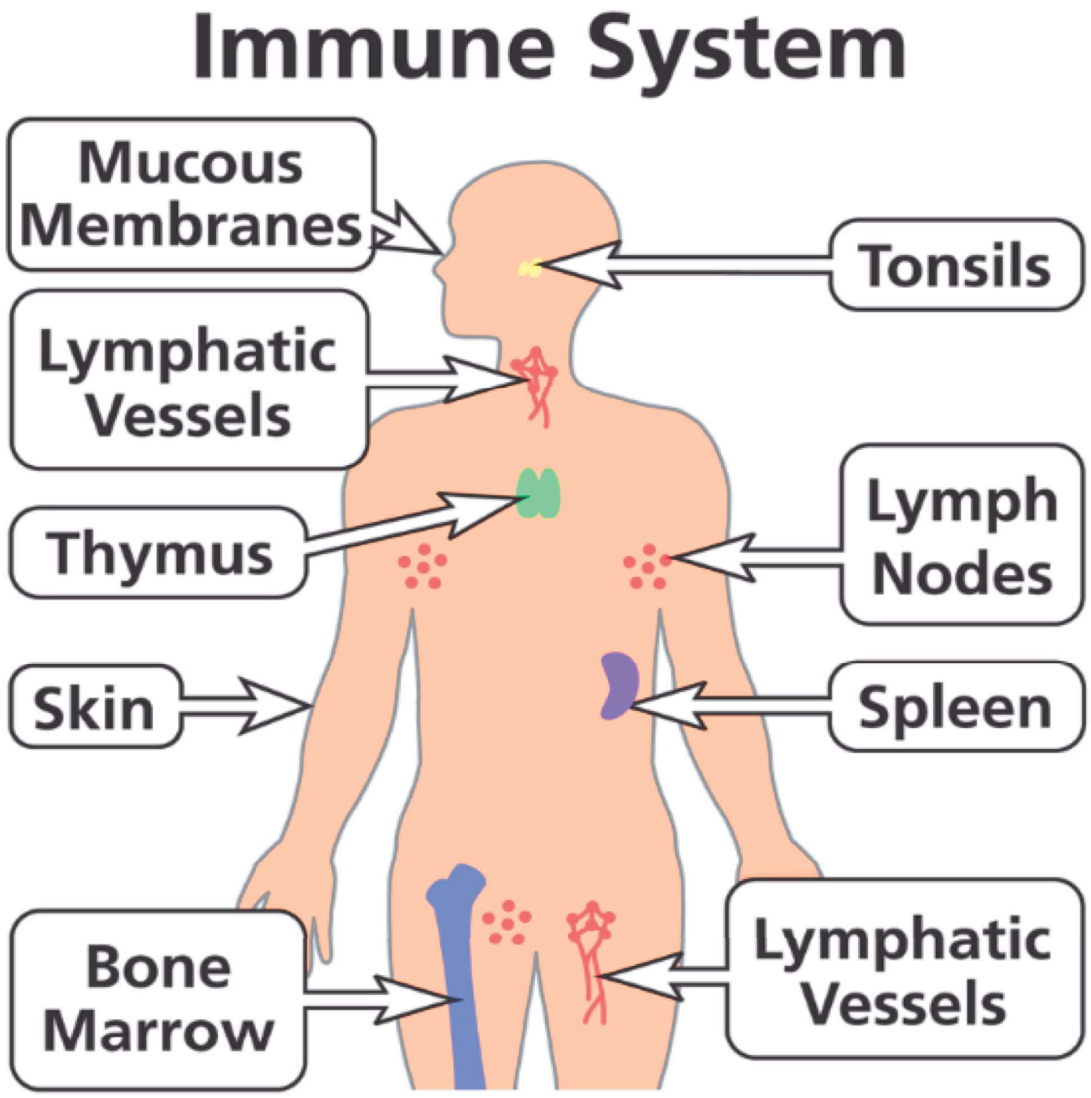
Infection and Immunity

- The immune system refers to a collection of cells, chemicals and processes that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins

- The immune system can be simplistically viewed as having two “lines of defense”:

- **innate immunity** and
- **adaptive immunity**

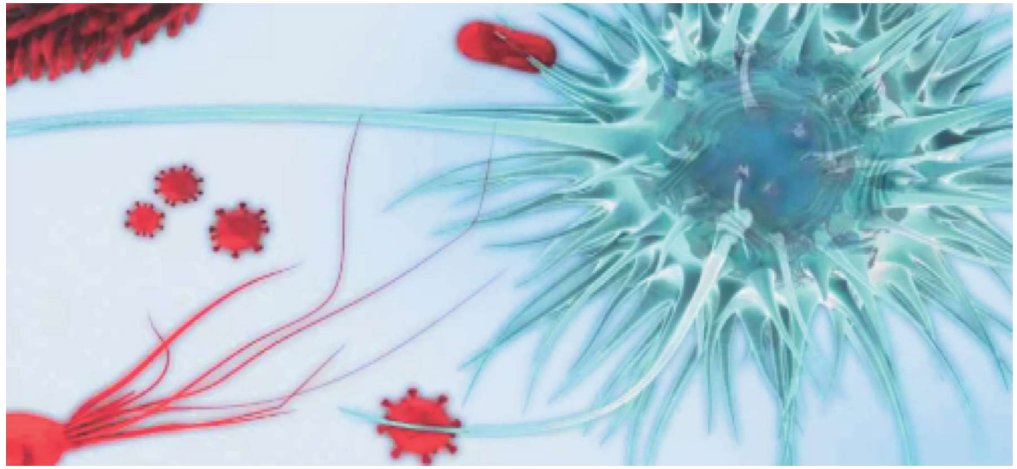


Innate immunity - First line of host defense

- Innate immunity is an **antigen-non specific defense mechanisms** that a host uses immediately or within several hours after exposure to almost any microbe

- The innate immune system is a rapid non specific reponse that helps the body prevent infection from pathogens never encountered before, prior to an antibody and T cell response being developed

- The innate immune response **has no immunologic memory** and, therefore, it is unable to recognize or “memorize” the same pathogen should the body be exposed to it in the future



Adaptive (acquired) immunity

- Adaptive (acquired) immunity **refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen**
- The hallmark of **adaptive immunity is the capacity for memory** which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen
- During adaptive immunity, antigens are transported to lymphoid organs where they are recognized by naive B-lymphocytes and T-lymphocytes
- These activated B- and T-lymphocytes subsequently proliferate and differentiate into effector cells
- **Adaptive immune responses are the basis for effective immunization against infectious diseases**

