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Lecturer Affiliation:

Ammar M. Al-Aalim

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Summary:

1- Learning the interferons
2- The importance and function
3- Pathways of its activation
4- The role it plays in virus infection

Innate Immunity

Rapid, powerful innate immune responses limit many viral infections. Interferons are especially important in this process. Lysozyme can destroy some viruses, as can many intestinal enzymes and bile. C-type lectins bind to viral glycoproteins and block virus interaction with host cells. For example, conglutinin, mannose binding lectin and the surfactant proteins A and D can all inactivate influenza viruses. Defensins from leukocytes and mucosal epithelial cells play a dual role in antiviral defenses since they can act both on the virus and in the host cell. Thus, defensins can inactivate enveloped virions by disrupting their envelopes or by interacting with their glycoproteins. Some defensins can block intracellular signalling pathways in infected cells and interfere with transcription of viral RNA. Finally, cells invaded by viruses may undergo premature apoptosis, preventing successful viral invasion and replication.

Pattern-Recognition Receptors

Viruses, unlike bacteria and fungi, do not contain easily recognizable microbe-specific structures since they are constructed from host-derived components. For this reason, animal cells have evolved the ability to recognize their only virus-specific components, their nucleic acids.

Three pattern-recognition receptor systems recognize viral nucleic acids. One system consists of RIG like receptors found within the cytosol of all nucleated cells. These proteins detect viral dsRNA and then signal through several adaptor proteins to activate the interferon-β (IFN-β) gene. The second system is mediated by toll-like receptors (TLRs). TLR3 recognizes dsRNA. TLR7 and TLR8 recognize single-stranded RNA viruses such as vesicular stomatitis and influenza viruses. TLR9 detects unmethylated CpG motifs in DNA. These motifs are common in both DNA viruses and bacteria. Mice deficient in either TLR7 or TLR9 or their adaptor protein MyD88 have a reduced ability to defend themselves against viruses. Plasmacytoid dendritic cells (pDCs) use a specialised signalling pathway that links TLR7 and TLR9 to the production of very large amounts of type I interferons. The third PRR system uses receptors with nucleotide binding oligomerization (NOD)-like domains.

Interferons

Interferons protect cells against viral, bacterial, and protozoan invasion. They are glycoproteins of 20 to 34 kDa classified into three types: I, II, and III. Type I interferons include multiple forms of IFN- α and IFN-β, as well as single gene products such as IFN-ω, -δ, -ε, - ν, -τ, -κ, and -ζ. There are 18 isoforms of IFN-α in humans, 12 in pigs and cattle, 4 in horses, and 2 in dogs. IFN-α is produced in large quantities by pDCs and in much smaller amounts by lymphocytes, monocytes, and macrophages. IFN-β can be produced by almost any virus-infected cell. (There are five isoforms in cattle and pigs and one in dogs and humans.) IFN-ω is produced by lymphocytes, monocytes, and human, horse, pig, rabbit, and dog trophoblast cells (eight functional genes in pigs, one in humans, two in horses, 15–20 in cattle, 13 in cats, and none in dogs or mice). Another type I interferon, IFN-τ, is found in the ruminant trophoblast (3–5 genes). IFN-δ is found in the placental tissues of pigs, sheep, and horses (two in horses). IFN-δ is only distantly related to the other type I interferons. IFN-κ is produced by keratinocytes. Bovine IFN-κ has been characterized and acts through JAK/STAT pathways like the other type I interferons. IFN-ζ is found in mice where it is also called limitin. IFN-ε is a member of the type I family whose expression is limited to reproductive and brain tissues. It plays a role in protecting the female reproductive tract. In most cases, these molecules act on virus-infected cells to inhibit viral growth. The trophoblast interferons also regulate the maternal immune response to the fetus.

There is only one type II interferon, IFN-γ, produced by antigen-stimulated Th1 cells. It is also produced in the pig trophoblast.Four type III interferons have been identified, IFN-λ1, -2, and -3 (also known as interleukin-29 [IL-29], IL-28A, and IL-28B) and IFN- λ4. They are mainly restricted to epithelial cells such as those on mucosal surfaces. (Pigs lack IFN-λ2.) They signal through a unique receptor complex consisting of IL-10Rβ and IL-28Rα. While structurally unrelated to type I interferons, they induce similar intracellular signals and a similar gene expression profile. Their effects are most apparent at intestinal and respiratory epithelia and at the blood-brain barrier.

