immune responses. Rather many viruses will have evolved mechanisms to attenuate innate immunity only to such a degree that their spread to new hosts is facilitated.

See also: Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity; Innate Immunity: Introduction.

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Innate Immunity: Introduction

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Glossary

Apoptosis A form of programmed cell death.

Apoptotic bodies Remnants of cells which underwent apoptosis.

Complement system A pathogen-triggered cascade of biochemical reactions involving more than 20 soluble and cell-bound proteins. Complement activation results in opsonization, priming of humoral immune responses, and perforation of membranes.

Cytokines Proteins which mediate cell–cell communication related to pathogen defense. Secreted by immune cells or tissue cells.

Innate immunity Physical and chemical barriers, cells, cytokines, and antiviral proteins which exclude, inhibit, or slow down infection with little specificity and without adaptation or generation of a protective memory.

Interferons (IFNs) Cytokines mediating antiviral activity. Distinguished into type I (IFN-α/β), type II (IFN-γ), and type III (IFN-λ). Type I and type III IFNs directly mediate antiviral activity in responding cells, whereas type II IFN is more immunomodulatory.

Interferon-stimulated response element (ISRE) A promoter element common to all type I IFN-stimulated genes.

Opsonization Tagging of infected cells or pathogens for destruction by phagocytic cells.

Pathogen-associated molecular patterns (PAMPs) Molecular signatures of pathogens used by the innate immune system to distinguish self from non-self. Often highly repetitive patterns.

Pattern recognition receptors (PRRs) Intracellular and extracellular receptors recognizing specific PAMPs.

Phagocytosis Uptake of particles by cells.

Introduction

Viruses attempting to conquer a mammalian body are faced with an array of problems. 'Innate immunity' in a wider sense means all sorts of factors which exclude, inhibit, or slow down infections in a rapid manner but with little specificity and without adaptation or generation of a protective memory. Many of these efficient and not at all primitive defenses are evolutionarily old and can be found in all metazoans. For the sake of brevity, however, the discussion in this article is restricted to mammals as these are the best investigated organisms in that respect. RNA interference, the innate immune system of plants and nonvertebrates, is not covered here.

Mammalian innate immune defenses against virus infections can be divided into several distinct parts such as mechanical and chemical barriers (not further mentioned here), complement system, phagocytic/cytolytic cells of the immune system which act in a nonspecific manner, and cytokines (most prominently the type I interferons).

The Complement System

The complement system (which 'complements' the adaptive immune system in the defense against pathogens) primes the adaptive immune response and is also directly effective against pathogens. Complement activation is achieved by specific receptors recognizing pathogens or immunocomplexes. Three different pathways are being distinguished which are termed the classical pathway (triggered by antigen–antibody complexes), the mannan-binding lectin pathway (triggered by lectin binding of pathogen surfaces), and the alternative pathway (triggered by complement factor C3b-coated pathogen surfaces). They all activate a cascade of reactions involving more than 20 soluble and cell-bound proteins, thus resulting in a rapid and massive response. The complement system is able to (1) tag infected cells and pathogens for destruction by phagocytic cells (opsonization), (2) prime humoral immune responses, and (3) perforate membranes of infected cells by the membrane-attack complex. In response, viruses have evolved effective countermeasures such as incorporation of cellular complement-regulatory proteins into particles or expressing specific inhibitors in infected cells.

Cellular Innate Immunity

Macrophages/monocytes, granulocytes, natural killer cells, and dendritic cells belong to the cellular branch of the innate immune system. Monocytes circulate in the bloodstream for several hours before they differentiate into macrophages. These potent phagocytic cells either continue patrolling or they permanently settle in particular tissues (i.e., the Kupffer cells of the liver), being able to rapidly remove viral particles and apoptotic bodies. Activated macrophages also synthesize inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, thus triggering an adaptive immune response. Granulocytes are also able to remove viral particles and apoptotic bodies by phagocytosis. They are rapidly attracted to inflammatory sites and enter the tissue by transendothelial migration. Both macrophages and granulocytes cleave the ingested viral proteins into fragments and present them to T lymphocytes.