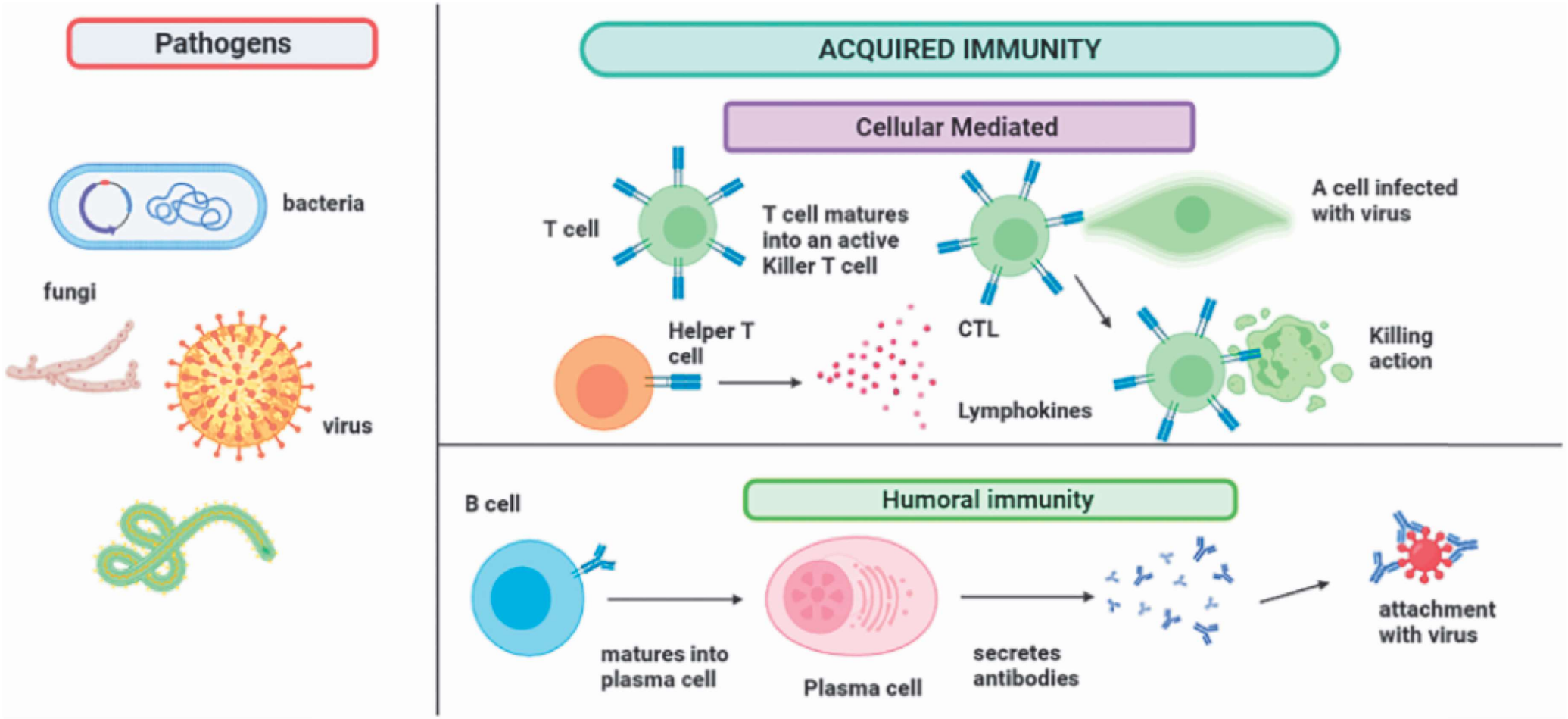
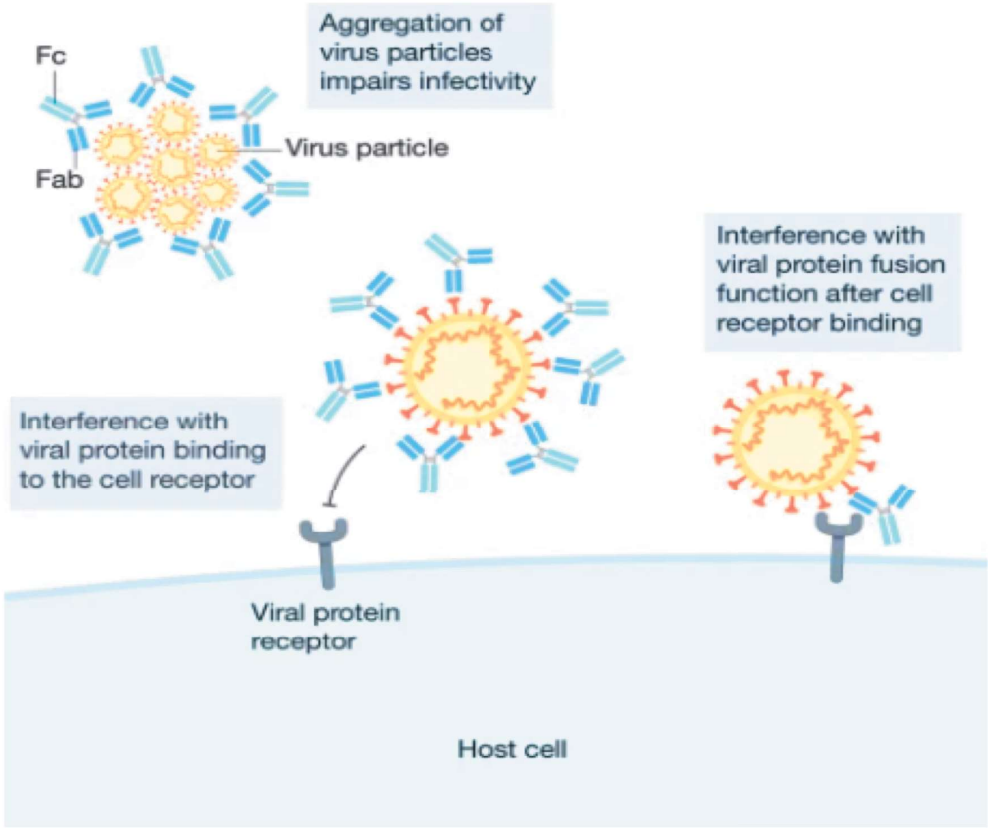


FIGURE 4 | Acquired immunity: immune cells show two types of responses. Humoral immunity involves the B cells that mature into plasma cells that secrete antibodies and attach to the pathogen. B cells also produce memory cells for the future attack of the same pathogen. Cytotoxic T lymphocytes (CTLs) are immune cells that play an important role in the fight against infections and tumor immunology. T cell matures into an active killer T cell called cytotoxic T lymphocytes, which attach to the infected cell and kill it. The lymphokines attract additional immune cells for stimulation and cascade the effect.

- Mechanisms of antibody-mediated neutralization of viruses by functions of the IgG Fab fragment that block binding to cell surface receptors and inhibit infectivity by aggregating viral particles and inhibiting steps in the viral life cycle, such as fusion

- Binding of antibodies with certain properties may enable changes in the viral entry protein that accelerate fusion



Barrier	Mechanism
Anatomic	
Skin	• Mechanical barrier retards entry of microbes • Acidic environment (pH 3–5) retards growth of microbes
Mucous membrane	• Normal flora compete with microbes for attachment sites • Mucous entraps foreign microbes • Cilia propel microbes out of body
Physiologic	
Temperature	• Body temperature/fever response inhibits growth of some pathogens
Low pH	• Acidic pH of stomach kills most undigested microbes
Chemical mediators	• Lysozyme cleaves bacterial cell wall • Interferon induces antiviral defenses in uninfected cells • Complement lyses microbes or facilitates phagocytosis
Phagocytic/endocytic barriers	
	• Various cells internalize (endocytosis) and break down foreign macromolecules • Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill and digest whole organisms
Inflammatory barriers	
	• Tissue damage and infection induce leakage of vascular fluid containing serum protein with antibacterial activity, leading to influx of phagocytic cells into the affected area

The innate immunity consists of components that are already at the location of infection and respond immediately with a generalized response

The innate immune system is composed of tissue barrier, innate immune cells, innate immune molecules, and cytokines

Innate immunity can be viewed also as comprising four types of defensive barriers: anatomic (skin and mucous membrane), physiologic (temperature, low pH and chemical mediators), endocytic and phagocytic, and inflammatory

Innate immunity includes different types of defensive barriers: anatomic (skin and mucous membrane)

Barrier	Mechanism
Anatomic	
Skin	• Mechanical barrier retards entry of microbes • Acidic environment (pH 3–5) retards growth of microbes

The skin

- **The skin, the largest organ of the body, is a very strong physical barrier, that many pathogens find impenetrable.**
- **Alongside the physical barrier, the skin is also inhospitable to many pathogens, containing antimicrobial peptides that can kill pathogens.**
- **It is also covered in commensal skin flora that promote wound healing and restrict the growth of other microbes**

Innate immunity includes different types of defensive barriers: anatomic (skin and mucous membrane)

Barrier	Mechanism
Anatomic	
Skin	• Mechanical barrier retards entry of microbes • Acidic environment (pH 3–5) retards growth of microbes
Mucous membrane	• Normal flora compete with microbes for attachment sites • Mucous entraps foreign microbes • Cilia propel microbes out of body

The mucosal surfaces

Key entry point for viruses and other pathogens into the body include the mucosal surfaces

This includes the mouth, nose, eyes, respiratory tract, gastrointestinal tract, urinary tract and reproductive tract.

With the respiratory and gastrointestinal tract being the main sites of viral entry.

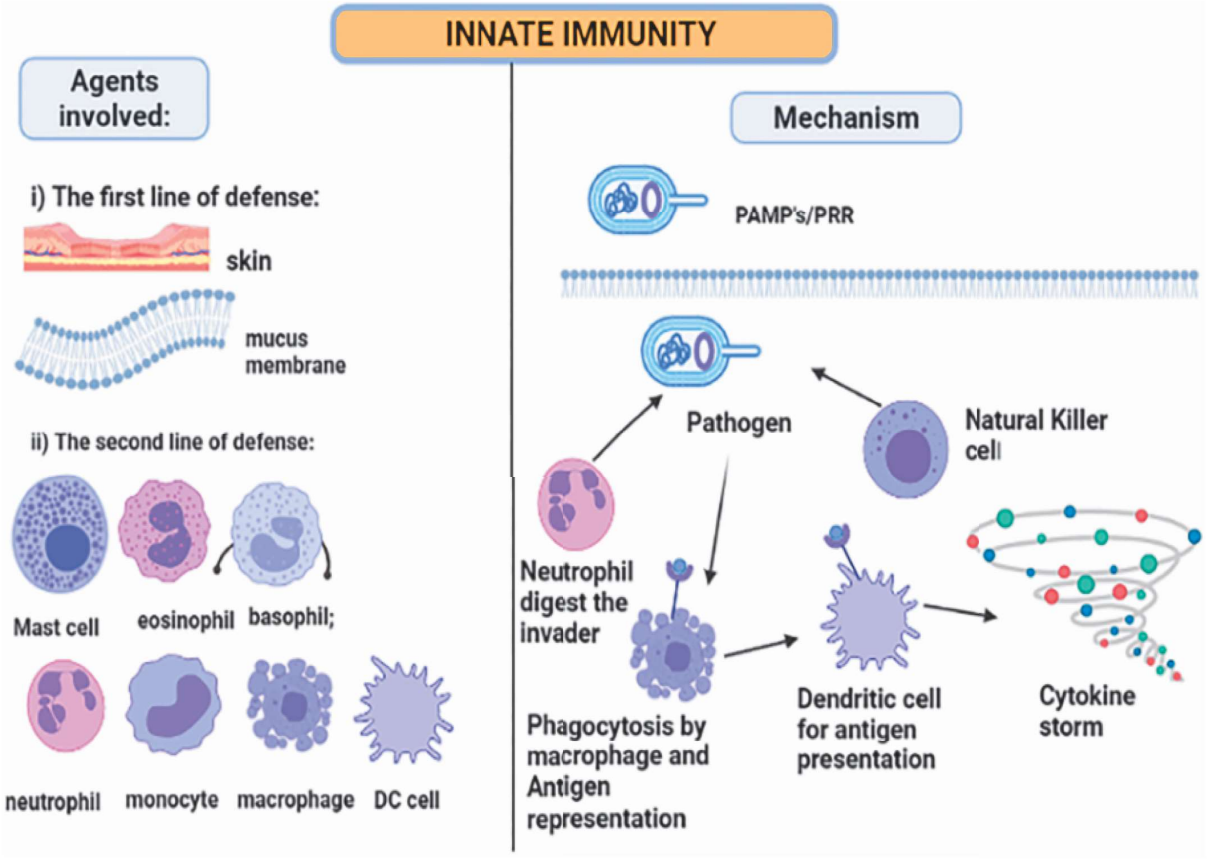
All these sites have an arsenal of antimicrobial agents, specialist cells types, mucosal antibody (mainly secretory IgA) and some have inhospitable conditions (low pH).