Table 1. Provides an overview of the different subtypes of IFN found in humans, key references and main characteristic is listed.

Type	Subtypes	Receptor	Comments	References
Type I IFNs				
IFN-α	IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8, -α10, -α13, -α14, -α16, -α17, -α21	IFN-αR1/ IFN-αR2	IFN-α is primarily produced by pDCs	Cella *et al* (1999), Barchet *et al* (2002), Dai *et al* (2004), Hardy *et al* (2004), Jaks *et al* (2007)
IFN-β		IFN-αR1/ IFN-αR2	IFN-β is produced by most infected cells	Platanias (2005), Khaitov *et al* (2009), Ioannidis *et al* (2013)
IFN-ε		IFN-αR1/ IFN-αR2	IFN-ε is associated with the female reproductive tract	Fung *et al* (2013), Marks *et al* (2019)
IFN-κ		IFN-αR1/ IFN-αR2	IFN-κ is selectively expressed in keratinocytes	LaFleur *et al* (2001)
IFN-ω		IFN-αR1/ IFN-αR2	One of the least studied IFNs but the presence of neutralizing auto-antibodies against it in severe COVID-19 patients suggests its role in antiviral immunity may be underappreciated	Hauptmann and Swetly (1985), Bastard *et al* (2020)
Type II IFNs				
IFN-γ		IFN-γR1/ IFN-γR2	Not discussed here	
Type III IFNs				
IFN-λ	IFN-λ1, -λ2, -λ3, -λ4	IFN-λR1/ IL-10R2	IFN-λ is produced by infected cells at barrier tissues such as epithelial cells in the respiratory tract	Meager *et al* (2005), Sommereyns *et al* (2008), Jewell *et al* (2010), Mordstein *et al* (2010), Crotta *et al* (2013), Wack *et al* (2015), Ye *et al* (2019)

Antiviral Activities

The two major type I interferons (IFN-α and IFN-β) are produced within a few hours of viral invasion, and high concentrations are achieved long before adaptive immunity develops. For example, in cattle infected with bovine herpesvirus-1 (BHV-1), peak interferon levels in serum are reached 2 days later and then decline, but they are still detectable by 7 days (Fig. 27.3). In contrast, antibodies are not usually detectable in serum until 5 to 6 days after the onset of a virus infection.

IFN-α and IFN-β are produced when viral nucleic acids bind TLRs-7 and -9 or RIG-1. Both bind to receptors on nearby cells and activate JAK/STAT signaling pathways. These pathways turn on at least 300 genes, many of which encode antiviral proteins. The result is the development of an “antiviral state” within a few minutes, which peaks at 5 to 8 hours.

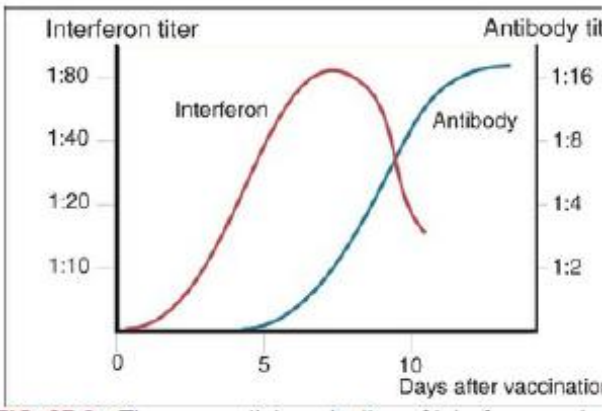


FIG. 27.3 The sequential production of interferon and a following intranasal vaccination of calves with infectious rhinotracheitis vaccine. (From data kindly provided by Dr. M. S