Natural killer (NK) cells are able to recognize infected cells in an antigen-independent manner and destroy them by their cytotoxic activity. Also, they rapidly produce large amounts of IFN-γ to activate the adaptive immune system. NK cells are regulated by a fine balance between stimulatory and inhibitory receptors. One of their prominent features is their ability to destroy cells which lack MHC I molecules on their surface. As many viruses downregulate MHC expression in order to avoid an adaptive immune response, NK surveillance represents an important early warning and attack system against virus infections.

A key connection between the innate and the adaptive immune system is provided by dendritic cells (DCs). These specialized immune cells sample antigen at the site of infection, activate themselves and the surrounding tissue cell by cytokine synthesis, and then migrate to secondary lymphatic organs in order to mobilize T cells against the presented antigen. The differentiation into efficient antigen-presenting cells (APCs) is achieved by cytokine production which, in turn, is triggered by stimulation of receptors recognizing pathogen-specific molecular patterns (PAMPs). Two main types of DCs are being distinguished: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are an early split-off of the myeloid bone marrow precursors, that is, the stem cells which are also giving birth to macrophages/monocytes and granulocytes, among others. Depending on the location, several subsets of mDCs such as Langerhans cells or interstitial cells are being distinguished. pDCs, which are not segregated into subpopulations, are thought to be derived from lymphatic precursor cells. Both mDCs and pDCs can sense viral infection by several intra- and extracellular PAMP receptors (see below). Depending on the DC type, high levels of interleukins or interferons are being produced which coin the subsequent immune reaction. pDCs are potent producers of the main antiviral cytokines, the type I interferons.

Antiviral Cytokines: The Type I Interferons

Isaacs and Lindenmann discovered in 1957 that cells which had been in contact with virus particles secrete a soluble factor which confers resistance to influenza viruses, a phenomenon called 'interference'. In the subsequent years, it became more and more clear that the so-called type I interferon (IFN-α/β) system is our primary defense mechanism against viral infections. In fact, humans with genetic defects in the IFN signaling pathway have a bad prognosis as they die at an early age of viral diseases which would otherwise pose little problems. Similarly, knockout mice with a defective IFN system quickly succumb to viral pathogens of all sorts although they have an intact adaptive immune system.

In response to virus infection, pDCs are particularly well equipped to synthesize and secrete IFN-α/β, but in principle all nucleated cells are able to do so. In an autocrine and paracrine manner, IFNs trigger a signaling

chain leading to the expression of potent antiviral proteins which limit further viral spread. In addition, IFNs initiate, modulate, and enhance the adaptive immune response. The signaling events which culminate in the direct IFN-dependent restriction of virus growth can be divided into three steps, namely (1) transcriptional induction of IFN synthesis, (2) IFN signaling, and (3) antiviral mechanisms.

Interferon Induction

A number of pattern recognition receptors (PRRs) recognize conserved PAMPs of viruses and initiate induction of IFN genes (see **Figure 1**). PRRs can be divided into the extracellular/endosomal toll-like receptors (TLRs) and the intracellular receptors RIG-I, MDA-5, and PKR. The main PAMPs of viruses appear to be nucleic acids, namely double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and double-stranded DNA (dsDNA).

dsRNA is an almost ubiquitous transcriptional by-product of RNA and DNA viruses. It is recognized by TLR3, the related RNA helicases RIG-I and MDA-5, and the protein kinase PKR. A third dsRNA-binding member of the RIG-I helicase family, LGP2, acts as a negative-feedback inhibitor.