They are resident to an array of immune cells that patrol these entry points to prevent pathogen invasion and infection.

- The innate immune system is composed of tissue barrier, innate immune cells, innate immune molecules, and cytokines.

- The innate immune cells including macrophages, DCs, Natural Killer Cells (NKs) and innate-like lymphocytes have been proved to be essential for responses against pathogens

- When the pathogen invades the host cell, the neutrophil and natural killer cells start digesting the pathogen. However, the macrophages and DC phagocytose the pathogen and act as antigen-presenting cells, producing a cytokine storm for attracting other immune cells to amplify the response



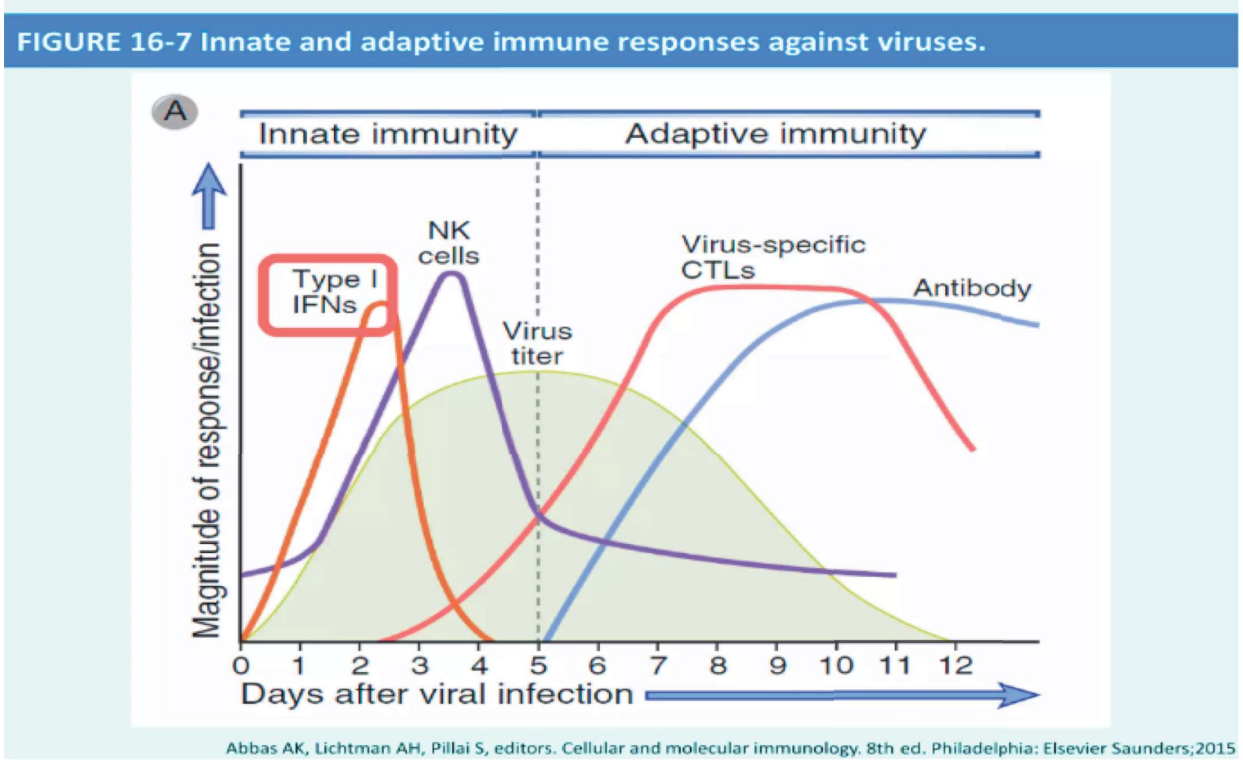
Phagocytic/endocytic barriers	
	• Various cells internalize (endocytosis) and break down foreign macromolecules • Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill and digest whole organisms

The innate immune response is the first line of defense against an invading pathogen

Two events required to trigger an effective anti-viral/microbial innate immune response are:

a) detection of the invading virus/bacteria by immune system receptors; and

b) initiation of protein signaling cascades that regulate the synthesis of IFNs and cytokines



- Key component of the innate immune system are **pattern recognition receptors (PRRs)**

- These are present on the cell surface of a number of different cell types (immune cells, epithelial cells, endothelial cells etc), and **recognise components shared by many pathogens**, which are not present in the host, distinguishing self from non self (Janeway, 1989, Janeway, 2013; Takeuchi and Akira, 2010).

- These unique microbial molecules are **called pathogen-associated molecular patterns or PAMPS**

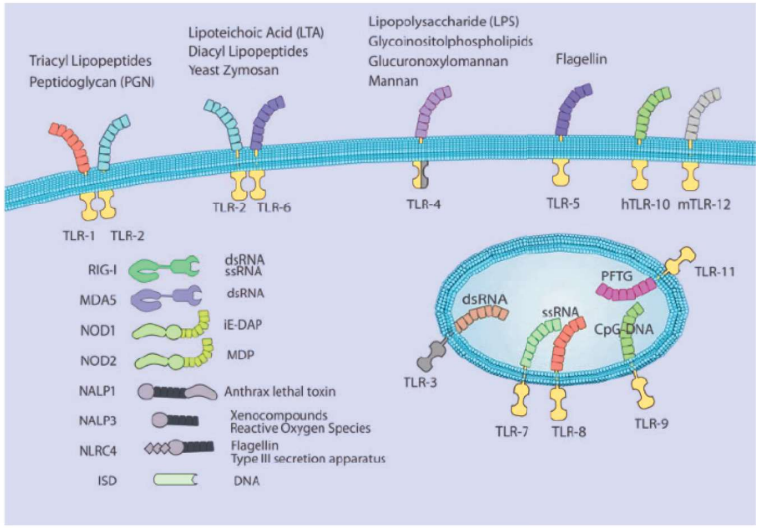
- **PAMPs** include things such as **lipopolysaccharide (LPS), flagellin, peptidoglycan**, which are present on the surface of different bacterial pathogens. They also recognise the genetic material of some viruses (**dsRNA/ssRNA**), and other ubiqutous components shared by other microorganisms (fungi, parasites) (Takeuchi and Akira, 2010).

- The ability to recognise different pathogens depend on which PRRs cells have, and therefore dictate the ensuing immune response. Pathogens will often activate multiple PRRs allowing cross talk between receptors and a more robust immune response

- However, the **host can produce some proteins and metabolites after being stimulated by its own tissue damage, cell necrosis, and other factors**. These molecules are called **damage-associated molecular pattern (DAMP). PRRs can also recognize such molecules, activate natural immunity, and cause inflammation**

There are five main groups of **PRRs**:

1. **Toll Like receptors (TLRs) are the most widely studied,**
2. **C-type lectin receptors (CLRs),**
3. **Retinoic acid inducible gene-I (RIG-1) like receptors (RLRs)**
4. **Nucleotide-binding oligomerisation domain-like receptors (NLRs).**
5. **Cytosolic DNA sensors**



Pattern recognition receptors and their cognate ligands. TLRs 3, 7, 8, 9 and 11 have been reported to exhibit endo- somal or intracellular localization while NOD1, NOD2, RIG-I, MDA-5, NALP1, NALP3, NLRC4, and the intracellular DNA sen- sor (ISD) function in the cytoplasm. Only a partial list of ligands or classes of ligands for each receptor is given.