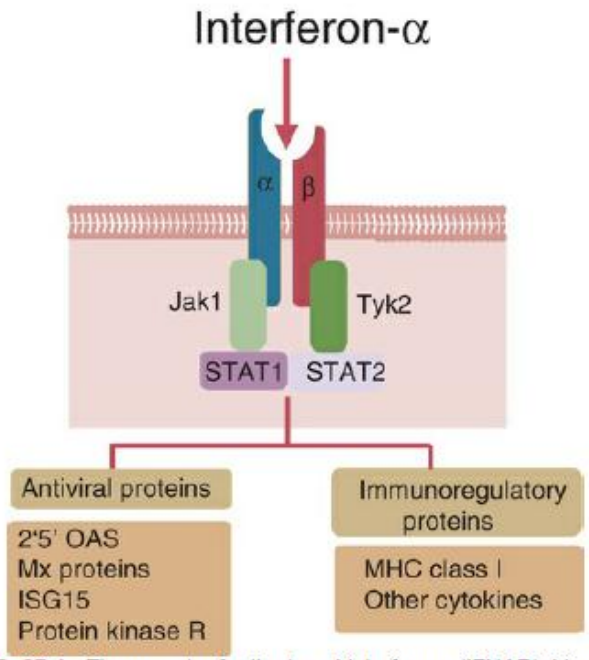
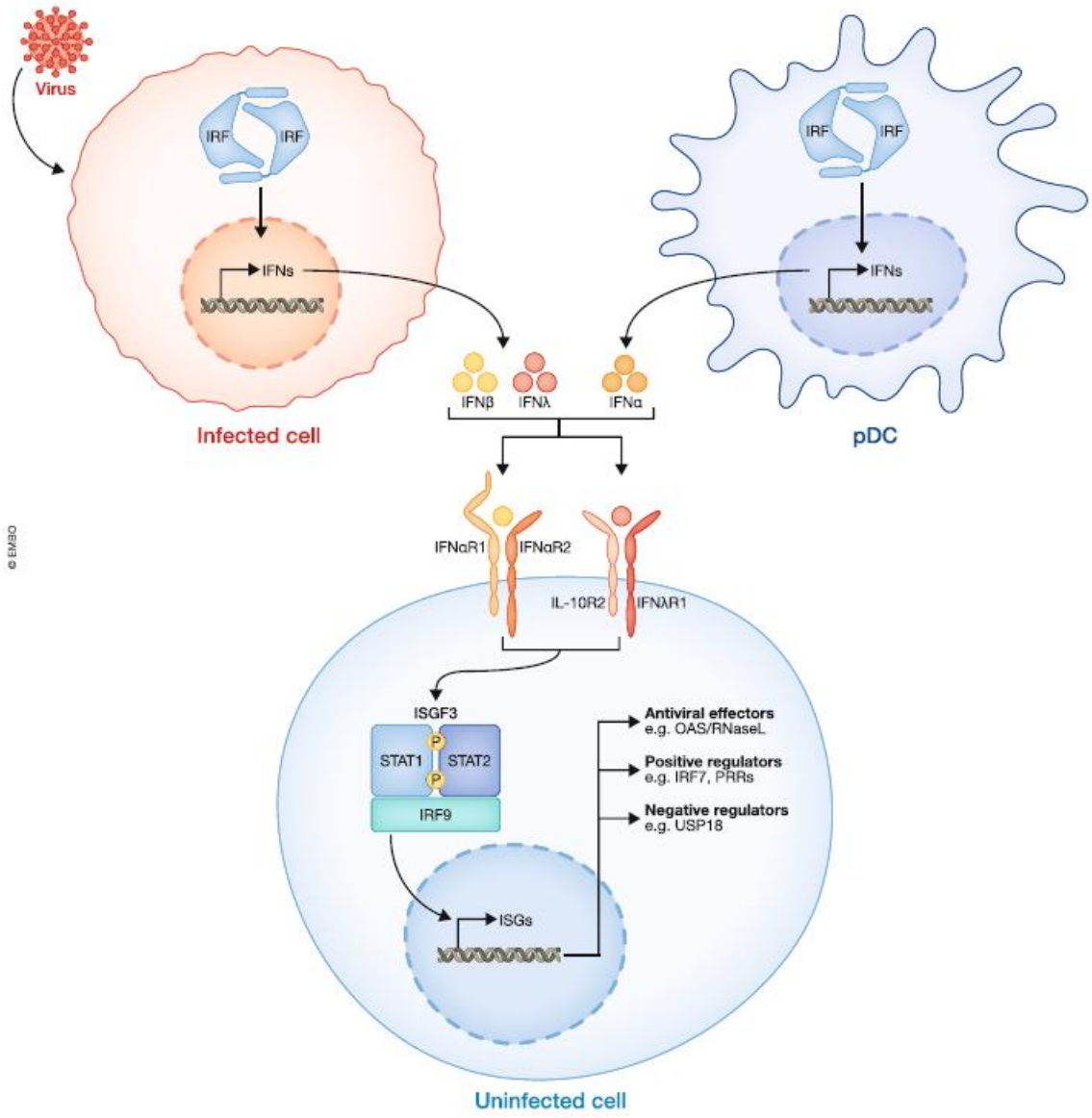


