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Type I Interferons in Host Defense

Review

Daniel B. Stetson[1,*] and Ruslan Medzhitov[1,*]
[1] Howard Hughes Medical Institute
Section of Immunobiology
Yale University School of Medicine
New Haven, Connecticut 06520

Summary

Type I interferons (IFNs) are a family of cytokines specialized to coordinate immunity to viruses and other intracellular infections. In the past several years, many of the receptors and signaling pathways that link pathogen detection to induction of type I IFNs have been identified and characterized. An integrated picture has emerged in which type I IFNs have essential functions in several seemingly disparate processes: they restrict viral spread by engaging machinery that ultimately cripples and kills infected cells, yet they are also positively linked to the activation and expansion of lymphocytes that are important for control of intracellular infections. These advances highlight the context-specific actions of type I IFNs and clarify the multiple points at which they are integrated into both innate and adaptive immunity.

Introduction

Viruses are the most abundant and diverse pathogens on earth. One look at the genome of any organism confirms that viruses have been around for a very long time. It is estimated that up to 40% of the human genome is derived from fragments of ancestral viral sequence, and approximately 1% of the genome consists of intact endogenous retroviruses (Sverdlov, 2000). The impact of viruses on our genes cannot be overstated: viruses are likely responsible for generating much of the variation that fueled selection of new traits and the evolution of our species, including exon shuffling, gene duplication, and diversification of noncoding regulatory elements.

Viruses can also be formidable pathogens and pose a unique challenge for the host's detection systems. It is now well established that immune recognition focuses on highly conserved, invariant structures of microbes that are distinct from the host (Janeway, 1989). Unlike bacteria and fungi, which contain easily distinguishable microbe-specific structures, viruses are made entirely of host-derived components. With no microbe-specific "pattern" to latch onto, the receptors that detect viruses have instead evolved to recognize the one feature that is shared without exception among all viruses: a genome composed of nucleic acids. Importantly, nucleic acid recognition is in principle an imperfect way for cells to distinguish self from non-self for obvious reasons, yet most (if not all) ways for a cell to signal the presence of viral infection are based on it. The receptors that accomplish this are generally very successful at identifying viral nucleic acids in the midst of an abundance of host-derived RNA and DNA. Because there is little structural basis for discrimination between viral and host nucleic acids, it appears that pattern recognition in this case is aided by the compartmentalization of antiviral sensors and by activities of host nucleases that help eliminate self nucleic acids from the extracellular fluids and from lysosomes (Barton et al., 2006; Napirei et al., 2000; Yoshida et al., 2005). Additional mechanisms that help such discrimination undoubtedly exist and await elucidation; insight into this fundamental question is attainable now that we know the identity of many of the receptors involved, as discussed below.

All organisms are susceptible to viral infections and all have a dedicated system to detect and prevent nucleic acid parasitism. These systems range from restriction enzymes and antiphage defense in prokaryotes to the multilayered and complex networks of antiviral responses in higher metazoans. Each of these systems directs responses that vary according to the organism's physiology and needs. Jawed vertebrates, which evolved an adaptive immune system of T and B cells, also developed the type I interferon (IFN) cytokine family that is dedicated to signaling the presence of intracellular infection and facilitating communication among the cells that provide defense against viruses and intracellular bacteria. All vertebrates examined to date have a gene that encodes IFN-β and at least two that encode IFN-α, together with natural killer (NK) cells, T cells, and B cells; there is no known organism that has one of these components without all of the others. The preservation of type I IFNs throughout the vertebrate lineage suggests an intimate functional linkage to adaptive immunity that has become increasingly apparent with recent experimental insights.

In this review, we will discuss the major receptor systems that detect viral infection and activate type I IFNs in vertebrates, with an emphasis on how these systems complement each other in host defense. We will attempt to integrate important recent developments into the well-established framework of IFN function that is discussed in the accompanying reviews (Honda et al., 2006; van Boxel-Dezaire et al., 2006; Banchereau and Pascual, 2006) and other papers (Honda et al., 2005b; Garcia-Sastre and Biron, 2006; Levy et al., 2003; Stark et al., 1998). These exciting advances clarify the mechanisms by which IFNs are activated, provide insight into their multiple and context-specific roles in antiviral immunity, and may suggest new directions for therapeutics and vaccine development.

Two Pathways for Type I IFN Induction: Toll-like Receptors

Prior to the year 2000, no mammalian receptors that linked viral recognition to activation of type I IFNs had been identified. After several years of remarkable progress, it is now clear that two complementary receptor systems account for most virus detection. A general principle that has emerged is that all of these receptors

*Correspondence: daniel.stetson@yale.edu (D.B.S.); ruslan.medzhitov@yale.edu (R.M.)

detect nucleic acids. Furthermore, these receptors fall into two categories: one class of receptors (exemplified by RIG-I and MDA5 proteins) is expressed ubiquitously and is localized to the cell's cytosol where it detects viral nucleic acids produced upon infection. The second class of receptors (members of the Toll-like receptor [TLR] family) detects viral nucleic acids in endosomes and only in specialized cell types. This latter mode of recognition does not require that the IFN-producing cells are infected themselves and hence need not be ubiquitous.