Table 1. Common PRRs in innate immunity

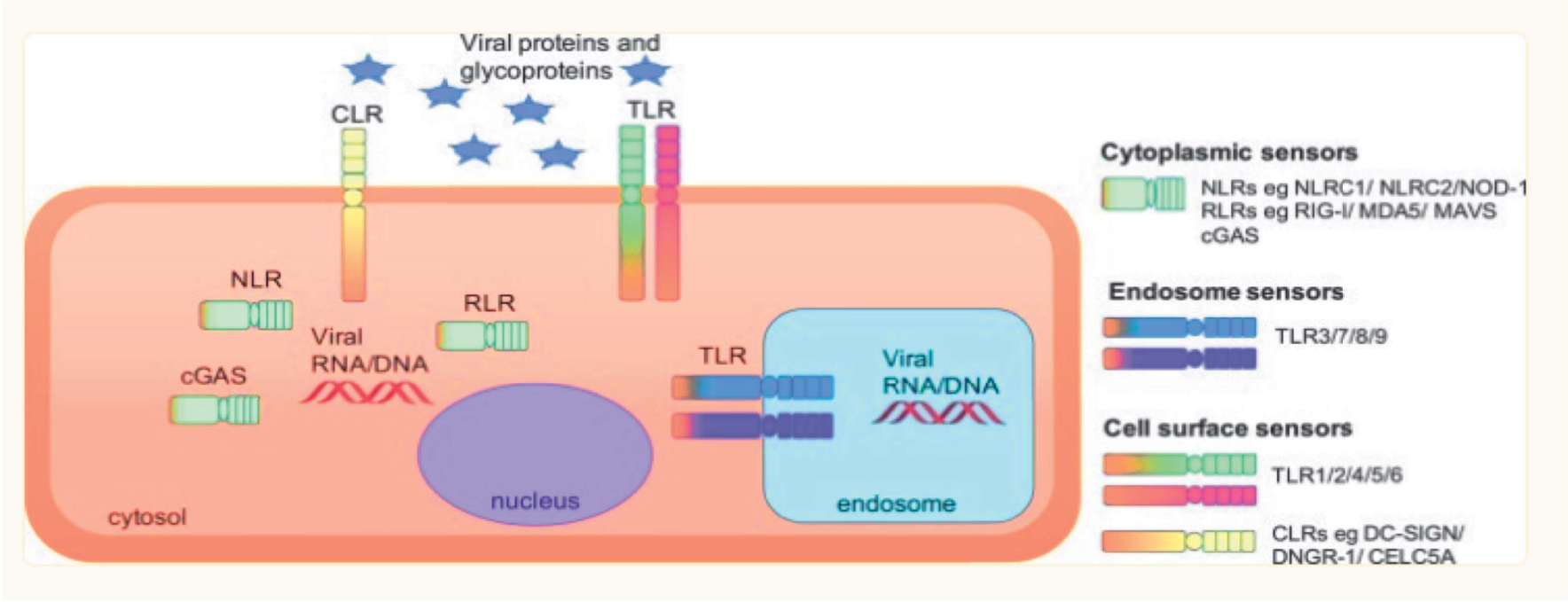
Items	PRR	Domains	Cellular distribution	PAMP	Sources	Signaling pathways
Toll-like receptors (TLRs)	TLR1 (TLR1–TLR2)	LRR domain–transmembrane domain–TIR domain (extracellular to intracellular)	Mo, DC, Ma, Eo, Ba	Triacyl lipopeptide	Bacteria	Most TLRs: MyD88-dependent pathways; TLR3: TRIF-dependent pathways; TLR4: MyD88-dependent pathways and TRIF-dependent pathways
	TLR2 (TLR1–TLR2, TLR2–TLR6)		Mo, DC, Ma, Eo, Ba	Lipoteichoic acid	Bacteria	
				Arabinomannan	Mycobacterium	
				Peptidoglycan	Bacteria	
				Zymosan	Fungi	
				Lipoprotein	Mycoplasma	
				Pore protein	Neisseria	
	TLR3		Mφ, DC, IEC	dsRNA	Virus	
	TLR4 (MD-2/CD14)		Mφ, DC, Ma, Eo	Lipopolysaccharides	Bacteria	
				Heat-shock proteins	Host	
	TLR5		IEC	Flagellin	Bacteria	
	TLR6 (TLR2–TLR6)		Mo, DC, Ma, Eo, Ba	Lipoteichoic acid	Bacteria	
				Peptidoglycan	Bacteria	
	TLR7		pDC, Mφ, Eo	ssRNA	Virus	
				Imidazoquinoline	Artificially synthesized	
	TLR8		Mφ, N	ssRNA	Virus	
	TLR9		pDC, Eo, Ba	Non-methylated CpG DNA	Bacteria, Virus	
	TLR10 (human)		pDC, Eo, Ba	dsRNA	Virus	
	TLR11 (mouse)		Mφ, DC	Profilin and related proteins	*Toxoplasma gondii*	
	TLR12 (mouse)		DC	Profilin and related proteins	*Toxoplasma gondii*	
	TLR13 (mouse)		Unknown	23s ribosomal RNA	Bacteria	
Nucleotide-binding oligomerization domain-like receptors (NLRs)	NOD1	LRR domain–NBD–effector domains	IEC, cytosol of Mφ	iE-DAP	Gram negative bacteria	RIP2-TAK1-NF-κB pathways
	NOD2			MDP	Gram-negative bacteria, Gram-positive bacteria	
RIG-I-like receptors (RLRs)	RIG-I	(RD)-CTD-DexD/H helicase domain–CARD	Cytosol	5′-triphosphorylated RNA, short-chain dsRNA	Virus	MAVS-TRAF6-NF-κB/TBK1 pathways
	MDA5			poly IC, long-chain dsRNA	Virus	
	LGP2			dsRNA	Virus	
C-type lectin receptors (CLRs)	Dectin-1	CTLD–ITAM	DC, Mφ	β-Glucan	Fungus	Tyrosine kinase-dependent and non-tyrosine kinase-dependent pathways
	Dectin-2			α-Mannan	Fungus	
Absent in melanoma-2-like receptors (ALRs)	ALRs	HIN-200-PYD	Cytosol	dsDNA	Bacteria	Inflammasome–pyroptosis

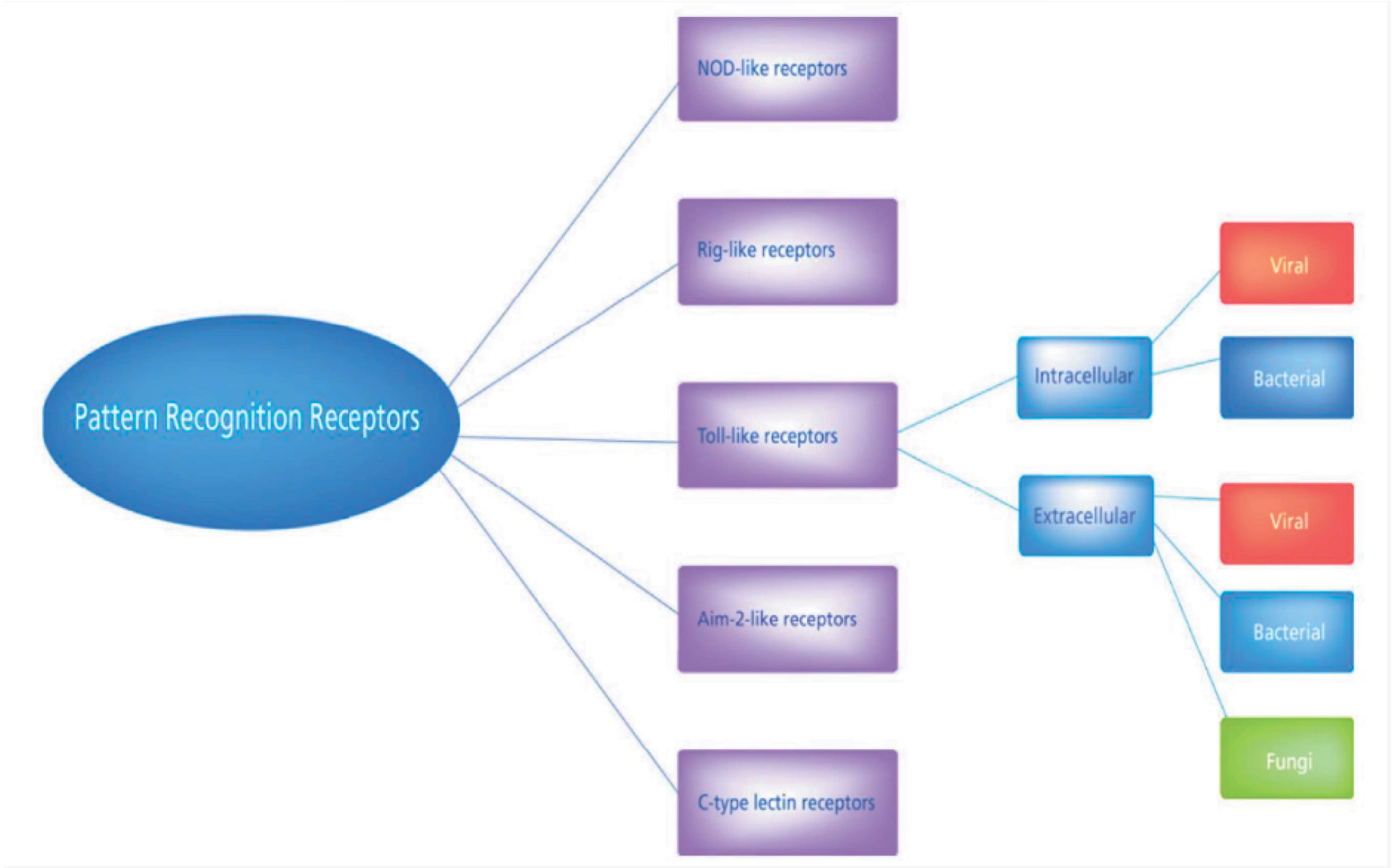
LRR leucine-rich repeat, *TIR* Toll/IL-1R domain, *NBD* nucleotide-binding domain, *RD* repressor domain, *CTD* C-terminal domain, *CARD* caspase activation and recruitment domain, *CTLD* C-type lectin-like domains, *ITAM* immunoreceptor tyrosine-based activation motif, *PYD* pyrin domain, *Mo* monocyte, *DC* dendritic cell, *Ma* mastocyte, *Eo* eosinophils, *Ba* basophils, *pDC* plasmacytoid dendritic cell, *IEC* intestinal epithelial cell, *N* neutrophil, *dsRNA* double-stranded RNA, *ssRNA* single-stranded RNA, *iE-DAP* γ-D-glu-meso-diaminopimelic acid, *MDP* muramyl dipeptide, *MyD88* myeloid differentiation factor 88, *TRIF* TIR domain-containing adaptor protein-inducing interferon β, *RIP2* receptor-interacting serine–threonine protein 2, *TAK1* transforming growth factor-β-activated kinase 1, *NF-κB* nuclear factor κB, *MAVS* mitochondrial antiviral signaling protein, *TRAF6* tumor necrosis factor receptor-associated factor, *TBK1* TANK-binding kinase 1

