap essential viral components and subsequently degrade them [74,75]. The enzyme 2′,5′-oligoadenylate synthetase 1 (OAS1) accumulates after signaling through the IFNAR by type I IFN. When exposed to dsRNA, this enzyme gains activity that eventually leads to the activation of ribonuclease L (RNAseL), concomitantly enabling cleavage of cellular and viral RNAs [69,76]. Protein kinase R (PKR) is also initially inactive. Type I IFN induces accumulation of PKR and dsRNA activates PKR to inhibit translation [77]. For a more detailed overview of the ISG function, we would like to refer to the excellent review recently published by Sadler *et al*. [69].

Interferons also induce antiviral proteins termed restriction factors. A good example is the bone marrow stromal antigen-2 (BST-2) protein, which restricts the release of fully formed progeny virions from infected cells. This tetherin protein showed activity against various viruses, including HIV [78–80]. Another restriction factor is apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G), which leads to degradation of HIV DNA [81,82]. The restriction factor tripartite motif (TRIM) 5α seems to counteract capsid formation by HIV (reviewed by Sastri *et al*.) [83].

In addition, many proteins stimulated by type I IFN are involved in IFN signaling (IRF7, RIG-I, MDA5, TLRs), thereby amplifying the IFN response (positive feedback). IFNs also induce or modulate

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adaptive immune responses by upregulating MHC class I and II, to facilitate T and B cell stimulation [84,85]. Finally, IFNs promote leukocyte accumulation at sites of infection by promoting vascular adhesion molecule expression and induction of chemokines, which are essential in leukocyte recruitment [86].

Recently, a new type I IFN-dependent antiviral pathway has been suggested. Pedersen *et al.* demonstrated that IFNβ rapidly induced the expression of several microRNAs (miRNAs) both in a hepatocarcinoma cell line (Huh cells) and primary hepatocytes [87]. These small non-coding RNA molecules are post-transcriptional regulators that inhibit gene expression by translational repression, mRNA cleavage and deadenylation [87,88]. Intriguingly, eight of these IFNβ-induced miRNAs showed sequence-predicted targets within the HCV genomic RNA. Moreover, application of synthetic miRNA-mimics resulted in antiviral effects similar to those induced by IFNβ, whereas anti-miRNA markedly reduced the IFNβ-mediated antiviral effect [87]. In addition, it has recently been shown that hepatic miRNA expression might be a useful tool for predicting the therapeutic outcome of a pegylated IFN/ribavirin combination therapy, further emphasizing the potential role of miRNAs in IFN-mediated antiviral effects [89].

In conclusion, the presence of a wide variety of PRRs enables the detection of multiple viral ligands present during infection. Activation of the PRR-DC-type I IFN axis (and especially the TLR7/9-pDC-IFNα axis) induces a rapid response to the virus. The many ISGs and the diversity of the type I IFNs that can be stimulated or produced, respectively, enables a coordinated response to the various viral infections, leading to control or elimination of the viral intruder.

WEAKNESSES

In the previous section, we described how well-equipped the immune system is to protect the host against viral infections. Nevertheless, viruses can evade or influence the immune response by targeting certain weaknesses of the immune system resulting in (severe) disease.

Modulation of the type I interferon response by viruses

Because of the strong antiviral and immuno-regulatory role of type I IFN, viruses developed a large variety of anti-type I IFN mechanisms. Consequently, nearly all steps of the type I IFN pathway can be blocked or manipulated by different viruses for their own benefit (Table 1) [90,91]. For example, PRR signaling can be suppressed by inhibition of downstream signaling or by sequestration of typical viral nucleic acids like dsRNA [90]. In this way, viral recognition is inhibited. Alternatively, viruses interfere with the production of type I IFN by targeting the transcription factors IRF3 and IRF7. The proteins involved in IRF activation are inactivated or IRF mimics are synthesized, which compete with the host IRFs [90,92,93]. Also, binding of IFN to IFNAR can be prevented by a virally-encoded type I IFN receptor, as observed during vaccinia virus infection [94,95]. Finally, the antiviral or immuno-regulatory effects of type I IFN are inhibited by targeting various ISGs and thereby facilitating viral replication and preventing immune recognition [96–99].