The Toll-like receptors (TLRs) have been the subject of intense investigation as prototypical signaling receptors that activate immunity in response to microbial stimuli (Akira et al., 2006; Iwasaki and Medzhitov, 2004). Several of the mammalian TLRs recognize different types of nucleic acids, and together they provide enough coverage to detect most types of viruses. TLR3 recognizes double-stranded RNA, a common feature of both DNA and RNA viruses that until recently was thought to be the principal (or even only) form of viral nucleic acid recognized by the immune system (Alexopoulou et al., 2001). TLR7 and TLR8 detect single-stranded RNA (ssRNA) and viruses that contain ssRNA genomes (Diebold et al., 2004; Heil et al., 2004; Lund et al., 2004). TLR9 detects unmethylated CpG motifs in DNA that are common in DNA viruses and also bacteria (Hemmi et al., 2000; Krug et al., 2004b; Lund et al., 2003).

The only known exception to the rule that all receptors that activate type I IFNs detect nucleic acids is TLR4, which detects lipopolysaccharides (LPS) derived from gram-negative bacteria and activates type I IFNs in conventional dendritic cells (DCs) and macrophages through a signaling pathway that utilizes the adaptor protein Trif (Oshiumi et al., 2003; Yamamoto et al., 2002). It is completely unclear at present why the TLR4-Trif pathway links recognition of bacterial cell walls to cytokines that generally signal the presence of a viral infection. By comparison, TLR2 also recognizes cell wall components (peptidoglycans) but does not activate IFNs (Takeuchi et al., 1999; Yang et al., 1998). It is likely that the TLR4-Trif axis arose to harness some of the functions of IFNs in enhancing T cell differentiation and NK cell function, as discussed below.

TLRs are primarily expressed on key sentinel cells of the innate immune system: macrophages and dendritic cells (DCs). TLR3 is expressed by conventional DCs, whereas TLRs 7 and 9 are expressed by both conventional and plasmacytoid dendritic cells (pDCs). pDCs have a specialized signaling pathway that links activation of TLRs 7 and 9 to the production of copious amounts of type I IFNs, and they are primarily responsible for the systemic concentrations of IFNs detected during many experimental viral infections (Colonna et al., 2004; Krug et al., 2004a). One feature shared by the four TLRs that sense nucleic acids is their intracellular localization: unlike all other TLRs, which are located at the cell surface, TLRs 3, 7, and 9 signal from within an acidified endosomal compartment. Consequently, inhibitors of endosomal acidification block the function of these three TLRs but none of the others (Hacker et al., 1998; Heil et al., 2003; Lee et al., 2006). This intracellular localization is important for the ability of these TLRs to discriminate between self and non-self nucleic acids: relocating TLR9 to the cell surface abolishes its ability to respond to virus-encapsulated DNA but enables recognition of self-derived genomic DNA in the extracellular milieu (Barton et al., 2006).

A key feature of TLR-dependent nucleic acid recognition is directly tied to their intracellular location: TLRs sample material entering cells from the outside and thus do not detect the presence of infection from within. This material often consists of viral particles that become uncoated or degraded upon endosomal acidification, thus exposing their nucleic acids for recognition. Recent evidence indicates that TLR3 also samples apoptotic cells for viral dsRNA as they are engulfed and degraded in the phagosome (Schulz et al., 2005). Considering the strong link between viral infection and apoptosis in vertebrates and the role of TLRs in antigen presentation, this mechanism would provide an efficient means for viral antigens within dead cells derived from any tissue to be presented to T cells by uninfected DCs, a process known as crosspresentation. The separation of viral infection from viral recognition in DCs has another important consequence: most viruses encode proteins that potently antagonize type I IFN expression and signaling within infected cells, but uninfected DCs would be refractory to such inhibition.

Two Pathways for Type I IFN Induction: Cytosolic Receptors

Although some TLRs can activate IFNs in a subset of specialized cells, almost all nucleated cells respond to viral infection by producing type I IFNs. Fujita and colleagues advanced our understanding of this more broadly expressed virus-detection system by identifying the caspase recruitment domain (CARD)-containing RNA helicases retinoic acid inducible gene-I (RIG-I) and melanoma differentiation antigen 5 (MDA5) as sensors that link detection of dsRNA to activation of type I IFNs (Yoneyama et al., 2004). Both RIG-I and MDA5 use the CARD-containing adaptor protein MAVS (also known as IPS-1, VISA, or CARDIF) to activate the IFN-β gene (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). The details of the RNA-activated signaling pathways have been reviewed elsewhere (Kawai and Akira, 2006; McWhirter et al., 2005), and recent developments will also be discussed in the accompanying review (Honda et al., 2006).