Viruses with a negative-strand ssRNA genome (e.g., influenza virus) are unique in that they do not produce substantial amounts of intracellular dsRNA. Their genomic ssRNA is recognized in the endosome by TLR7 and -8. Interestingly, in the cytoplasm, RIG-I recognizes
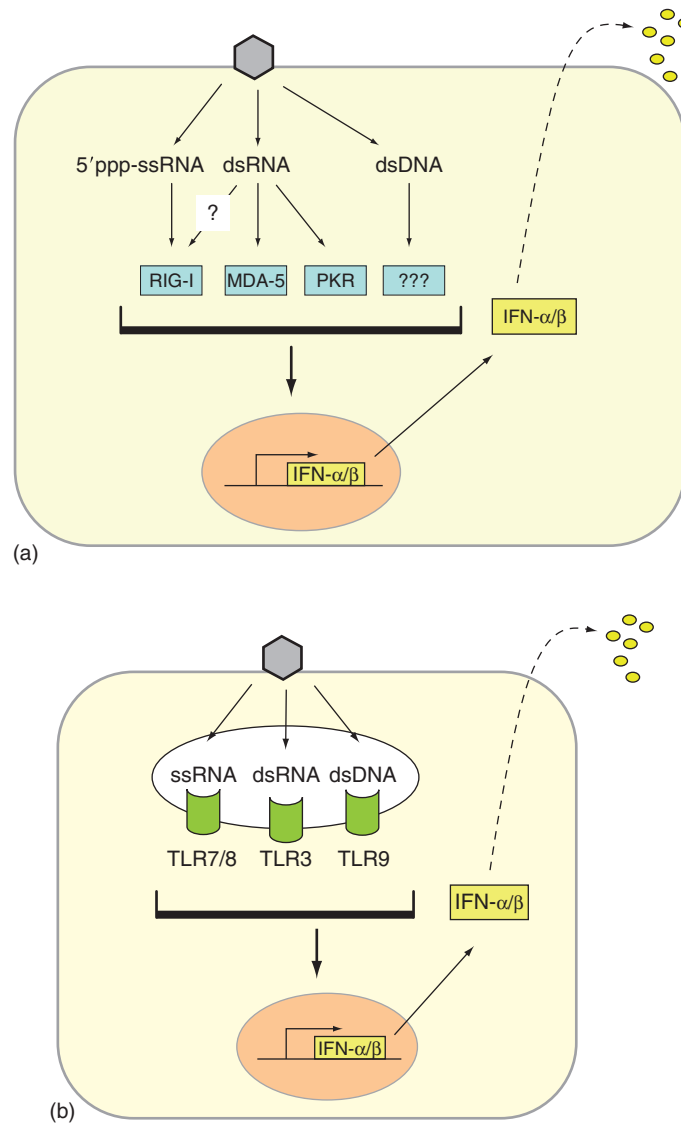

Figure 1 Depending on the virus, ssRNA, dsRNA, dsDNA, or combinations thereof represent characteristic by-products of infection which lead to induction of IFN-α/β genes. (a) These signature molecules are recognized by the intracellular PRRs RIG-I, MDA-5, PKR, and an unknown receptor for viral dsDNA. RIG-I recognizes dsRNA, but was shown to be important for recognition of 5′-triphosphate-containing ssRNA *in vivo* (see text). (b) Intracellular PAMP recognition is mirrored by the endosomal TLR pathways recognizing the same characteristics, except that ssRNAs do not need to be 5′-triphosphorylated.

the influenza virus genome in a 5′-triphosphate-dependent manner. The question how much the well-documented dsRNA-binding and unwinding activity of RIG-I contributes to its 5′-triphosphate-dependent recognition of viral genomes remains to be solved.

The third important PAMP, viral dsDNA, is again recognized both by an endosomal receptor, TLR9, and an unknown intracellular receptor. Thus, for all three nucleic acid-based PAMPs of viruses, there are specific PRRs present both in the endosomal and the intracellular compartment.

Besides nucleic acids, some viral proteins can provoke a TLR response such as the envelope proteins of respiratory syncytial virus and measles virus by activating TLR4 and TLR2, respectively.

