Review

Innate Antiviral Defenses Independent of Inducible IFNα/β Production

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The type I interferons (IFNs) (IFNα and IFNβ) not only have potent antiviral activities, but also have pathological functions if produced at high levels or over a long time. Recent articles have described antiviral immune mechanisms that are activated in response to virus infection at epithelial surfaces independently of IFNα and IFNβ. This may allow the host to exert rapid local antiviral activity and only induce a full-blown, and potentially pathological, type I IFN response in situations where stronger protective immunity is needed. Here, I describe the emerging understanding of early antiviral defenses, which are independent of type I IFN responses, and also discuss how this enables tissues to exert rapid antiviral activities and to limit type I IFN production.

Immune Responses to Viral Infections

Viruses are major causes of disease, including influenza, hepatitis, encephalitis, and AIDS, and there is still an incomplete understanding of the determinants governing susceptibility towards infections. The immune system is essential for defense against viral infections and, in recent years, it has emerged that the innate immune response in particular is pivotal for optimal early defense against viruses [1–4]. Indeed, there is accumulating evidence demonstrating that patients who develop overt pathology in response to viral infections have genetic defects in the innate immune system [5–8]. Type I IFNs (see Glossary) are a large group of virus-induced cytokines that includes the subtypes IFNα (includes 13 human IFNαs), β, κ, λ, and ω. Type I IFNs are central components of host defense against these infections, and mice unable to respond to this class of cytokines have elevated susceptibility to viral infections [9–11]. However, type I IFNs also have potentially pathological functions [12], and excessive IFN production during infection or sterile conditions leads to tissue damage, and is associated with disease [13–16]. Among the type I IFNs, IFNα and β are by far the most studied, and are the two subtypes demonstrated to have both beneficial and deleterious roles in defense and disease. Therefore, in this review, I concentrate on IFNα/β and refer to these cytokines when the term ‘Type I IFN’ is used, unless otherwise specified.

A series of recent articles now suggest that the host has antiviral defense systems that act before the induction of type I IFNs, and also that the IFN response, once initiated, is mounted in a gradual manner [17–23]. Here, I present a model of how the infected tissue uses antiviral responses, which are independent of inducible type I IFN production, to exert immediate antiviral defenses, thus reducing the requirement for strong type I IFN production. This allows the organism to exert local antiviral activity rapidly after infection, and to launch a full-blown type I IFN response only when the virus is establishing infection.

Type I IFN Responses

Most, if not all, nucleated cells have the capacity to produce and respond to type I IFNs [24]. Type I IFNs are induced by virus infections to high levels, but are also expressed constitutively at

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very low levels [25]. The tonic IFN signaling leads to constitutive low expression of IFN-stimulated genes (ISGs), which impacts homeostatic balances in the immune system and also sets the threshold for immune responses to infections (Figure 1A). The processes affected by tonic IFN signaling include some of the early immune reactions discussed in this review, such as restriction factors (RFs), which are ISGs. The virus-induced expression of IFNα/β is stimulated by pattern recognition receptors (PRRs), most notably, PRRs that detect nucleic acids [26]. For instance, Toll-like receptor 3 (TLR3) detects double-stranded RNA in endosomes, retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) detect RNA structures in the cytoplasm, and cyclic GMP-AMP synthase detects double-stranded DNA in the cytoplasm [27–29]. Following the detection of nucleic acids, PRRs activate signaling pathways that lead to the downstream activation of transcription factors of the IFN regulatory factor (IRF) family. IRF transcription factors bind to specific promoter elements in the IFN genes to induce their transcription (Figure 1A). The type I IFN proteins are secreted cytokines and act via binding to the IFNα receptor (IFNAR) complex, which comprises the IFNAR1 and IFNAR2 chains [30]. IFNAR signals through the kinases TYK2 and JAK1 to activate the trimeric transcription factor complex ISG factor 3 (ISGF3), comprising signal transducer and activator of transcription (STAT)1, STAT2, and IRF9, to stimulate expression of ISGs. Several hundred ISGs have been reported [31–33], and they have an array of functions (Figure 1B). A large subset of the ISGs has direct antiviral activity through their capacity to block specific steps in the viral life cycle [34]. This includes, for instance, IFIT1, which binds viral 5’-triphosphate RNA, thus sequestering the DNA from the eukaryotic translation initiation factors [35,36]; Other ISGs serve to orchestrate the cellular innate immune response. This includes, for instance, chemokines, such as CXCL10, which promote leukocyte recruitment [37], and granule components, such as granzymes, which prime the cytotoxic activity of natural killer cells [38–41]. In addition, type I IFNs are involved in shaping the development of the T helper 1 cell response, which is important for the control of viruses. At the mechanistic level, this includes IFN-mediated activation of STAT4 [42,43].