Knockout mice lacking RIG-I and MDA5 have dramatic phenotypes when challenged with RNA viruses in vitro and in vivo. RIG-I-deficient cells fail to activate type I IFN production when challenged with several classes of viruses, including vesicular stomatitis virus (VSV), Newcastle disease virus, Sendai virus, influenza virus, and Japanese encephalitis virus (Kato et al., 2005, 2006). Interestingly, pDC-derived type I IFN production in response to these viruses requires MyD88-dependent TLR signaling but not RIG-I, highlighting the fact that TLRs and cytosolic sensors can independently activate type I IFNs in vivo (Kato et al., 2005). MDA5 is the primary cytosolic receptor for the RNA polymer poly I:C and is required for detection of the picornavirus encephalomyocarditis virus (EMCV) (Gitlin et al., 2006; Kato et al., 2006). Together, these studies establish that RIG-I and MDA5 detect different viral RNA structures, although the precise nature of these structures and the biochemical basis for this differential recognition remains unknown.

The generation of MAVS-deficient mice demonstrated that both RIG-I and MDA5 use this adaptor protein to activate type I IFNs (Kumar et al., 2006; Sun et al., 2006). Consequently, the phenotype of MAVS-deficient mice resembles the sum of RIG-I and MDA5 deficiency when challenged with RNA viruses that are detected by these helicases. Importantly, MAVS-deficient mice are highly susceptible to infection with these viruses despite an intact TLR-dependent recognition system. This interesting observation formally demonstrates that although the two receptor systems can independently activate the type I IFN response, they are not functionally redundant. As discussed below, this also implies that type I IFNs are not sufficient for antiviral defense and that these cytosolic receptors may also control essential antiviral functions in an IFN-independent manner.

In addition to intracellular recognition of viral dsRNA, which has been known to exist for many years, foreign DNA is also recognized by an intracellular sensor that triggers type I IFN production (Ishii et al., 2006; Okabe et al., 2005; Stetson and Medzhitov, 2006). This DNA-recognition system presumably evolved to detect DNA derived from DNA viruses during infection. Thus, similar to RIG-I and MDA5, DNA recognition is a cell-autonomous and, presumably ubiquitous virus-recognition system. The sensor for intracellular viral DNA is currently unknown. Interestingly, however, the signaling pathway induced by this sensor is distinct from RIG-I and MDA5 signaling in that it does not result in activation of NF-κB transcription factor or MAP kinases, but does require IRF3 for the induction of interferon genes (Stetson and Medzhitov, 2006). Consistent with the different downstream signaling pathway, DNA-induced interferon production is MAVS independent (Kumar et al., 2006; Sun et al., 2006). The reason why dsRNA and DNA trigger different signaling pathways presumably has to do with the induction of distinct effector responses to RNA and DNA viruses. These two types of viruses differ dramatically with regards to their size and infection strategies, and so it is not surprising that different antiviral responses need to be induced to interfere with stages of the infection cycle unique to RNA or DNA viruses.

Interestingly, a potent type I interferon response is also induced by several intracellular bacterial infections. This has been most clearly demonstrated in the case of *Listeria monocytogenes* (O'Riordan et al., 2002), although other intracellular bacteria like *Shigella flexneri* and *Legionella pneumophila* induce interferon responses as well (Hess et al., 1987; Stetson and Medzhitov, 2006). It appears that the induction of IFN-β in *Listeria*-infected macrophages may be mediated by the DNA-recognition pathway (Stetson and Medzhitov, 2006). Consistent with this possibility, IFN-β induction by *Listeria* is TLR and nucleotide binding oligomerization domain (NOD) protein independent, but requires invasion of the host cell's cytosol (O'Connell et al., 2005; O'Riordan et al., 2002; Stockinger et al., 2004). In the case of *Listeria*, IFN-β production and type I IFN-activated signaling in lymphocytes makes the host more susceptible to infection (Auerbuch et al., 2004; Carrero et al., 2004, 2006; O'Connell et al., 2004). It is important to note that although type I IFNs appear to be detrimental to the host during *Listeria* infection, they are likely only one facet of the response activated by the sensor(s) that detect cytosolic *Listeria*; this pathway probably induces other cytokines and effector mechanisms, some of which might be beneficial to the host. Nonetheless, this finding is a dramatic example of context-dependent effects of type I IFNs in the course of infection, which appears to be a common theme in IFN biology, as discussed below. In the case of other bacteria that invade the host cytosol, it remains to be seen whether IFNs have a protective or detrimental effect at different stages of infection.