All PRR-triggered signaling pathways eventually culminate in a strong activation of type I IFN transcription. The 'classic' intracellular pathway of IFN-β gene expression involves RIG-I and MDA-5 which both contain two N-terminal caspase-recruiting domain (CARD)-like regions and a C-terminal DExD/H box RNA helicase domain (**Figure 2(a)**). RNA binding to the helicase domain
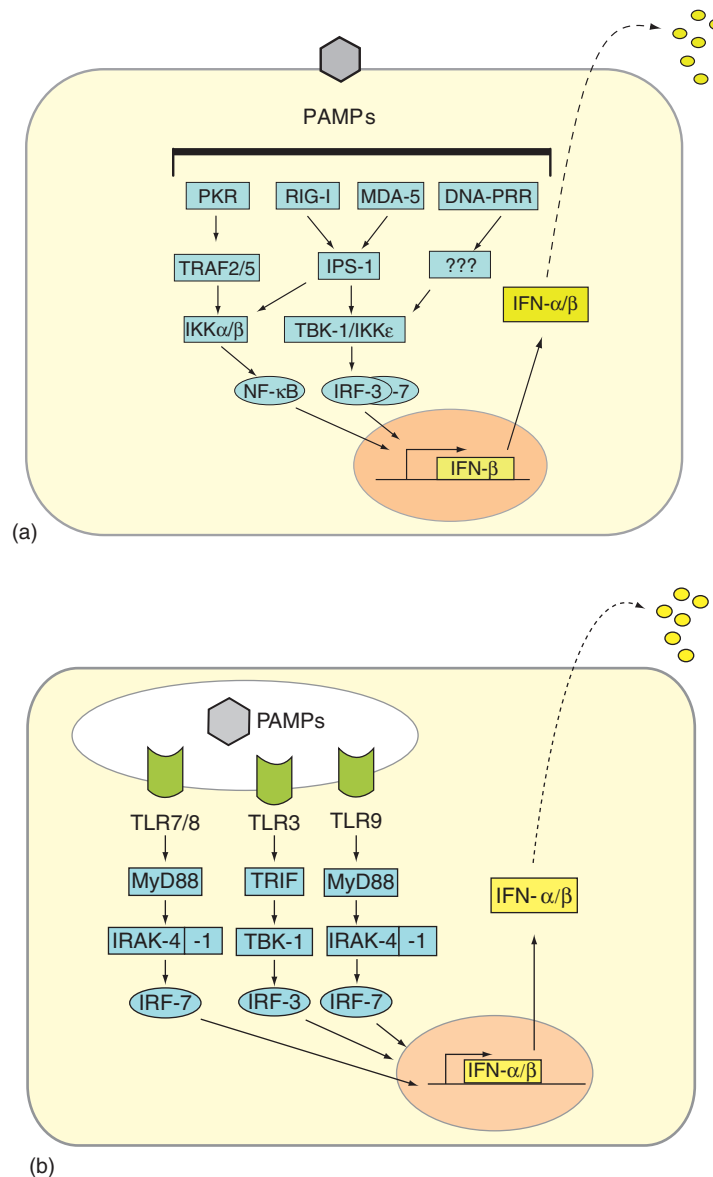

Figure 2 (a) PAMP recognition by intracellular PRRs leads to activation of the transcription factors NF-κB, IRF-3, and AP-1 (not shown). The cooperative action of these factors is required for full activation of the IFN-β promoter. IRF-3 is phosphorylated by the kinases TBK-1 and IKKε which in turn are activated by RIG-I and MDA5 via IPS-1. NF-κB is activated by the PKR pathway as well as by IPS-1. The IFN-induced IRF-7 later enhances IFN gene transcription, but is also essential for immediate early IFN-β transcription. (b) PAMP recognition by endosomal PRRs. IRF-7 is activated by IRAK-1, which in turn is phosphorylated by IRAK-4 in an MyD88-dependent manner. Both TLR7/8 and TLR9 use the MyD88 adaptor, whereas TLR3 activates IRF-3 via TRIF and TBK1.

induces a conformational change which liberates the CARD domain to interact with the signaling partner IPS-1 (also called Cardif, MAVS, or VISA). This adaptor mediates RIG-I and MDA-5 signaling and needs to be located at the mitochondrial membrane. IPS-1 has a CARD-like domain which binds to RIG-I and MDA5 and a C-terminal region which activates the kinases IKKε and TBK-1. These kinases are known to phosphorylate the transcription factor IFN regulatory factor (IRF)-3, a member of the IRF family. Phosphorylated IRF-3 homodimerizes and is transported into the nucleus. In addition, the transcription factor nuclear factor-kappa B (NF-κB) is recruited in a PKR/TRAF- and IPS-1-dependent way. Together, IRF-3 and NF-κB strongly upregulate IFN gene expression. This leads to a 'first wave' of IFN production (IFN-β and IFN-α4 in mice) which triggers the expression of the transcription factor IRF-7. Recent evidence has shown that IRF-7 is a master regulator of IFN gene expression and that IRF-3 seems to cooperate with IRF-7 for full activity. IRF-7 can be activated in the same way as IRF-3 and is responsible for a positive-feedback loop that initiates the synthesis of several IFN-α subtypes as the 'second-wave' IFNs.