Alternatively, virus-related morbidity and mortality are not only due to virus-induced immune evasion, which facilitates extensive viral replication, but may also result from a concomitant, an inappropriate, and an exaggerated response of

Table 1. Viral inhibition of the type I IFN pathway

General target	Specific target	Virus examples	References
PRR signaling	almost all proteins	Ebola, influenza, HCV	[90,91]
Transcription	IRF3, IRF7,	Paramyxoviruses, Rabies	[90,92,93]
Cytokine receptors	IFNAR	Vaccinia	[94,95]
ISGs	ISG15, mx1, OAS1, PKR, for example	SARS, influenza, HCV	[96–99]

PRR, pattern recognition receptor; ISGs, interferon-stimulated genes; IRF, interferon response factor; IFNAR, interferon α/β receptor; SARS, severe acute respiratory syndrome.

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the immune system with devastating consequences for the host. A typical example of a combination of efficient inhibition of the type I IFN response together with an exaggerated immune response is provided by the highly pathogenic avian H5N1 influenza strain. The non-structural 1 (NS1) protein of H5N1 is an effective antagonist of the type I IFN pathway [100–102]. This results not only in high viral replication but also in an inflammatory response characterized by high levels of cytokines like TNFα [103]. This "hypercytokinemia" or "cytokine storm" results in excessive infiltration of inflammatory cells into the lungs [103–106]. Also, higher plasma levels of inflammatory mediators were detected in deceased H5N1 patients compared with survivors [107]. The deregulation of type I IFN by H5N1 is also observed in the highly virulent 1918 H1N1 influenza strain and the Ebola and Marburg viruses [108–112], in which both viral and immune pathology result in severe disease [6]. Thus, the increased resistance to the antiviral effects of IFN enhances viral replication and evokes an aberrant proinflammatory response characterized by high levels of cytokines and chemokines, which induces the pulmonary injury observed in H5N1 patients.

Likewise, the devastating effects of an HIV infection may also results from such a combination. HIV infection results in progressive immune deficiency, impaired adaptive responses, low CD4 T cell counts and increases susceptibility to opportunistic infections. One of the earliest findings during the AIDS epidemic was a deficient IFNα production in HIV-infected patients. Next to a lower number of IFN-producing cells, also each cell produced less IFNα in response to HIV [113,114]. The decrease in IFNα can be due to the Vpr protein of HIV, which strongly inhibits type I IFN production by pDCs [115]. In addition, the effects of IFNα are antagonized by the HIV protein Vpu, which induces degradation of the restriction factor BST2 [79,116]. However, during the chronic phase of HIV infection, it is hypothesized that IFNα contributes to the decline of the immune system by inducing apoptosis of CD4 T cells. Because of the non-infectious interaction between the HIV-bound gp120 protein and the CD4 receptor on pDCs, IFNα is produced and this results in killing (possibly by pDCs) of uninfected CD4 T cells [114–117]. Thus, although apoptosis of infected cells is usually a protective mechanism to prevent viral spread [118,119], here, it results in a distinct advantage for the virus due to the decreased immune control by CD4 T cells.

Thus, despite the strength of the type I IFN system, viruses have evolved mechanisms to evade or manipulate the system to guarantee their survival. Among others, this is predominantly accomplished through interfering with PRR signaling, inhibition of IRF3 and IRF7 activation and targeting ISGs.

OPPORTUNITIES

The search for therapies has led to the development of vaccines and antiviral drugs, which resulted in an impressive reduction in virus-related morbidity and mortality. Unfortunately, both vaccination and antiviral drugs are not sufficient to prevent or control all viral infections, which make it imperative to develop novel therapies. As a result, immune-based therapies are currently under development as new treatment methods. This may provide new opportunities for the treatment of acute or chronic viral infections (Figure 2).