In summary, two distinct IFN-inducing systems are now known to exist: one is restricted to endosomes of specialized sentinel cells and mediated by TLRs, and the other is a ubiquitous cytosolic viral detection system mediated by RIG-I and MDA5 and a DNA sensor(s). In principle, any type of virus would be vulnerable to detection by both a TLR and a cytosolic sensor; this breadth of coverage is remarkable considering the staggering diversity of viruses and the comparably tiny number of receptors charged with sensing them. It would be interesting to determine whether retroviruses, which generate both RNA and DNA during their infectious cycle, are detected by sensors of both of these nucleic acids or whether one type of receptor predominates. Based on the recent insights into the receptor systems that sense nucleic acids and the generation of knockout mice lacking specific components of each pathway, we are now able to evaluate the relative contributions of each of these systems to antiviral immunity. What is beginning to emerge from these studies is a more integrated picture of the different ways in which type I IFNs facilitate antiviral defense through both cell-intrinsic and non-cell-autonomous mechanisms.

Cell-Intrinsic Antiviral Defense: The "Antiviral State"

The activity of type I IFNs was originally described almost 50 years ago as a soluble factor produced by cells treated with inactivated, nonreplicating viruses that blocked subsequent infection with live virus (Isaacs and Lindenmann, 1957; Isaacs et al., 1957; Nagano and Kojima, 1958). Interestingly, one group used the RNA influenza virus whereas the other used the DNA vaccinia virus, implying the existence of cellular sensors of both types of viral nucleic acids even in the first reports describing type I IFNs. As indicated in these initial studies, type I IFNs produced by infected cells act in an autocrine and paracrine manner to signal the presence of viral infection. By signaling through the type I IFN receptor, IFNs activate the inducible expression of hundreds of genes that together establish the "antiviral state" (van Boxel-Dezaire et al., 2006; Stark et al., 1998). Antiviral state is a generic term and perhaps belies the fact that we still have only an elementary understanding of what exactly it is. Indeed, there are entire families of IFN-inducible genes with completely unknown function, including several large GTPases and a series of proteins with tetratricopeptide repeat domains (IFITs) (Samuel, 2001). Although the function of each of these and other IFN-inducible genes awaits elucidation, it is safe to say that they act to cripple every stage of the viral life cycle. Some IFN-inducible proteins have broad antiviral effects. For example, 2′-5′ oligoadenylate synthases (OAS) are activated by viral dsRNA and produce 2′-5′ oligoadenylates, which in turn activate the latent nuclease,

RNase L, resulting in the degradation of viral RNA transcripts as well as host RNAs (Stark et al., 1998). Another well-studied IFN-induced antiviral effector is PKR, a member of the eukaryotic initiation factor 2α (eIF2α) kinase family. Activation of PKR by dsRNA leads to eIF2α phosphorylation with a consequent blockade of translation of most cellular and viral mRNAs. Interestingly, translation of mRNAs containing upstream open reading frames in their 5′ UTRs is enhanced upon eIF2α phsophorylation. Most known examples of proteins encoded by such mRNAs are restricted to the unfolded protein response and nutrient deprivation or stress conditions that activate other members of the eIF2α kinase family (Harding et al., 2002). Conceivably, these proteins may indirectly contribute to the antiviral state. However, one would expect that some of the bona fide antiviral effectors, including perhaps type I IFNs themselves, may also be translated preferentially when PKR is activated and eIF2α phosphorylated.

In addition to the relatively nonspecific antiviral activities of PKR and OAS-RNaseL systems, certain IFN-inducible genes may also exhibit specificity for distinct classes of viruses as implied by the existence of distinct sensors and signaling pathways that detect DNA or RNA within infected cells. Recent studies demonstrate that there are indeed IFN-inducible genes that are able to intercept specific types of viruses. The MX and guanylate binding proteins are large, IFN-inducible GTPases of the dynamin family. The MX proteins have been shown to have a protective effect against two RNA viruses, influenza and VSV (Arnheiter et al., 1990; Pavlovic et al., 1993). Although detailed mechanisms of MX and GBP functions await elucidation, their structural similarity with dynamin suggests that these proteins may interfere with viral assembly and trafficking in the cell.