Given these many functions of type I IFNs, it is no surprise that this class of cytokines has strong antiviral activities. However, accumulating evidence suggests that type I IFNs also contribute to the pathology of viral infections. For instance, in the lungs of influenza A virus-infected mice, IFNα and IFNβ induce expression of the death receptor DR5 on epithelial cells and of TNF-related apoptosis-inducing ligand (TRAIL) on inflammatory monocytes [44]. This in turn stimulates TRAIL-DR5-dependent cell death and lung pathology [44]. Moreover, in HIV-1 infection, circulating IFNα levels are an important prognostic indicator of HIV-1 clinical progression [45], and there are data suggesting roles for type I IFN in both T cell death and senescence in HIV infection [46,47]. In two studies addressing the impact of prolonged type I IFN production on the antiviral activity of T cells, it was found that type I IFN production during chronic viral infection led to lymphoid tissue disorganization and expression of the negative immune regulatory molecules interleukin (IL)-10 and programmed death ligand 1 [48,49]. This was reduced by the inhibition of IFNAR signaling, which led to enhanced virus clearance in a manner dependent on CD4 T cells and IFNγ production [48,49]. Beyond viral infections, there is an increasing understanding of type I interferonopathies, which are sterile inflammatory diseases with an underlying etiology involving the dysregulation of nucleic acid metabolism [13]. This includes diseases such as systemic lupus erythematosus and Aicardi–Goutières syndrome [12]. Finally, well-described adverse effects of clinical treatment with IFNα are influenza-like symptoms, neuropsychiatric effects, and bone marrow suppression [50]. Therefore, type I IFNs have not only strong antiviral activity, but also the potential to promote disease development.

Early Antiviral Defense Independent of the Inducible Type I IFN Response

Given the potentially pathological functions of type I IFNs, it is conceivable that the host seeks to limit the expression of these cytokines. There are now known to be several host antiviral mechanisms acting before the type I IFN response. Since the discovery of the direct antiviral
activity of specific ISGs, it has been noted that most ISGs are expressed at a basal level in many cell types, at least partly due to tonic IFN signaling. This allows cell-autonomous antiviral activity by ISGs independent of virus-induced IFN production. This phenomenon has been termed ‘restriction’, and the proteins exerting these activities are termed ‘RFs’ [51]. For instance, it is well established that the ISGs APOBEC3B, Tetherin, and SAMHD1 can directly block HIV-1 replication [52–54], and that IFITM proteins restrict several human pathogenic viruses, including influenza A virus [51]. Through this means, cells have tools to immediately counteract viruses as they enter the cell. Therefore, successful action of RFs serves a dual role, by both directly blocking viral replication before establishment of infection and limiting the accumulation of pathogen-associated molecular patterns (PAMPs) and, hence, type I IFN expression. An example of this is provided by studies on the lentivirus murine leukemia virus in APOBEC3-deficient mice [55]. These mice have a higher viral load, which leads to elevated expression of IFNβ stimulated by viral reverse transcription products [55].