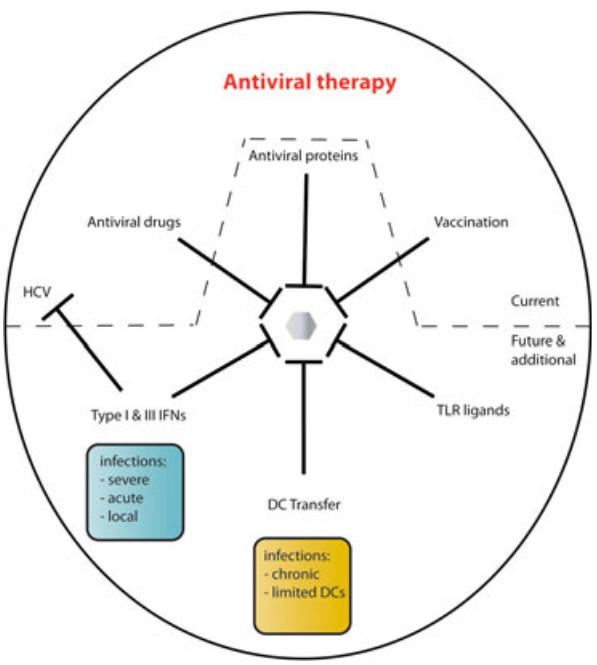

Figure 2. Antiviral therapy options. Current therapy involves antiviral drugs, vaccination, and IFNα therapy for treatment of HCV patients. In addition to these therapies, treatment with type I and III IFNs can counteract acute and local infections, TLR ligands have shown to be beneficial in various viral infections, and DC transfer could be attractive where dysfunctional or limited numbers of DCs contribute to the pathogenesis

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Interferon therapy revisited

A plausible approach to treat virally infected patients is the administration of type I IFN. Indeed, pegylated IFNα in combination with the antiviral drug ribavirin is commonly used in treating patients with a chronic HCV infection. Although this therapy is effective in nearly 50% of the cases, the administration of pegylated IFNα is associated with severe side effects [120–122]. Normally, during viral infections, type I IFN gives the "sick-signal" that results in fever. Patients treated with type I IFN have to endure these feverish periods for prolonged periods of time. In addition, hematologic and psychological problems have been frequently reported during treatment periods. Also with respect to HIV, positive effects of IFN-treatment have been reported both *in vitro* [123,124], as in clinical trials [125–129]. On the other hand, (excessive) IFNα can contribute to the immunopathogenesis (reviewed by Herbeuval *et al.*) [117]. Thus, it remains controversial whether IFNα is beneficial or detrimental in HIV, because both underproduction and overproduction of IFNα can induce severe effects in the host.

Nonetheless, because of their strong antiviral effects, type I IFNs remain attractive drugs for antiviral therapy. In particular during acute (respiratory) infections, IFNs may be an interesting therapy. This requires no systemic and chronic application of IFNs as observed in HCV patients, which may, therefore, significantly reduce the observed side effects. Local application, for example, by a nasal spray, has been shown to be effective in the prevention of seasonal respiratory infections without causing severe side effects [86,130]. This administration route might be, particularly, attractive for the prevention of virus-induced exacerbations in chronic obstructive pulmonary disease and asthmatic patients in which impaired IFN production may be an important mechanism contributing to virus-induced exacerbations [131,132]. IFNα also showed promising effects in severe acute respiratory syndrome (SARS) [133–135] and can be very important to induce an adequate immune response and possibly suppress excessive inflammatory responses observed in SARS [136–139]. Interestingly, also other members of the IFN family can be used to prevent or treat viral respiratory infections. The recently discovered type III IFNs (or IFNλ1 and IFNλ2/3) show strong antiviral effects against respiratory viral infections [131,140–142], especially when given prophylactically [142,143].

Toll-like receptor ligands

Because stimulation of TLRs by antigenic microbial epitopes is sufficient to induce a full-blown immune response, TLRs seem a likely target for antimicrobial therapy. Indeed, synthetic variants of the microbial structures have been shown to induce natural responses without the need for infection, and this quality has been used extensively to improve the efficacy of vaccines. For example, vaccines composed of a mixture of TLR ligand and antigens have been shown to be more effective than antigens alone [144–148]. Moreover, TLR ligands covalently linked to peptides are even superior in their ability to induce specific CD8+ T cells [149].