FIG. 27.4 The receptor for the type I interferons (IFNAR). Ligand binding triggers the JAK-STAT transduction pathway and eventually activates both antiviral and immunoregulatory pathways.



IFN-stimulated genes act through many different pathways and have diverse effects on viruses. Some broadly inhibit viral growth while others target specific viruses. They may target different stages of viral replication such as viral entry, envelope uncoating, genome replication, protein assembly, or viral release. The existence of diverse IFN-α isoforms, even though they signal through a common receptor, suggests that they have different functional roles.

Interferons also target cells to promote viral clearance or induce apoptosis. These include increased neutrophil survival, activation of macrophages, and regulation of natural killer (NK) cells, DCs, B cells, CD8+T cells, and Th1 cells. They are, in effect, broad-spectrum antivirals.

Here are six of the most important pathways.

1- *The 2′5′ A pathway:* Type I interferons upregulate transcription of the genes coding for 2′5′-oligoadenylate synthetases (2′5′-OAS). These enzymes are then activated by exposure to long

dsRNA from viruses in the cytoplasm. They act on adenosine triphosphate (ATP) to form 2′5′ adenylate oligomers. These oligomers bind and activate a ribonuclease called RNAase L. The RNAase L degrades viral RNA and so inhibits viral growth.

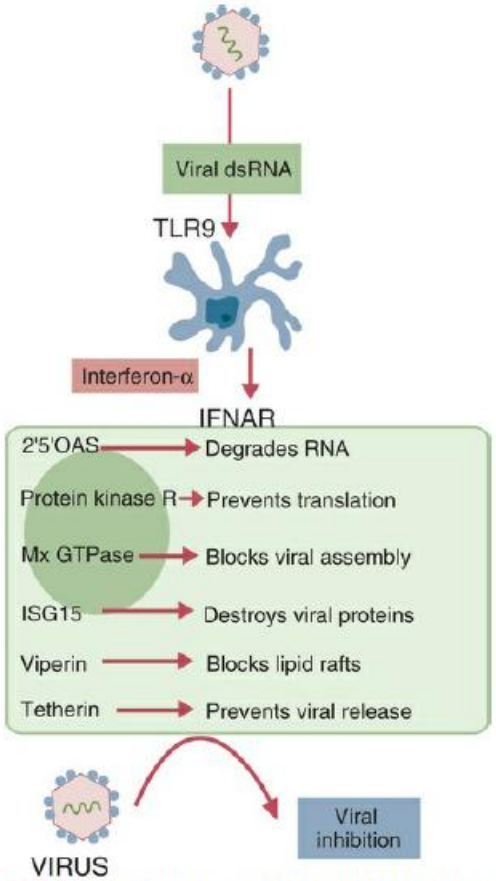


FIG. 27.5 Some of the mechanisms by which the interferons can exert their antiviral activities. IFNAR: Interferon alpha/beta receptor. OAS: Oligoadenylate synthetase. ISG: Interferon-stimulated genes.