Most infections are acquired through mucosal surfaces and, given the frequent exposure of these surfaces to infectious agents and other environmental substances, it appears essential for the immune system to be able to act locally and only mount stronger immune responses with systemic effects if infections are established. Interestingly, type III IFNs (IFNλs), which are induced through the same pathways as type I IFNs and induce largely the same set of genes [33], act preferentially on epithelial surfaces, due to the restricted expression of the IFNλ receptor chain IL28RA by epithelial cells [56,57]. Mice infected in the airways with influenza A virus or in the
gastrointestinal tract with reovirus or rotavirus rely on IFNα to fully control the infection [58–61]. In agreement with this, treatment with recombinant IFNα on mucosal surfaces efficiently controls replication of a broad range of both RNA and DNA viruses [58,60–62]. Recently, further details were provided on the mechanism that governs IFNα-dependent antiviral control at mucosal surfaces (Figure 2). It was reported that IFNα produced by intestinal epithelial cells acts synergistically with IL-22 produced by group 3 innate lymphoid cells to induce STAT1 activation in intestinal epithelial cells; this leads to the subsequent induction of ISGs in intestinal epithelial cells and control of rotavirus infection [18]. The production of IL-22 was stimulated by IL-1α, which was released from intestinal epithelial cells [18]. Release of IL-1α from cells can occur through a controlled process or during cell lysis [63]. Thus, it is possible that the synergistic action of IFNα and IL-22 at epithelial surfaces integrates the sensing of viruses and cellular damage to potentiate a local antiviral response at the site of infection. Given the restricted expression of IFNα receptors, the type III IFNs do not appear to induce much immunopathology [44]. Therefore, IFNαs have a pivotal role in limiting viral replication at epithelial surfaces and, hence, the accumulation of viral IFN-stimulating PAMPs, without overt systemic effects. Future studies should investigate how the elevated viral load observed during some viral infections in IL28RA-deficient mice impact the levels and duration of type I IFN production.

Restricted biological action of a cytokine can be achieved by modest and local expression of the cytokine or restricted expression of the receptor chains. Since IFNα and -β are highly inducible and the receptor is ubiquitously expressed, production of these cytokines gives rise to systemic effects, as already discussed. The case of IFNα described above provides an example of restricted expression of the receptor chains as a means to focus the biological activity. An example of localized and modest expression of a cytokine at the site of action is provided by IFNs, which is a type I IFN constitutively expressed by epithelial cells in the female reproductive tract, lungs, and gut [64,65]. IFNs: is not induced by virus infection or after stimulation of PRRs, but is regulated by hormones, such as estrogen [64]. The constitutive expression of IFNs: stimulates a basal level of ISGs, which was demonstrated to set the immunological tone in the female reproductive tract [64]. Hence, Ifne−/− mice are more susceptible to genital herpesvirus infection. Interestingly, as was also observed for the APOBEC3-deficient mice after infection with murine leukemia virus [55] (see discussion above), the elevated viral load in Ifne−/− mice led to increased expression of IFNβ [64].

Recently, the existence of an antiviral pathway that acts before the induction of IFNs at epithelial surfaces was reported [17]. Using a mouse model for genital herpes, it was observed that a subset of NF-κB-inducible genes was induced in epithelial cells of the infected genital tract before IFNs were produced [17]. The genes induced encoded an array of immune mediators that included the CXCR3-binding chemokines CXCL9 and CXCL10, and mucins. CXCL9 and CXCL10 were found to recruit neutrophils, which in turn exerted antiviral activity. The induction of these CXCR3 chemokines before IFNs occurred independently of known virus-sensing PRRs and was also observed following physical damage of the mucus layer, or treatment with bacterial mucin-digesting enzymes. These results suggest that disturbance of the integrity of mucosal surfaces is detected by the tissue to stimulate influx of neutrophils with antimicrobial activity and also production of mucins, which can facilitate rapid restoration of the mucus layer. Moreover, mice with impaired ability to activate this novel antiviral program, which is independent of virus-induced type I IFN production, showed elevated inflammatory responses at later time points during infection, and these inflammatory pathways were activated in a TLR-dependent manner [17]. This suggests that the early control of viruses limits the accumulation of viral PAMPs that can stimulate pathological inflammatory responses.

Collectively, there is now accumulating data to suggest that cells and tissues have evolved systems to control viruses before the well-described action of type I IFNs, and that these systems also aid the organism to resolve the infection without causing excessive inflammation.
Scaling Up the Production of Type I IFNs

The above-described action of RFs and recruited neutrophils occurs before virus-induced IFNα/β starts to act, and IFNAs preferentially act locally on epithelial cells. However, as soon as viruses enter host cells, the sensing and signaling events leading to IFN production are initiated. This section describes how the production of IFNα/β is tightly regulated and only gradually increased. It should be emphasized that IFNAs are induced by similar mechanisms as IFNα/β, but are not discussed here, given the superior role of type I IFNs in immunopathology.