- **The location of PRRs varies within the cell, some are present on the cell surface (plasma membrane) and recognise extracellular pathogens.**

- These are mainly present on immune and epithelial cells.

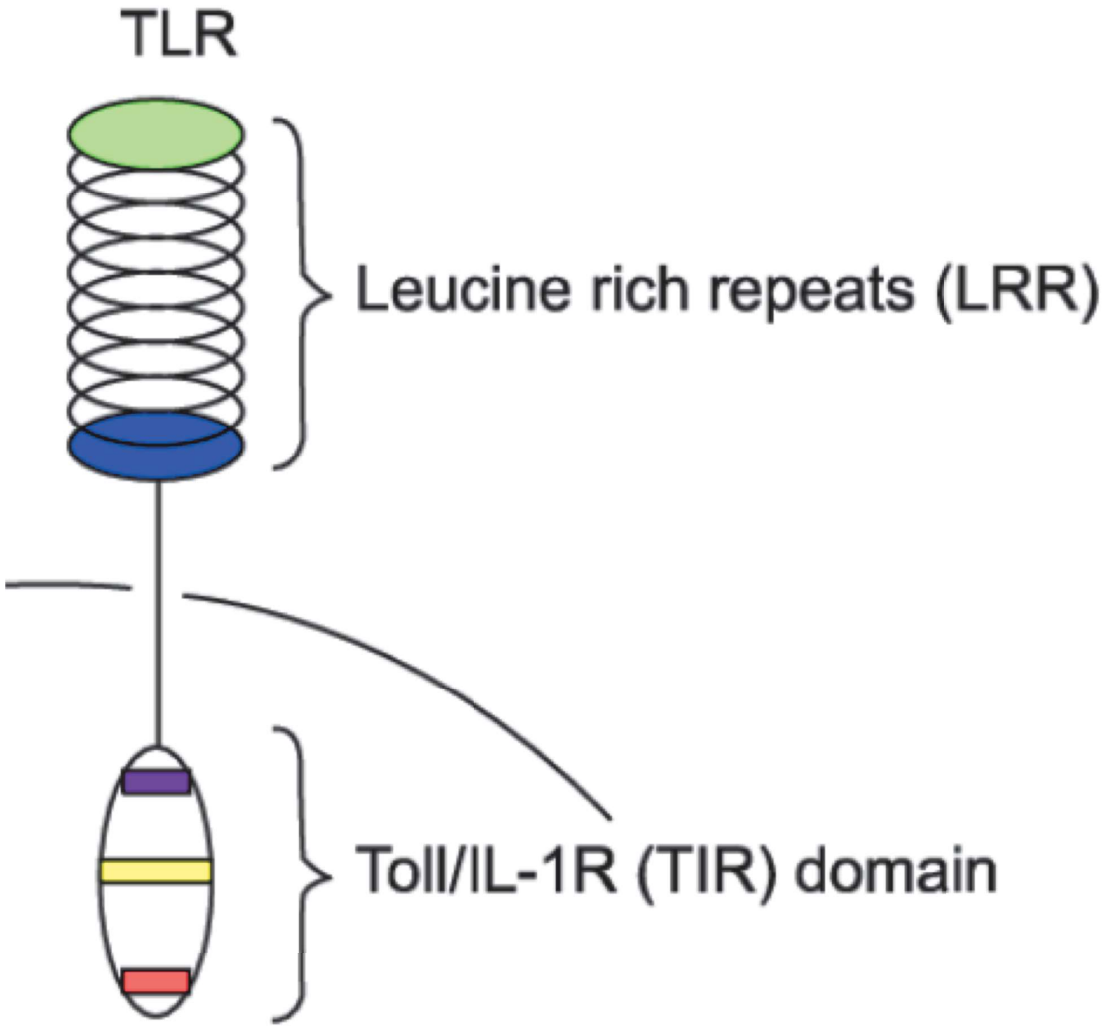
- Other **PRRs are present within the cells** and recognise intracellular pathogens, such as viruses (endosome membrane/cytoplasm). Intracellular **PRRs can also be membrane bound and present within the endosome membrane or free within the cytoplasm.** These are often found in all cell types




Pattern Recognition Receptors
NOD-like receptors
Rig-like receptors
Toll-like receptors
Aim-2-like receptors
C-type lectin receptors
Intracellular
Extracellular
Viral
Bacterial
Viral
Bacterial
Fungi

TLRs consist of a extracellular domain that recognises PAMPs and an intracellular domain that has cell signalling capabilities, known as the Toll Interleukin Receptor (TIR) domain.

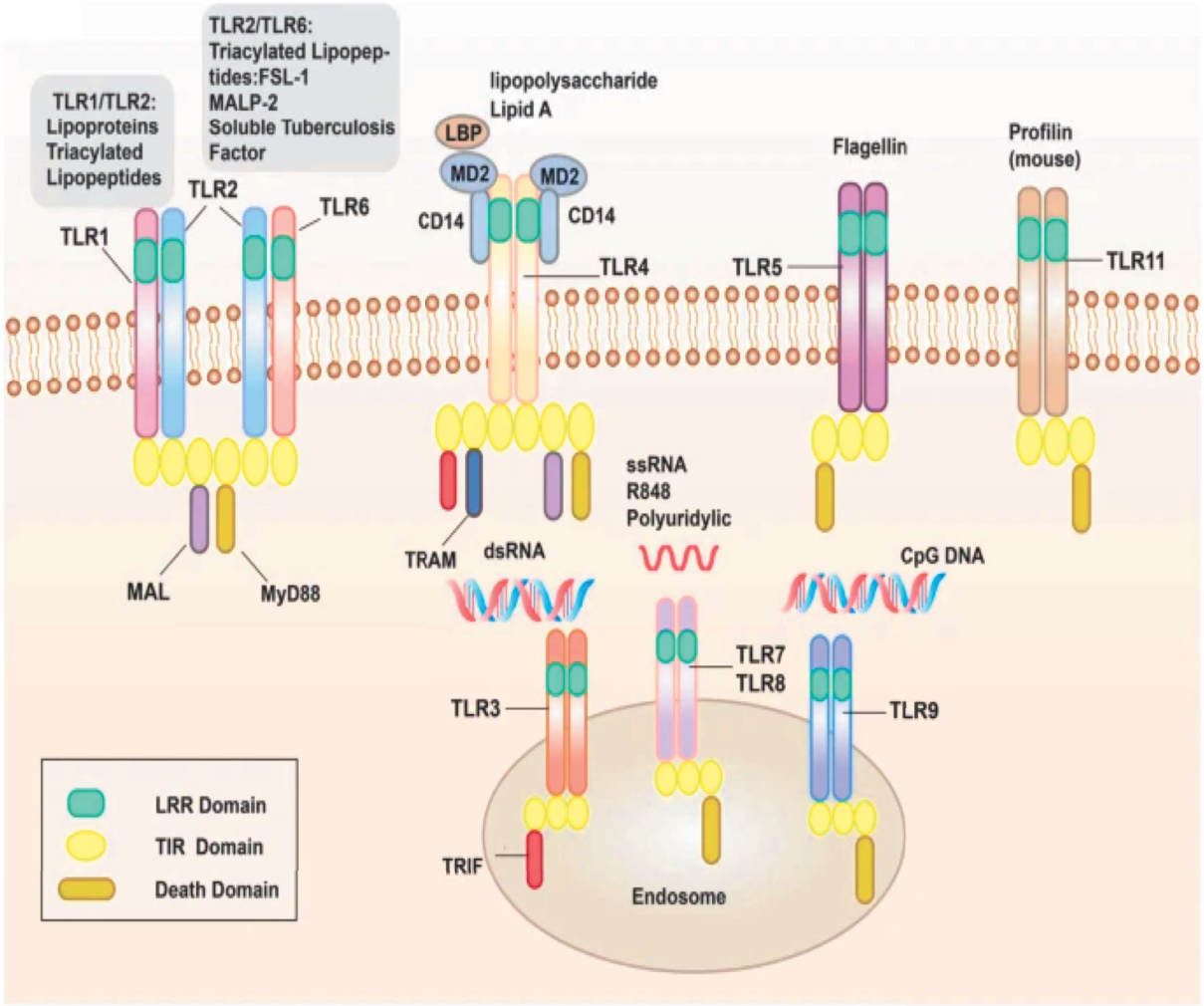
Although all TLRs share similar extracellular LRRs, they recognize very different microbial signatures