When a direct antiviral response is required, the use of synthetic TLR3, TLR7, TLR8, or TLR9 ligands can be considered. Both *in vitro* and *in vivo* studies have shown that prophylactic treatment with the dsRNA mimic polyinosinic:polycytidylic acid (poly (I:C)) and CpG oligodeoxynucleotides (CpG ODNs) specific for TLR3 and TLR9, respectively, is protective during viral infection [150–153]. Depending on virus and cell type, different types of CpG ODNs can be applied to initiate an appropriate response [154–156]. Also, TLR7 and TLR8 may be therapeutic targets. For stimulation of these TLRs, imidazoquinolones (e.g. resiquimod and imiquimod) are the best known ligands, and these small molecular weight compounds have indeed been shown to possess antiviral properties [15,16,157–160], although their immunostimulatory and antiviral effect may be limited compared with poly (I:C) and CpG ODNs [161]. Interestingly, the use of imiquimod as a cream to treat human papillomavirus-induced genital warts has already been approved [16,145,162]. TLR ligands can also reduce HCV viremia [163–165] and even HIV could be targeted [166]. Besides stimulation of type I IFN production, TLR ligands also initiate immunoregulatory mechanisms [167]. This is particularly important for the generation of the adaptive immune response and immunological memory. Nonetheless, at this time, few TLR ligands have been approved for clinical application in treating viral disease [13,148].

Dendritic cell transfer

During various viral infections, pDCs (and cDCs) are less functional or are present in lower numbers [168–170]. This is, for example, observed in HCV-infected [171,172] and HIV-infected patients, where the number of pDCs (partially) predicts the clinical

outcome [173–175]. Therefore, adoptive transfer of pDCs (and cDCs) can be used to reach the required level of pDCs and the subsequent initiation of the type I IFN response. Moreover, this will increase the efficacy of TLR ligands as they require their appropriate receptors that are predominantly present on DCs. As shown by Wang *et al.*, adoptive transfer of pDCs was used to successfully activate the antiviral response and limit RSV replication [176]. Thus, the administration of (stimulated) pDCs (in concert with cDCs) to restore DC function and/or numbers can activate the innate immune system to reach the required level of immune activation to control the viral infection, but this is probably dependent on the individual, the type of viral infection (chronic) and the stage of infection.

Other options

As observed in many viral infections, the (concomitant) proinflammatory response can contribute significantly to the disease. Therefore, anti-inflammatory drugs [177] are attractive to suppress symptoms during viral disease. Also, the use of antiviral drugs for specific inhibition of viral replication remains attractive as therapy, especially in combination with other treatments (like IFNα treatment and ribavirin in HCV patients). Furthermore, although TLR ligands and IFNs can induce production of restriction factors, these might also be applied directly to limit viral replication. On the other hand, IFN-inhibitor proteins of viruses can be targeted to restore immune functions [178] and make additional restriction factors or immunotherapy more effective.

Taken together, although viruses are well able to subvert or manipulate the type I IFN response, the IFN system can also be used or stimulated to strengthen the response towards viral infections. IFNs themselves are already used in HCV treatment, and promising effects have been shown in respiratory viral infections. Moreover, the therapeutic use of TLR ligands is currently under intense investigation as they have shown to have great potency to stimulate those immune cells critically involved in the antiviral immune response. This stimulates the production of antiviral proteins or inhibitors of viral evasion proteins, which can also be used independently of TLR stimulation or IFN application. Finally, the transfer of (stimulated) pDCs for gradual production of type I IFN and other cytokines (in combination with cDCs for induction of adaptive immunity) might be an option to limit symptoms or even control virus replication.

THREATS

In the previous section, we revealed among others the opportunities related to TLR ligands as potential antiviral drugs. Yet, although promising results with TLR ligands have been reported during the last decade, there are also several **threats**.