2- *The Mx guanosine triphosphatase (GTPase) pathway:* Mx proteins are large interferon-induced GTPases that accumulate as oligomers on intracellular membranes. Following viral infection, Mx monomers are released. These bind and trap viral nucleocapsids and other essential viral components and so block the assembly of new viruses. Mx proteins are expressed in many different cell types such as hepatocytes, endothelial cells, and immune cells. They inhibit a wide range of RNA viruses, including the influenza viruses.

3- *The protein kinase R (PKR) pathway:* PKR is induced by type I interferons. The inactive kinase accumulates in the cell nucleus and cytoplasm, where it is activated by viral RNA. Activated PKR regulates several cell signalling pathways and phosphorylates an initiation factor called eIF2α, which then prevents translation initiation by viral mRNA.

4- *The ISG15 pathway: ISG15* codes for a ubiquitin-like protein that binds to many different proteins and enhances their destruction. It is not known how this results in increased antiviral resistance and reduced viral replication.

5- *The viperin pathway:* Viperin is a protein that has direct antiviral activity. It is induced by all three classes of interferon, double-stranded DNA and RNA, and by many different viruses. It appears to act on cellular lipids and interferes with lipid raft formation at different stages in the viral life cycle, depending upon the virus.
6- *Tetherin.* This is an interferon-stimulated gene that encodes a small membrane protein. It physically crosslinks (tethers!) virions to the plasma membrane and thus inhibits the release of enveloped viruses from the cell surface.

The ability of cells to produce interferons varies. Virus-infected leukocytes, especially pDCs, produce large amounts of IFN-α; almost any virus-infected cell can produce IFN-β; and antigen-stimulated T cells are the major source of IFN-γ.

NK cells can kill virus-infected cells. NK cell cytotoxicity is stimulated by type I interferons and, as a result, is important early in a virus infection. Indeed, NK cells provide a first line of defense against many viruses. NK cells also produce large amounts of IFN-γ and perforins, and these too have direct antiviral effects. NK cells may therefore reduce the severity of viral infections long before the development of adaptive immunity and the appearance of specific cytotoxic T cells. IFN-α not only activates NK cells but most other immune cell populations as well. Thus, it stimulates the differentiation of monocytes into dendritic cells, as well as the maturation and activity of dendritic cells. IFN-α stimulates memory T cell proliferation, activates naïve T cells in chronic viral diseases, and enhances antigen-specific T cell priming. It promotes B-cell functions such as IgG production and MHC expression.

RNA Interference

RNA interference (RNAi) is an innate antiviral pathway that is important in plants and many invertebrates. It has recently been recognized in mammals. Viral double-stranded RNA (dsRNA) is broken up by an intracellular nuclease called DICER into small interfering RNAs (siRNAs). These siRNAs are loaded into an RNA-induced silencing complex (RISC) that then binds to the viral RNA and destroys it, thus preventing viral growth. These siRNAs have been detected in a mouse germ cell line. Conversely, they are absent from somatic cells of adult mice. Embryonic cell lines cannot produce type I interferons, but adult somatic cells can. It has been suggested, therefore, that germ cells and embryos rely on RNA interference, but as they develop, they switch to an interferon response.

Evasion of Interference With Antigen Processing Pathways

Many viruses interfere with the expression of MHC class I molecules and so inhibit antigen presentation. They use many different suppressive techniques, including reducing transcription of MHC genes, blocking transporter protein function and the transfer of peptides into the endoplasmic reticulum, inhibiting proteasomal degradation of viral proteins, inhibiting the intracellular transport of MHC class I α chains, preventing delivery of the loaded MHC to the cell surface, and ubiquinating and hence destroying MHC molecules. Thus bovine herpesvirus-1 suppresses the expression of MHC class I molecules by interfering with transporter protein functions and downregulating the expression of mRNA for MHC class I molecules. Other viruses may cause MHC class I molecules to be retained within a cell; they may prevent molecules such as ICAM-1, CD4, and CD28. peptide binding to transporter proteins, prevent proteasomal degradation, redirect MHC molecules to lysosomes for degradation, or even encode inhibitors that block caspase activity. Influenza A viruses can block macrophage differentiation into dendritic cells. Other viruses may downregulate the expression of co-stimulating