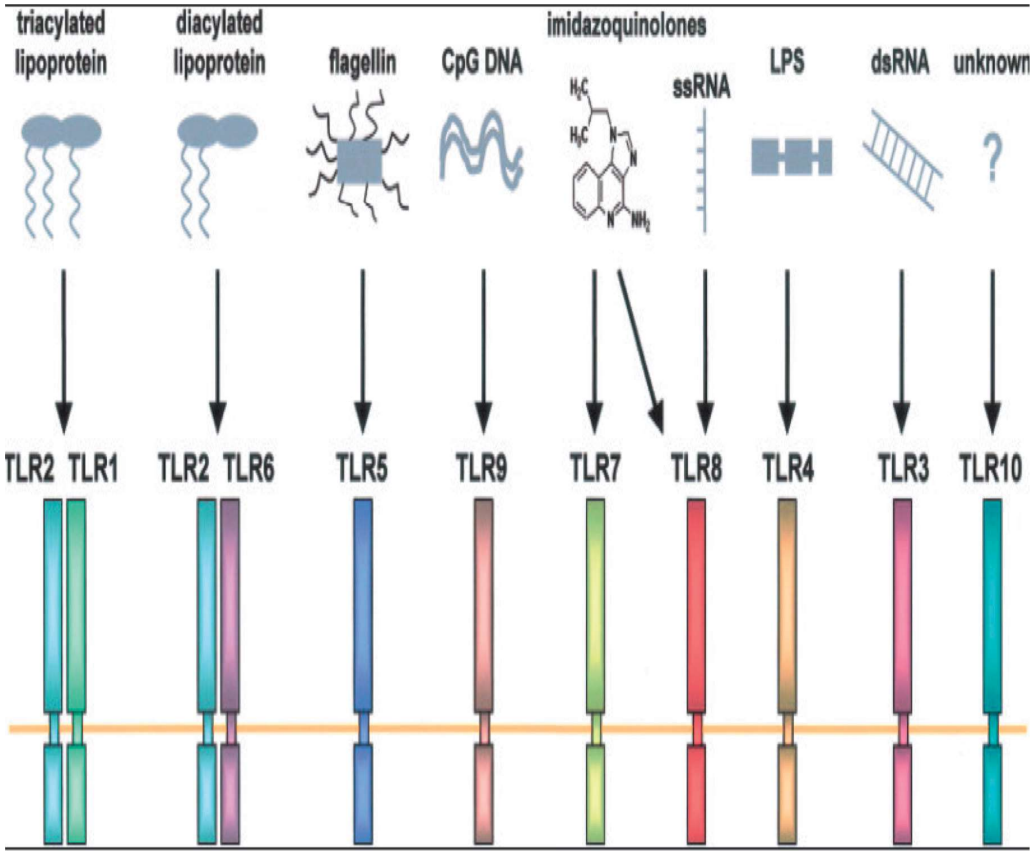
Toll like receptors (TLRs)

- In humans there are 10 functional TLRs (Medzhitov, 2001; Takeda and Akira, 2005).
- These are found as heterodimers or homodimers in the plasma membrane or endosome membrane (Lester and Li, 2014).
- TLR1, 2, 4, 5, 6, 10 are found in the plasma membrane, and TLR3, 7, 8, 9 in the endosome membrane (recognise intracellular pathogens)
- TLRs (TLR1, 2, 4, 5, 6, 10) expressed on the surface of cells, mainly recognize the membrane components of pathogenic microorganisms, such as lipids, lipoproteins, and proteins; TLR 3, 7, 8, 9 are expressed on endosome membrane and recognize the nucleic acids of microorganisms

Fig. 1



Toll-like receptors (TLRs) 3, 7, 8 and 9 are the major PRRs that recognize distinct types of virally-derived nucleic acids and activate signaling cascades that result in the induction of IFNs and cytokines

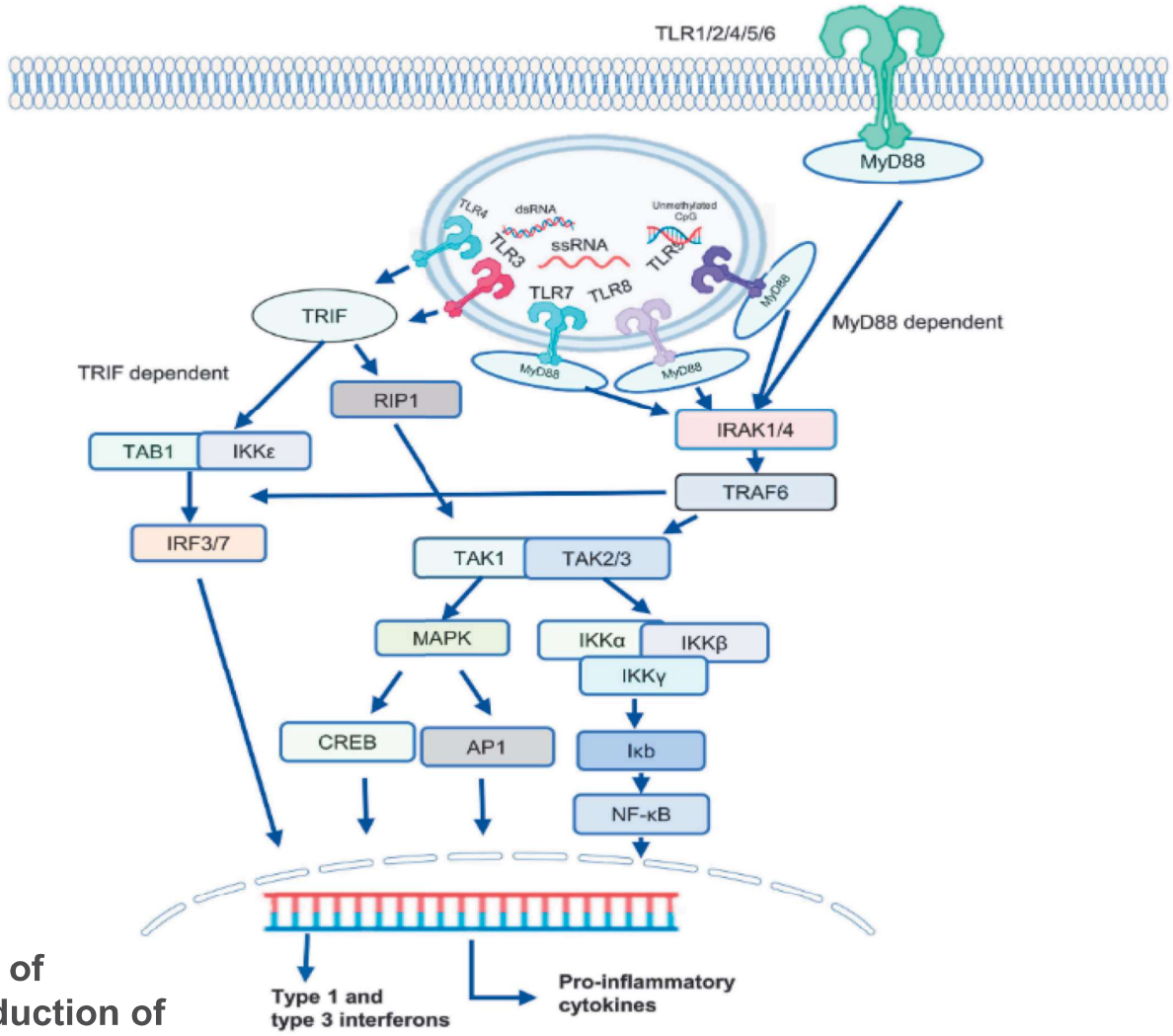


Toll-like receptor	Ligand
TLR1	Lipopeptides
TLR2	Lipoprotein, lipoteichoic acid, others
TLR3	Double-stranded RNA
TLR4	Lipopolysaccharide (LPS)
TLR5	Flagellin
TLR6	Lipoprotein, lipoteichoic acid, others
TLR7	Viral RNA
TLR8	Viral RNA
TLR9	Unmethylated CpG-containing DNA
TLR10	Unknown
TLR11	Profillin

There are currently over ten known Toll-like receptors that bind microbial associated molecular patterns (MAMPs) from bacteria, fungi and viruses.