Perhaps the best example of virus-specific functions of IFN-inducible genes and the most promising recent development in the search for novel HIV therapeutics is the recently discovered APOBEC3 family of cytidine deaminases. It was established several years ago that HIV deficient in the virion infectivity factor (vif) is only able to form infectious viral particles in certain cell lines (termed permissive) and not in human T cells and several other cell lines (termed nonpermissive) (Fisher et al., 1987; Gabuzda et al., 1992). The phenotype of the nonpermissive cells was interesting: they supported a single round of virus production, but the newly formed virions were unable to infect even permissive cells. Fusion of permissive and nonpermissive cells established that the nonpermissive phenotype was dominant and suggested that a cellular factor expressed only in nonpermissive cells could suppress infectivity of vif-deficient HIV (Madani and Kabat, 1998). Malim and colleagues used subtractive cDNA analysis to identify CEM15 (also known as APOBEC3G) as this factor and showed that it is incorporated into vif-deficient, but not wild-type, virions (Sheehy et al., 2002). Virion-incorporated APOBEC3G deaminates cytosine to uracil in reverse-transcribed retroviral DNA, generating catastrophic mutations and rendering viral DNA susceptible to cleavage by uracil DNA glycosylase and apurinic-apyrimidinic (AP) endonuclease enzymes (Harris et al., 2003; Mangeat et al., 2003). Interestingly, the deaminase activity of APOBEC3G is not required for its antiretroviral function (Newman et al., 2005), and cellular (non-virus-incorporated) APOBEC3G potently blocks vif$^+$ HIV infection in resting CD4$^+$ T cells (Chiu et al., 2005), suggesting that the regulation of APOBEC3G function may be more complex than initially suggested. Nonetheless, these findings provide a remarkable example of IFN-inducible genes targeting a specific class of viruses.

Although the effector proteins induced by type I IFNs are clearly an essential component of the antiviral state, mounting evidence suggests that these cannot mediate antiviral immunity on their own and instead cooperate with numerous other signaling pathways to activate the full complement of antiviral functions. For example, MAVS-deficient mice infected with VSV suffer severe mortality despite a strong systemic type I IFN response provided by TLR-dependent recognition in pDCs (Sun et al., 2006). Moreover, fibroblasts lacking Fas-associated death domain protein (FADD), an important signaling molecule in the RIG-I and MDA5 pathways, are unable to curtail VSV replication in vitro, even when pretreated with recombinant type I IFNs (Balachandran et al., 2004). Thus, although the paracrine function of type I IFNs is a very efficient way for infected cells to disseminate an alarm signal, these findings suggest that IFN-inducible effectors on their own are not sufficient to confer immunity and highlight the other key roles that type I IFNs play in coordinating the antiviral response.

Cell-Intrinsic Antiviral Defense: Type I IFNs and Apoptosis

It is well known that treatment of cells with type I IFNs sensitizes them to apoptosis upon subsequent viral infection (Stark et al., 1998). The rationale for this response is fairly simple: because viruses require host cell machinery to replicate, elimination of an infected cell would shut down this machinery and thus prevent viral spread. Many viruses have evolved mechanisms to interfere with host cell apoptosis, reflecting the importance of this process in antiviral defense (Seet et al., 2003).

How do interferons sensitize cells to apoptosis? Several overlapping mechanisms likely exist. PKR is required for apoptosis of cells in response to polyI:C transfection, which activates MDA5 (Balachandran et al., 2000). Paradoxically, PKR-deficient fibroblasts are far more sensitive to apoptosis when infected with VSV; this apoptotic response in PKR-deficient cells is correlated with an inability to curtail VSV replication and can be reversed by pretreatment of the cells with type I IFNs (Balachandran et al., 2000). The tumor suppressor p53 also may play an important role in virus-induced apoptosis. p53 is interferon inducible, p53-deficient cells are defective in VSV-induced apoptosis, and p53-deficient mice are highly susceptible to in vivo infection with VSV (Takaoka et al., 2003). It is also possible that signaling from the cytosolic nucleic acid receptors might cooperate with type I IFN signaling to activate apoptosis in infected cells. The observation that the RIG-I and MDA5 signaling adaptor MAVS is localized to the outer membrane of mitochondria suggests the interesting possibility that this pathway may interact with Bcl2 family members, which modulate apoptosis from the mitochondrial membrane (Seth et al., 2005).

The strong link between type I IFNs and cell-intrinsic apoptosis may be an important clue to the evolutionary

significance of why the type I IFN system exists in vertebrates and not in any other class of organisms. Perhaps it is because vertebrates are unique in the animal kingdom in that they use apoptosis as a potent form of host defense. When would the benefits of apoptosis (removal of an infected cell) outweigh the costs (loss of an important cell) enough to make it a useful form of defense? Only when tissue renewal can prevent impairment of physiological functions caused by irreplaceable cell loss. Most cells of adult invertebrate animals are postmitotic, which would make the deliberate removal of even a few key cells in invertebrates highly deleterious. The few examples of renewable tissue in invertebrates, such as the tentacles of octopi and starfish and the segments of earthworms, are clearly secondary adaptations and occur by de novo regeneration instead of by continuous replacement of individual cells over time. In contrast, most cells in vertebrate animals are renewable and continually replenished throughout adult life. There are exceptions to this general rule, including certain postmitotic neuronal populations, and the rate of cell renewal varies widely among tissues, with epithelial cells and hepatocytes at the faster end of the spectrum and tissues like heart muscle at the slower end. It would be interesting to determine whether a cell's utilization of the type I IFN system for apoptosis is directly proportional to its "renewability." This would imply that a postmitotic, irreplaceable neuron would respond to type I IFNs quite differently than an easily renewable epithelial cell; even the signaling downstream of the widely distributed cytosolic nucleic acid sensors might vary considerably depending on the type of infected cell. A deeper understanding of this specificity in the type I IFN system would have important implications for clinical treatment of viruses with cell type-specific tropism.