Gradual Build-Up of Type I IFN Production

Since type I IFN production is stimulated after sensing of viral PAMPs by PRRs, there is generally a correlation between viral load and IFN induction, as discussed above [55,64]. In addition, there

Figure 2. Different Stages in the Early Antiviral Response at Mucosal Surfaces. The figure illustrates early antiviral events occurring both independent of and dependent on type I interferons (IFNs), and also shows how the type I IFN response is gradually increased over time. Black arrow: viral infection, cytokine production, or leukocyte migration; purple arrow, cytokine action on a receptor; red line with blunt end: antiviral activity. Abbreviation: ILC, innate lymphoid cell; RF, restriction factor.
are several regulatory steps in the induction of type I IFN production, which need to be activated before a full IFN response is triggered. Therefore, a full IFN response builds up over time, as seen, for instance, during an influenza A virus infection in the lungs [66]. It is likely that, in most infections, the full IFN response is not activated due to control of the virus by the first wave of type I IFNs or by efficient viral evasion mechanisms. The gradual build-up of type I IFN production with in-built amplification mechanisms may represent a system whereby the host can limit type I IFN production to the amount required to control the infection. These mechanisms are discussed below.

Mechanisms for Scaling Up IFN Production

Most PRRs sensing viral nucleic acids are expressed at relatively low levels constitutively, including TLR3, RIG-I, and cyclic GMP-AMP synthase (cGAS) [21–23]. Following infection, the activation of TLR3, RIG-I, and cGAS induces IFN production, which in turn upregulates expression of the genes encoding these innate receptors [21–23]. This elevated PRR expression correlates with enhanced IFNα/β induction by viral infections (Figure 2). It is not only the expression of the genes encoding PRRs that is under the control of IFNs, but also expression of proteins in the signaling pathway. Most notably, IRF7, which is essential for high IFNα expression [1], is an ISG. This phenomenon establishes a positive feed-back loop, where the first wave of type I IFN is driven by IRF3, which activates transcription of IFNβ and IFNα1 (α4 in mice), which in turn induces expression of IRF7 and priming for a full IFN response [67]. Consequently, mice lacking IFNAR or STAT1 produce less IFNα and -β after viral infection despite a higher viral load [66,68]. The importance of the IFR7-dependent positive feedback loop differs between cell types. Most cell types have very low basal expression of IRF7, but plasmacytoid dendritic cells (pDCs) express IRF7 at high levels constitutively, due to a mechanism involving self-priming [69], thus enabling this cell type to produce high levels of type I IFN immediately following sensing of viruses.

A second type of host system to induce low-grade IFNα/β expression at the initial stages of viral infection has been described for both enveloped and nonenveloped viruses. It was shown that the process of membrane fusion is sensed when enveloped viruses enter cells [19,70]. This occurs through a noncanonical STING-dependent pathway, which is independent of cGAS and cGAMP and stimulates low expression of IFNβ and IFNα4 [70]. A study by James and colleagues identified a complement-dependent pathway that enables the sensing of nonenveloped viruses and subsequent IFN induction. These authors showed that complement factor C3 is covalently attached to the capsid of the entering virus, and this induces signaling and IFNβ expression dependent on the RIG-I adaptor MAVS [20]. C3-binding also targets viruses for proteasomal degradation, thereby preventing their replication [20].

Third, the gradual build-up of IFNα/β expression also includes recruitment of leukocytes with the capacity to induce high levels of IFNs. Most work in this area has focused on pDCs and there are several studies that demonstrate recruitment of pDCs to virus-infected areas in mice leading to expression of type I IFNs in a TLR-dependent manner [71–73]. Moreover, in rhesus macaques infected with simian immunodeficiency virus, pDC are recruited to the ileum, colon, and rectum, and augment IFN expression in these areas [74]. Finally, studies of skin lesions from patients with varicella zoster virus infection, which is sensitive to type I IFNs [75], show that there is a strong recruitment of IFN-producing pDCs to these areas [76]. Thus, there is solid evidence for pDCs being recruited to areas of virus infection where they augment IFN production. More recently, data have emerged that other recruited cell types, mainly of the myeloid lineage, also contribute to mounting a full IFN response. For instance, Ly6C+ monocytes are recruited in a CCR2-dependent manner to sites of viral infection [77,78], and have been reported to be an important source of type I IFN [79]. Moreover myeloid DCs recruited into the lungs of influenza A virus-infected mice contribute to the production of type I IFNs [80].
The concerted action of the events described in this section ensures a gradual increase in the production of IFNα/β during infection. Eventually, the full IFN response is achieved with a strong impact on antiviral defense via direct antiviral action and promotion of cellular antiviral immune responses. However, at this stage, IFNα/β can also serve pathological functions, two of which are stimulation of cell death and priming of excessive inflammatory responses.