mDCs can sense dsRNA by the classic intracellular pathway and, in addition, by TLR3 (**Figure 2(b)**). dsRNA-induced triggering of endosomal TLR3 proceeds via TRIF and TRAF3 which activate the kinase TBK-1, leading to phosphorylation of IRF-3 and, subsequently, to the activation of IFN-β gene expression.

pDCs sense the presence of viral ssRNA or dsDNA by TLR7, TLR8, and TLR9 (**Figure 2(b)**). Upon activation, TLR7, -8, and -9 signal through their adaptor molecule MyD88, the IRAK kinases, and IRF-7 to transcriptionally activate multiple IFN-α genes. In contrast to other cell types, pDCs contain considerable amounts of constitutively expressed IRF-7. IRF-7 is further upregulated in response to IFN and generates a positive-feedback loop for high IFN-α and IFN-β production. Furthermore, TLR7 and TLR9 are retained in the endosomes of pDCs to allow prolonged IFN induction signaling.

Type I IFN Signaling

IFN-β and the multiple IFN-α subspecies activate a common type I IFN receptor (IFNAR) which signals to the nucleus through the so-called JAK–STAT pathway (**Figure 3**). The STAT proteins are latent cytoplasmic transcription factors which become phosphorylated by the Janus kinases JAK-1 and TYK-2. Phosphorylated STAT-1 and STAT-2 recruit a third factor, IRF-9, to form a complex known as IFN-stimulated gene factor 3 (ISGF-3) which translocates to the nucleus and binds to the IFN-stimulated response element (ISRE) in the promoter region of interferon-stimulated genes (ISGs). Specialized proteins serve as negative regulators of the JAK–STAT pathway. The suppressor of cytokine signaling
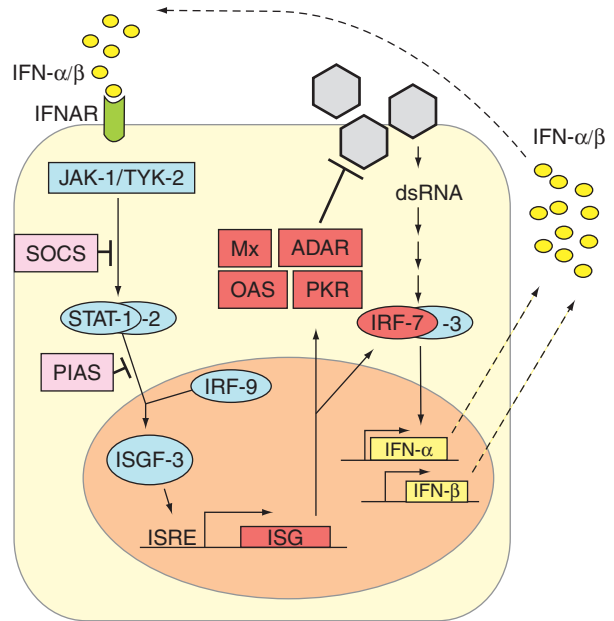

Figure 3 IFN-α and IFN-β bind to the type I IFN receptor (IFNAR) and activate the expression of numerous ISGs via the JAK/STAT pathway. IRF-7 amplifies the IFN response by inducing the expression of several IFN-α subtypes. Mx, ADAR, OAS, and PKR are examples of proteins with antiviral activity. Modified from Haller O, Kochs G, and Weber F (2006) The interferon response circuit: Introduction and suppression by pathogenic viruses. *Virology* 344: 119–130, with permission from Elsevier.

(SOCS) proteins prevent STAT activation whereas protein inhibitor of activated STAT (PIAS) family members function as small ubiquitin-like modifier (SUMO) E3 ligases and inhibit the transcriptional activity of STATs.