Interestingly, the other class of organisms that uses programmed cell death for defense also has renewable tissues: plants activate a hypersensitivity response to eliminate host cells surrounding sites of infection and prevent pathogen spread. Instead of type I IFNs, plants use inducible production of other types of rapidly diffusing soluble mediators to relay an alarm signal among their noncirculating cells (Nimchuk et al., 2003).

Non-Cell-Autonomous Roles of Type I IFNs

In addition to cell-intrinsic effects of type I IFNs that confer the antiviral state, the IFN system is linked to a variety of effector responses of the innate and adaptive immune systems. One characteristic shared by IFN-regulated effector responses is that their activation ultimately results in the elimination of infected cells. Thus, two major effector cell populations regulated by type I IFNs are natural killer (NK) cells and cytotoxic T cells (CTLs). In addition, type I IFNs have been shown to facilitate cross-presentation by DCs for presentation of viral antigens to $CD8^+$ T cells (Le Bon et al., 2003). Cytotoxic responses mediated by NK cells and CTLs are designed to eliminate infected cells, so it appears that a common feature of IFN activity is the defense against intracellular infections.

The mechanisms whereby type I IFNs contribute to NK and CTL responses are diverse and include production of chemokines that recruit the cytotoxic cells to the site of infection and induction of cytokines that positively regulate cytotoxic cell numbers and activities. For example, type I IFNs induce production of IL-15, which plays a critical role in proliferation and maintenance of NK cells and memory $CD8^+$ T cells (Nguyen et al., 2002a; Zhang et al., 1998). IFNs also directly activate NK cells to enhance their cytotoxic activity (Lee et al., 2000; Nguyen et al., 2002a).

A number of studies have demonstrated that type I IFNs also play an essential role in the differentiation and function of effector $CD8^+$ T cells. Murali-Krishna and colleagues explored the role of type I IFNs in the $CD8^+$ T cell response to infection with lymphocyte choriomeningitis virus (LCMV), a well-characterized model of $CD8^+$ T cell function and memory (Kolumam et al., 2005). They found that $CD8^+$ T cells lacking the type I IFN receptor were profoundly impaired in their ability to expand and differentiate into effector CTLs, demonstrating that type I IFNs provide a nonredundant costimulatory signal in vivo.

A study by Taniguchi and colleagues suggests a more context-specific role for type I IFNs in $CD8^+$ T cell differentiation. Knockout mice lacking interferon regulatory factor 7 (IRF7), a transcription factor required for TLR9-mediated type I IFN production in pDCs, were unable to mount a $CD8^+$ T cell response to protein antigens delivered with the TLR ligand CpG DNA as an adjuvant (Honda et al., 2005a). In contrast, $CD8^+$ T cell expansion and function in IRF7-deficient mice was normal when TLR2 ligands were used as the adjuvant. Acute depletion of pDCs in wild-type mice gave similar results and selectively impaired $CD8^+$ T cell responses when protein antigen was administered with TLR9 ligands but not when the same antigen was delivered with TLR2 ligands. TLR2 is expressed by conventional DCs and does not activate type I IFNs, so it is reasonable that the $CD8^+$ T cells that emerge after TLR2 activation would not require IFNs. However, TLR9 can also activate conventional DC maturation and production of inflammatory cytokines such as IL-12, yet this conventional DC-derived pathway is insufficient to compensate for loss of pDC-derived, TLR9-activated type I IFNs in IRF7-deficient mice. One interesting question raised by these findings is whether pDCs are the relevant antigen-presenting cells after protein and CpG DNA immunization or whether they provide type I IFNs in *trans* to $CD8^+$ T cells encountering peptide-MHC complexes on a different antigen-presenting cell. Another question with implications for vaccine development is whether this selective requirement for pDCs and type I IFNs revealed with TLR9 ligands extends to the RNA ligands for TLR7. If so, this would imply that TLR activation in pDCs drives the differentiation of a specialized type of $CD8^+$ effector T cells that is distinct from those that encounter antigens presented by conventional DCs. Together with the in vivo studies that use LCMV infection, it now appears that there are at least two types of $CD8^+$ T cells: those that require type I IFNs for expansion and function and those that do not. Thus, vaccines aiming to elicit protective $CD8^+$ T cell memory against viral infection should target the pDC-mediated TLR7 and TLR9 response, whereas vaccines against other pathogens like gram-positive bacteria should be designed to expand the type I IFN-independent subset of $CD8^+$ T cells. Because these two types of effector CTLs are generated by different pathways,