**Tissue-Specific Scaling of the IFN Response**

It is well recognized that tissues differ with respect to their sensitivity towards inflammation-mediated damage, including IFN-driven pathology [12]. For instance, lung, liver, and gut are relatively more resistant to immunopathology compared with the brain [81]. There is now emerging evidence suggesting that the contributions of the mechanisms discussed above for amplification of type I IFN responses differ among tissues. The classical IRF7-dependent positive feedback loop for IFN induction was found to be important for achievement of high type I IFN production in the lung, liver, and heart, but not in lymphoid organs [66]. The relatively limited role for the positive feedback loop in lymphoid organs was explained by the constitutive presence of IRF7-expressing pDCs. Thus, the organs frequently exposed to pathogens, such as epithelial surfaces, have little constitutive expression of IRF7 and no resident pDCs [66,68,82], possibly as a strategy to avoid ‘premature’ production of high amounts of type I IFN immediately after exposure to viruses. However, in response to infection and inflammation, pDCs are programmed to traffic to those organs frequently exposed to pathogens [83]. This is explained by the expression of CCR6 and CCR10 by a subset of pDC, thus enabling trafficking to inflamed skin and epithelial sites after local production of CCL20 and CCL27/28, respectively, leading to IFNα production at these sites [83]. The brain is sensitive to the pathological functions of type I IFN [12]. After intracranial infection with lymphocytic choriomeningitis virus, IFNβ, but not IFNα, was detected in the brain, and Irf7−/− mice did not have reduced expression of IFNβ [84]. Therefore, the IRF7-mediated positive feedback loop seems to be less active in the brain compared with peripheral tissues, possibly as a means to prevent high type I IFN levels in this organ. Recent studies on patients with severe viral infections demonstrated that loss-of-function mutations in abundantly expressed molecules in the IFN induction pathways give rise to narrow clinical phenotypes [5–8,85,86]. For instance, functional TLR3 deficiency leads to selective elevated susceptibility to herpes simplex encephalitis [5].

The results discussed in this section could suggest the existence of tissue-specific mechanisms to unfold the type I IFN response during viral infections, although more work is needed to consolidate this concept.

**Concluding Remarks and Future Perspectives**

Type I IFNs have been termed the first line of defense against viral infections [87,88]. With the recent identification of early antiviral activities occurring independent of, and even before, IFN induction, it is now emerging that there is an extra layer in the innate immune system to fight viral infections. In addition, there are several mechanisms to ensure that the production of IFNs is titrated to what is required to control the virus and what is tolerated by the tissue. Future work should identify the mechanisms involved and decipher how viruses try to evade these early defense mechanisms (see Outstanding Questions). It will also be interesting to learn how the type I IFN system interacts with the innate antiviral mechanisms that seem to operate independent of virus-induced type I IFN production.

It is now well established that type I IFN can contribute to the immunopathology of viral infections [44–47], and there are data to suggest that abolishment of specific IFNα/β-independent antiviral functions leads to elevated production of type I IFNs [55,64]. Such data also suggest that the existence of innate antiviral systems that operate independent of inducible type I IFN production allows the host to exert rapid local antiviral activity and spare the full IFN response for situations where the infection is established.

**Outstanding Questions**

What are the mechanisms that enable epithelial surfaces to sense the disruption of mucosal integrity? Are there additional yet-to-be-discovered type I IFN-independent antiviral mechanisms?

How important are the pre-type I IFN antiviral activities for defense against viral infections? Are individuals with inherited or acquired defects in these mechanisms more susceptible to viral infections?

Do viruses have mechanisms to evade the type I IFN-independent antiviral activities?

Are there interactions between the classical type I IFN antiviral response and those activated independent of type I IFN? Do such interactions lead to an IFN response (in strength and duration) that allows an optimal balance between antiviral activity and IFN-mediated pathology?
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