The Toll-like receptor (TLR) signaling pathway

TLRs are categorized into two groups depending upon whether they utilize a MyD88-dependent pathway or a TRIF-dependent pathway. TLRs 1,2, and 5–9 transmit signals through a MyD88-dependent pathway, whereas TLR3 signaling is mediated by a MyD88-independent pathway. By contrast, TLR4 can signal through MyD88-dependent and MyD88-independent pathways.



TLRs signal through the recruitment of specific adaptor molecules, leading to activation of the transcription factors NF-κB and IRFs, which dictate the outcome of innate immune responses

NF-kB, which controls the expression of an array of inflammatory cytokine genes, while IRFs the production of IFN

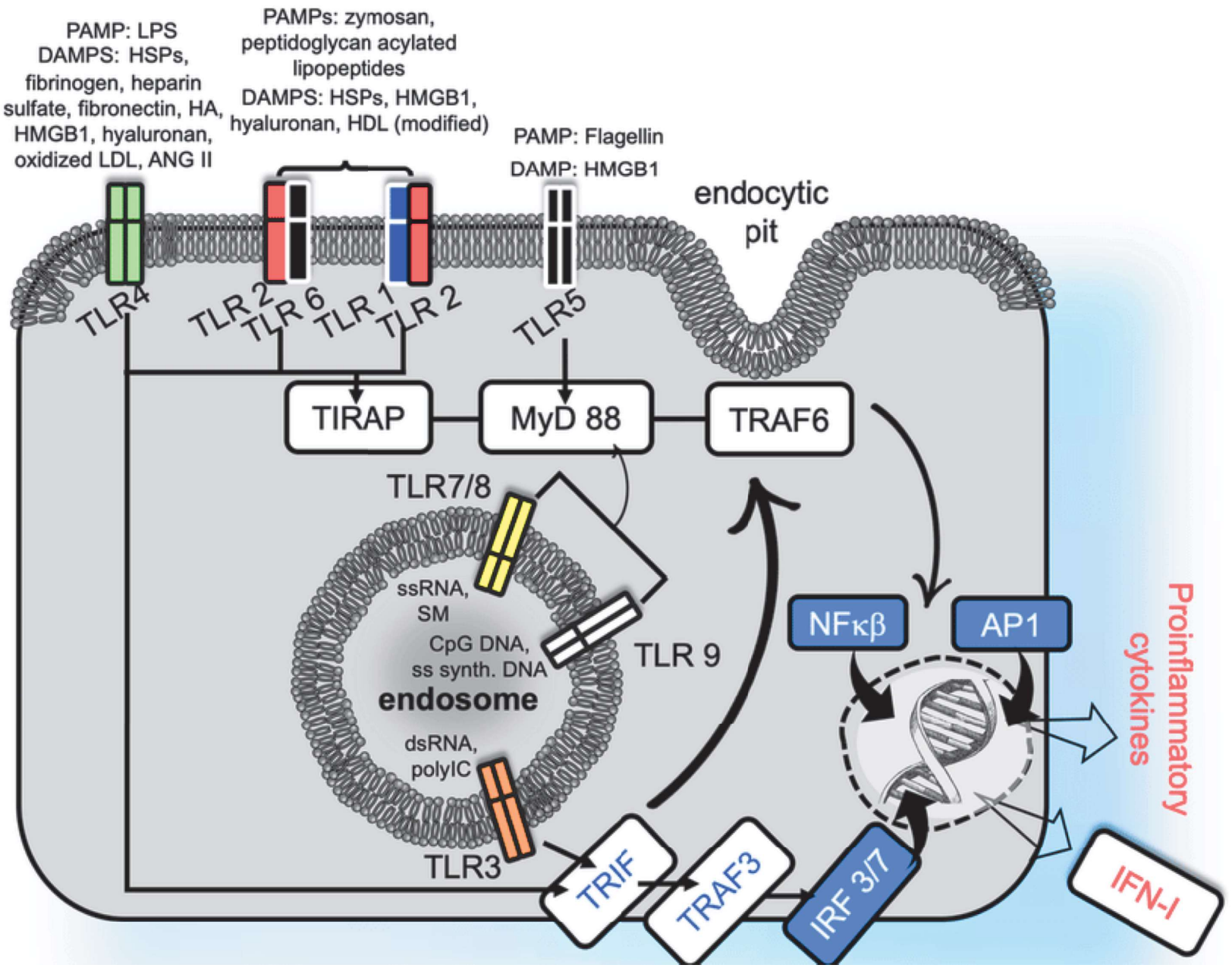
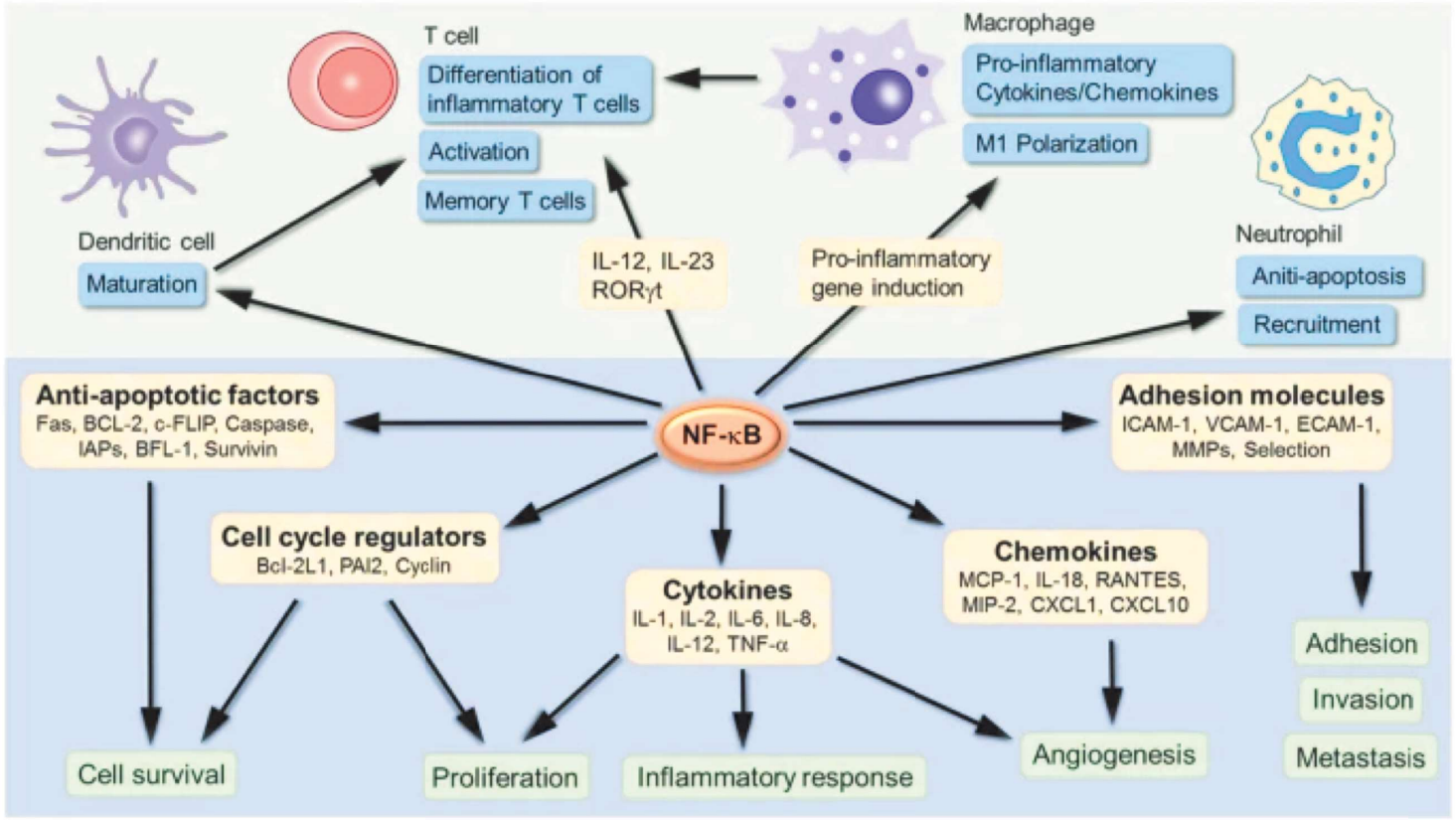
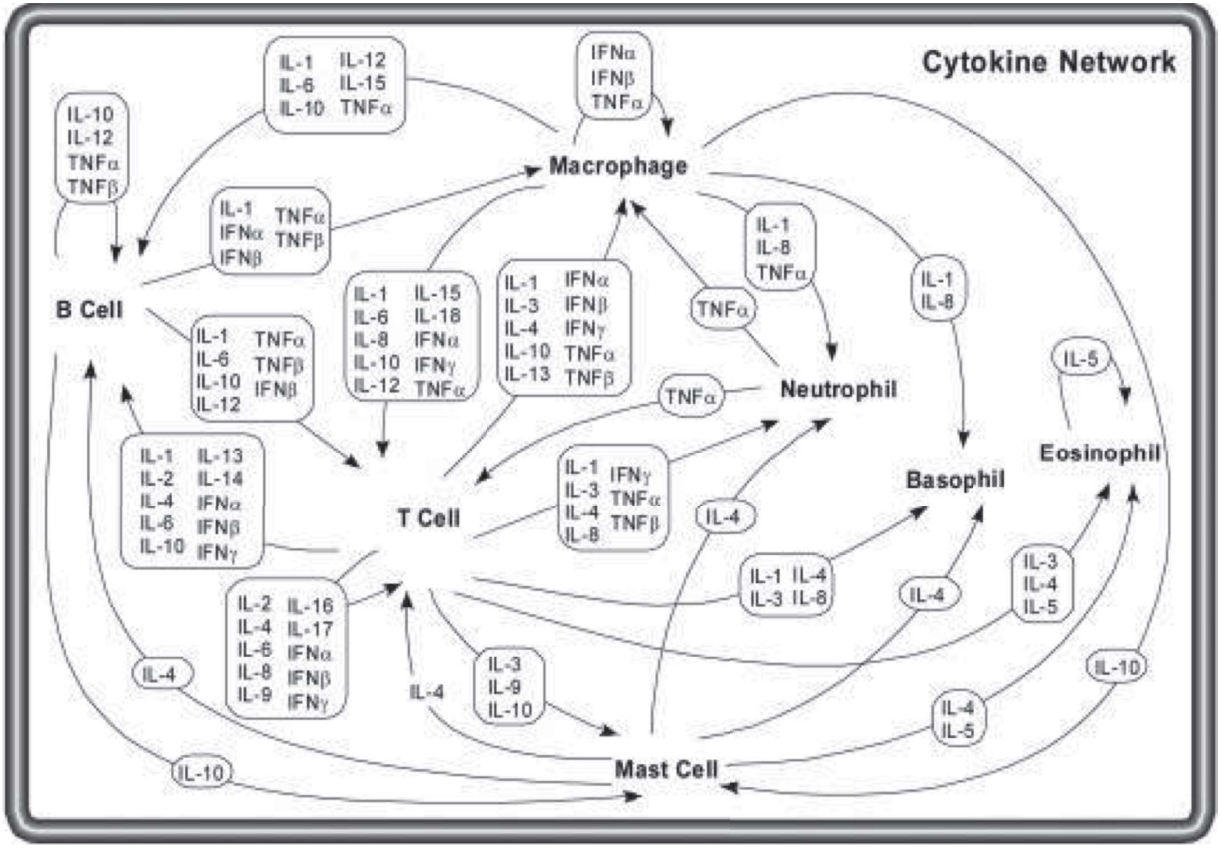