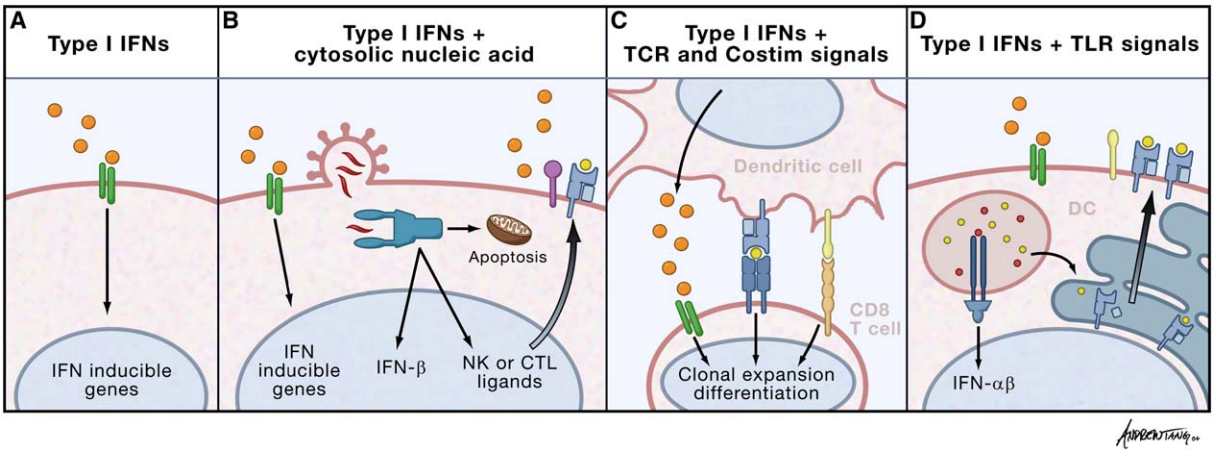

Figure 1. Context-Specific Actions of Type I IFNs

(A) In uninfected cells, type I IFN signaling in the absence of other cues would activate the expression of IFN-inducible genes that make cells more sensitive to detection and elimination of incoming virus.

(B) Infected cells integrate type I IFN signals with cytosolic nucleic acid recognition to activate cell-intrinsic apoptosis and the expression of ligands that allow NK cells and CTLs to distinguish an infected cell that must be eliminated from its uninfected neighbors.

(C) For $CD8^+$ T cells encountering MHC-peptide antigens in the context of viral infection, T cell receptor and costimulatory receptor signaling cooperates with type I IFNs to drive their clonal expansion and differentiation. This function of type I IFNs is quite different from its usual role in establishing the antiviral state, which slows host cell metabolism.

(D) In dendritic cells encountering nucleic acid TLR ligands, autocrine or paracrine type I IFNs facilitate crosspresentation of MHC class I peptides derived from endocytosed viral particles or apoptotic, virally infected cells.

it is likely that type I IFN-dependent memory CTLs may not be efficient against a pathogen that does not trigger type I IFN production; conversely, the IFN-independent CTLs would be unable to provide protective immunity to viral infection.

Interestingly, the effect of type I IFNs on $CD8^+$ T cells is dependent on the stage of $CD8^+$ T cell differentiation. Whereas type I IFNs limit nonspecific $CD8^+$ T cell expansion in a STAT1-dependent manner, they promote antigen-specific expansion and IFN-γ production by CTLs in a STAT4-dependent fashion (Nguyen et al., 2002b). Thus, the relative abundance of STAT1 and STAT4 in $CD8^+$ T cells determines the outcome of their exposure to IFNs. This context-dependent effect of type I IFNs is characteristic of their activity, which we will discuss in the next section.

Context-Specific Actions of Type I IFNs

An emerging principle in type I IFN biology is the realization that type I IFNs are part of a contextual language that can mean different things to different cells or even within the same cell depending on the presence of other cues. A simplified model that summarizes some of the contextual meanings of type I IFNs discussed above is depicted in Figure 1. A cell encountering type I IFNs in the absence of other cues will activate the classical antiviral state by upregulating expression of interferon-inducible genes (Figure 1A). These genes include nucleic acid sensors like RIG-I and MDA5; increased abundance of these sensors would make cells surrounding an infected cell more sensitive to detection of virus. Within an infected cell, type I IFN signaling can pair with cell-autonomous detection of viral nucleic acids (Figure 1B). It is likely that these two pathways collaborate to distinguish an infected cell from its uninfected neighbors in at least two ways. First, these two pathways may synergize to activate cell-intrinsic apoptosis in infected cells. Second, they may result in modulation of cell-surface ligands that instruct NK cells and CTLs to kill an infected cell that cannot commit apoptosis on its own. Importantly, the type I IFNs in this case need not be autocrine and could be supplied in *trans* by pDC-derived systemic IFNs; however, cell-intrinsic nucleic acid recognition must be a prerequisite for apoptosis and modulation of NK and CTL ligands.