Direct Antiviral Effects of Type I IFNs

Type I IFNs activate the expression of several hundred IFN-stimulated genes (ISGs) with multiple functions. To date, five antiviral pathways have been studied in great detail, namely the protein kinase R (PKR), the RNA-specific adenosine deaminase 1 (ADAR 1), the 2–5 OAS/RNaseL system, the product of the ISG56 gene (p56), and the Mx proteins. PKR, ADAR1, and 2–5 OAS are constitutively expressed in normal cells in a latent, inactive form. Basal mRNA levels are upregulated by IFN-α/β and these enzymes need to be activated by viral dsRNA. PKR is a serine-threonine kinase that phosphorylates – among other substrates – the α-subunit of the eukaryotic translation initiation factor eIF2. As a consequence, translation of cellular and viral mRNAs is blocked. PKR also plays a role in virus-induced NF-κB activation, as described above. ADAR 1 catalyzes the deamination of adenosine on target dsRNAs to yield inosine. As a result the secondary structure is destabilized due to a change from an AU base pair to the less stable IU base pair and mutations accumulate within the viral

genome. The 2–5 OAS catalyzes the synthesis of short $2'$–$5'$ oligoadenylates that activate the latent endoribonuclease RNaseL. RNaseL degrades both viral and cellular RNAs, leading to viral inhibition. P56 binds the eukaryotic initiation factor 3e (eIF3e) subunit of the eukaryotic translation initiation factor eIF3. It functions as an inhibitor of translation initiation at the level of eIF3 ternary complex formation and is likely to suppress viral RNA translation. Mx proteins belong to the superfamily of dynamin-like large GTPases and have been discovered as mediators of genetic resistance against orthomyxoviruses in mice. They most probably act by enwrapping viral nucleocapsids, thus preventing the viral polymerase from elongation of transcription.

The antiviral profiles of the IFN effectors listed above are distinct but often overlapping. Mx proteins, for example, mainly inhibit segmented negative-strand RNA viruses and also Semliki Forest virus, whereas the 2–5 OAS/RNaseL system appears more important against positive-strand RNA viruses. Moreover, only rarely the presence of one particular IFN effector determines host resistance. Rather, it is the sum of antiviral factors affecting, for example, genome stability, genetic integrity, transcription, and translation that confers the full antiviral power of IFN.

Indirect Antiviral Effects of Type I IFNs

Besides the effector proteins listed above, several ISGs contribute in a more indirect manner to the enhancement of both innate and adaptive immune responses. Virus-sensing (and in part antiviral) PRRs such as TLR3, PKR, RIG-I, and MDA5 are by themselves upregulated in a type-I-IFN-dependent manner. Similarly, IRF-7 and and STAT1, the key factors of type I IFN and ISG transcription, respectively, are ISGs. The strong positive-feedback loop mediated by the upregulation of these PRRs and transcription factors is counterbalanced by several negative regulators such as LGP2, SOCS, and PIAS, which are either ISGs or depend on IFN signaling for their suppressive action.

Type I IFNs can directly enhance clonal expansion and memory formation of CD8$^+$ T cells. Also, IFNs promote NK cell-mediated cytotoxicity and trigger the synthesis of cytokines such as IFN-γ or IL-15 which modulate the adaptive immune response, enhance NK cell proliferation, and support CD8$^+$ T-cell memory. Moreover, by upregulating TLRs, MHCs, and costimulatory molecules, IFNs enable APCs (most prominently DCs) to become competent in presenting viral antigens and stimulating the adaptive immune response.

Good Cop–Bad Cop

Given their massive impact on the cellular gene expression profile, it is quite expected that type I IFNs have not only antiviral, but also antiproliferative and immunomodulatory effects. Treatment with IFNs is an established therapy against several viral and malignant diseases such as hepatitis B, hepatitis C, Kaposi's sarcoma, papillomas, multiple sclerosis, and several leukemias and myelomas. However, the strong and systemic effects of IFNs do not come without a price. Administration of IFN can locally produce inflammation, and systemically cause fever, fatigue, malaise, myalgia, and anemia. It is no coincidence that these latter are 'flu-like' symptoms, since in many acute infections IFNs play a dominant role. The effects of IFN which are desired and beneficial if restricted to the site of first infection can turn into a life-threatening 'cytokine storm' if it becomes systemic. Severe acute respiratory syndrome (SARS) and human infections with H5N1 influenza viruses are examples of such out-of-control innate immune responses. Another 'dark side' aspect is that patients with autoimmune diseases have chronically elevated levels of IFNs, and that IFN therapy can aggravate autoimmune disorders. It is thought that pDCs (and in part B cells) are autostimulated by self-DNA via TLR9 and by small nuclear RNA complexes (snRNPs) via TLR7. Chronic production of IFNs causes maturation of mDCs, which in turn activate autoreactive T and B cells.