Autoimmunity

Endosomal TLRs usually only respond to DNA/RNA derived from pathogens while immune responses to host genetic material are prevented in different ways. First, DNA (and RNA) from apoptotic or necrotic host cells is removed by DNAses (and RNAses, respectively). Second, the nucleic acids from microbes are fundamentally different from host nucleic acids. Viral and bacterial DNA contain unmethylated CpG motifs, whereas in host DNA heavy methylation and fewer CpG motifs are common [179]. Furthermore, the TLRs that bind (microbial) nucleic acids are endosomally located [7,14]. Because of this intracellular localization, self-nucleic acids cannot stimulate these TLRs. Finally, regulatory receptors are present on pDCs, which limit type I IFN responses [180].

Sometimes, however, these barriers are not sufficient, and aberrant immune responses arise ultimately resulting in autoimmune diseases like systemic lupus erythematosus (SLE) [181–183], an autoimmune disorder that especially affects the skin. In SLE, it is assumed that apoptotic or necrotic material containing nucleic acids are phagocytosed by pDCs and cDCs. The pDCs respond with production of type I IFN and other cytokines resulting in activation of the cDCs, which then stimulate autoreactive T and B cells. After differentiation of B cells into plasma cells, autoantibodies are produced and complex with the nucleic acids from necrotic cells. Subsequent binding to the Fc receptor for IgG (FcγRIIa) on pDCs [184] and cDCs results in further type I IFN production and B-cell stimulation [185]. This vicious cycle can be evoked or aggravated by the administration of TLR ligands. The reason why these pDCs respond to the host-derived nucleic acids is still unclear.

Thus, concerns about instigating or enhancing autoimmune diseases are important reason why TLR ligands are not extensively administered in

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the clinic. Despite promising results in the last decade with these ligands in antiviral therapy, precautionary measures to prevent induced autoimmune responses are definitely necessary.

Species differences

Much of what we know comes from animal experiments, but translating experimental results from laboratory animals to humans is often problematic. This is also the case with the translation of our knowledge from the immune response of well-studied mouse models to humans. For example, the response to certain viruses can be entirely different in both hosts, due to adaption of the virus to its host [186]. Moreover, important differences in antiviral mechanisms between mice and humans have been observed.

First, there are differences in the TLR-induced response. Studies indicate that murine pDCs are able to produce IL-12p70 in addition to IFNα post-TLR9 stimulation, whereas human pDCs do not [60,62,63]. Secondly, the location of TLR9 is different in mice than in humans. In humans, TLR9 is exclusively expressed in pDCs and B cells [187] while mice express TLR9 on cDCs, B-cells, macrophages, and monocytes [188]. Thus, a TLR9 ligand can induce entirely different responses in both species. Another major difference is the function of TLR8. TLR8 stimulation induces IL-12 production in humans [189], but this receptor appears to be non-functional in mice, although this is still a matter of debate [190]. Finally, the cytokine flt-3 ligand is used to differentiate murine hematopoietic stem cells into DCs with a relatively high percentage of pDCs [191,192]. This does not reflect the human situation in which most experiments are performed with PBMCs, containing a very low number of pDCs [154–156,193] that are probably at a different stage of maturation.

Hence, as stimulation of the type I IFN response can improve immunity toward viral infection, it can also evoke or aggravate aberrant immune responses (autoimmunity), thereby limiting clinical application of TLR ligands and IFNs. Furthermore, although animal experiments have been extremely helpful in deciphering antiviral responses, these are not an exact representation of the human type I IFN response, further hindering clinical application.

CONCLUSION

In this review, we provided a condensed overview of the molecular pathways involved in the most potent antiviral part of the innate immune system, the type I IFN response. Moreover, we reviewed the cells and receptors that are intimately involved in this type I IFN system. Also, we evaluated the (im)possibilities of new ways to modulate the type I IFN response, for example, by TLR ligands or adoptive DC transfer, as promising future antiviral therapies. Nonetheless, although strong antiviral effects of IFNs, TLR ligands, DCs, and restriction factors have been shown by many studies, the clinical application of these immune-based therapies is unfortunately still limited, which might be related to concern for eventual undesired side effects like autoimmune diseases. Therefore, to be clinically successful, perhaps a more personalized approach is required. The application of these immune-based therapies can then be considered based on the individual, virus, stage of infection, and symptoms, thereby fine-tuning the type I IFN response and preventing side effects as much as possible.

CONFLICT OF INTEREST

The authors have no competing interest.

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