Paradoxically, in $CD8^+$ T cells receiving appropriate T cell receptor and costimulatory signals, type I IFN signaling is essential for the massive expansion and differentiation of virus-specific effector CTLs (Figure 1C). A similar situation exists in NK cells, where type I IFN signaling collaborates with activating NK receptors to enhance NK cell-mediated cytotoxicity. It is understandable that virus-specific CTLs would be placed under the control of cytokines that specifically announce the presence of virus. But what would happen if the $CD8^+$ T cells were themselves infected? This model suggests that cell-intrinsic viral detection would revert infected $CD8^+$ T cells to the state depicted in Figure 1B and implies that cell-intrinsic nucleic acid detection is dominant over activation and differentiation cues when paired with type I IFN signals. This cell-autonomous antiviral state would arrest the cell cycle, block host cell mRNA translation, and drive cells to commit apoptosis, thus preventing the unwanted scenario of infected cells increasing their numbers. Importantly, "bystander" activation of $CD8^+$ T cells by type I IFNs in the absence of a TCR and costimulatory signal would revert them to a conventional antiviral state.

Finally, type I IFN signaling in DCs, when paired with a TLR signal, would favor crosspresentation of antigens within the TLR-containing phagosome (Figure 1D). During crosspresentation, viral antigens from infected cells

or viral particles engulfed by DCs access the MHC class I loading machinery. The fact that type I IFNs both facilitate this presentation of virus-derived peptides and also are essential for differentiation of the $CD8^+$ T cells specific for these crosspresented antigens may provide a dual safeguard against inappropriate expansion of non-antigen-specific CTLs.

There is important clinical relevance to the contextual language of type I IFNs. For example, vaccines designed to combine a TLR signal with type I IFNs would likely have a different effect than vaccines that also activate a cytosolic nucleic acid sensor. Additionally, viruses are known to target both type I IFN receptor signaling as well as the signaling machinery that links cytosolic nucleic acid recognition to activation of type I IFNs (Foy et al., 2003; Garcia-Sastre and Biron, 2006). Based on the pairing of these two pathways in infected cells, therapies that would restore both of these functions would be far more effective than those that restore either IFN signaling or nucleic acid detection. In the case of hepatitis C virus, inhibition of the virus-encoded NS3/4A protease that cleaves and inactivates MAVS would reactivate both functions in infected cells (Loo et al., 2006; Meylan et al., 2005). As we continue to develop our knowledge of the different pathways that cooperate with type I IFNs to achieve diverse outcomes, we will be in a position to better understand the subtleties of this contextual language and use it for therapeutic benefit.

Conclusions and Perspectives

Several years of remarkable progress have illuminated the many different points at which type I IFNs are integrated into host defense. With this progress, a number of basic principles that govern type I IFN function have emerged. Two general features unite the receptors that activate production of type I IFNs. First, these receptors coordinate the immune response against intracellular pathogens, including viruses and probably cytosolic bacteria. Second, nucleic acid recognition appears to underlie most of an organism's ability to activate type I IFNs. We can say this with fair certainty for RNA virus detection based on the recent characterization of the RIG-I- and MDA5-signaling pathways. Whether the same principle holds true for recognition of DNA viruses and intracellular bacteria remains to be seen. If true, then the source of type I IFNs that play a pathogenic role in many autoimmune settings must be an aberrant recognition of self nucleic acids by these sensors, a cost of this imperfect recognition strategy that detects pathogen components that are also present in the host (the role of type I IFNs in autoimmunity will be discussed in an accompanying review; Banchereau and Pascual, 2006).

The sensors that activate type I IFNs probably have other important functions as well. This is certainly true for TLRs expressed by DCs, which are integrated into a number of key processes including antigen presentation (and crosspresentation) and inducible expression of other molecules that initiate the adaptive immune response. The cell-intrinsic cytosolic sensors might also have diverse functions beyond activation of type I interferons, including induction of apoptosis and modulation of ligands that flag infected cells for elimination by NK cells and CTLs. An important area of future research will be the precise elucidation of these IFN-independent functions and all of the signaling pathways activated by these receptors.

In conclusion, the pace of recent advances in our understanding of how type I IFNs operate in immunity suggests that major breakthroughs in vaccine development and clinical treatment of viral infections are more attainable now than ever before. The discovery of many of the receptors that activate type I IFNs along with the details of antiviral signaling pathways provides a fertile new ground for development of strategies to enhance or intercept these responses in the event of pathogen infection or autoimmunity, respectively.

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