Concluding Remarks

The concept of innate immunity certainly comprises more than the IFN system (see above), but type I IFNs represent a central part. These cytokines not only have direct antiviral effects but also orchestrate the first defense reactions and the subsequent adaptive immune response, thus determining the course of infection. The recent findings that basically every virus appears to have evolved one or several countermeasures for controlling the IFN response is testament to its importance. In addition, IFNs are not only antiviral, but also effective tumor suppressors. Tumor cells often eliminate the IFN system during the transformation process. The payoff is an increased susceptibility to infection, an Achilles heel which is exploited by the therapeutic concept of oncolytic viruses. Tumor selectivity of such viruses can be even more increased by using IFN-sensitive mutants. The inability of those mutants to fight the IFN response is complemented by the mutations of the tumor cells, thus allowing virus growth. At the same time, these viruses are unable to infect the IFN-competent body cells.

Recently, it became apparent that there exists a hitherto unnoticed parallel world called the type III IFN system. The cytokines IFN-λ1, -λ2, and -λ3 are induced by virus infection or dsRNA and signal through the JAK/STAT cascade, but use a separate common receptor. They are able to activate antiviral gene expression and have been shown to inhibit replication of several viruses.

Thus, the IFN response has a backup system to enforce the first line of defense against virus infections.

Future studies will have to address the relative contribution of type I and type III IFNs to antiviral protection and the coming years may have even more surprises in stock. The innate immune system may be old, but as long as there are viruses and tumors, it will never come out of fashion.

See also: Immune Response to viruses: Antibody-Mediated Immunity; Innate Immunity: Defeating; Interfering RNAs; Polydnaviruses: Abrogation of Invertebrate Immune Systems.

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Inoviruses

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Glossary

Everted DNA Inside-out DNA helix with phosphates at the center.
Integrative phage A phage capable of having its genome integrated into that of its host.
Productive state Of a bacterium actively exporting progeny filamentous phage without lysis.
Prophage state Of a bacterium with a repressed phage genome inserted in its genome or in one of its plasmids.

Discovery, Ecology, and Host Range

In 1960, T. Loeb reported finding phages that formed plaques on *Escherichia coli* carrying the F transfer factor but not on those without it. His finding was confirmed by workers around the world, and publications in 1963 by H. Hoffmann-Berling and D. A. Marvin, by Loeb and N. Zinder and co-workers, and by H. P. Hofschneider established that such plaque formers, independently isolated, were small spherical RNA phages (fr, f2, and M12), and small filamentous DNA phages (fd, f1, and M13), the latter now collectively referred to as Ff phage (**Figure 1**). The capital F is for the F factor specifying the host, and the small f stands for filamentous. These new DNA viruses had intriguing characteristics. Surprisingly, they did not lyse their hosts in culture yet formed the plaques on bacterial lawns, their filamentous virions contained DNA genomes that were both single stranded and circular, and it was observed, by E. Trenkner, that capsid protein of the infecting phage is incorporated into progeny virions. The fd, f1, and M13 genomes were among the first viral genomes sequenced. They each have either 6407 or 6408 nucleotides encoding the same 11 genes but fewer than 100 nucleotide differences even though the isolates were from different places in the world. This near constancy seemed remarkable, and has become more so in view of the plasticity of the Ff genome made evident in a plethora of subsequent mutagenesis studies.

Now over 50 different species of filamentous DNA phages are known, and their bacterial hosts occupy many habitats. Taxonomically the phages comprise the genus *Inovirus* in the family *Inoviridae,* the terms coming from the Greek word for filament. Infections by many