PAMP: LPS
DAMPS: HSPs, fibrinogen, heparin sulfate, fibronectin, HA, HMGB1, hyaluronan, oxidized LDL, ANG II
PAMPs: zymosan, peptidoglycan acylated lipopeptides
DAMPS: HSPs, HMGB1, hyaluronan, HDL (modified)
PAMP: Flagellin
DAMP: HMGB1
endocytic pit
TLR4
TLR 2
TLR 6
TLR 1
TLR 2
TLR5
TIRAP
MyD 88
TRAF6
TLR7/8
ssRNA, SM
CpG DNA, ss synth. DNA
endosome
TLR 9
dsRNA, polyIC
TLR3
TRIF
TRAF3
IRF 3/7
NFκβ
AP1
Proinflammatory cytokines
IFN-I

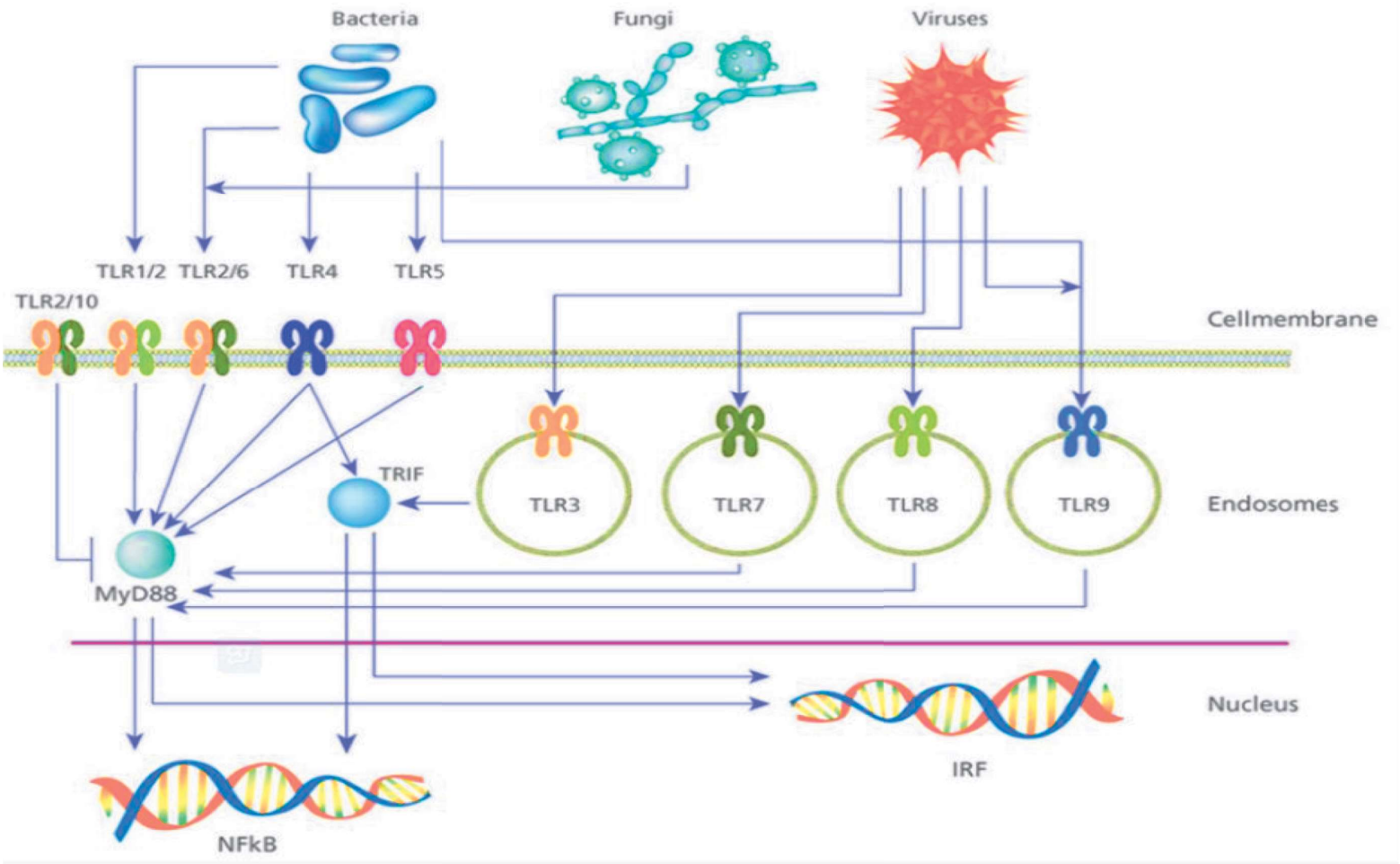
Figure 1



NF-κB target genes involved in inflammation development and progression. NF-κB is an inducible transcription factor. After its activation, it can activate transcription of various genes and thereby regulate inflammation. NF-κB target inflammation not only directly by increasing the production of inflammatory cytokines, chemokines and adhesion molecules, but also regulating the cell proliferation, apoptosis, morphogenesis and differentiation.



- Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells.
- Cytokines could be glycoproteins, polypeptides, or proteins and function as signalling molecules, facilitating and controlling the immune system and inflammation and the process of hematopoiesis
- Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action).
- There are both pro-inflammatory cytokines and anti-inflammatory cytokines.



Different pathogens activate different TLRs. TLRs signal through two different pathways using myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon β, leading to activation of NF-kB and IRF respectively NF-kB leads to DNA transcription and cytokine production, while IRF leads to interferon production. TLRs: toll like receptors; NF-kB: nuclear factor κ-light-chain-enhancer of activated B cells; IRF: interferon regulatory factor

Many viral infections, especially those of RNA viruses, result in the delivery and replication of viral RNA in the cytosol of infected host cells.

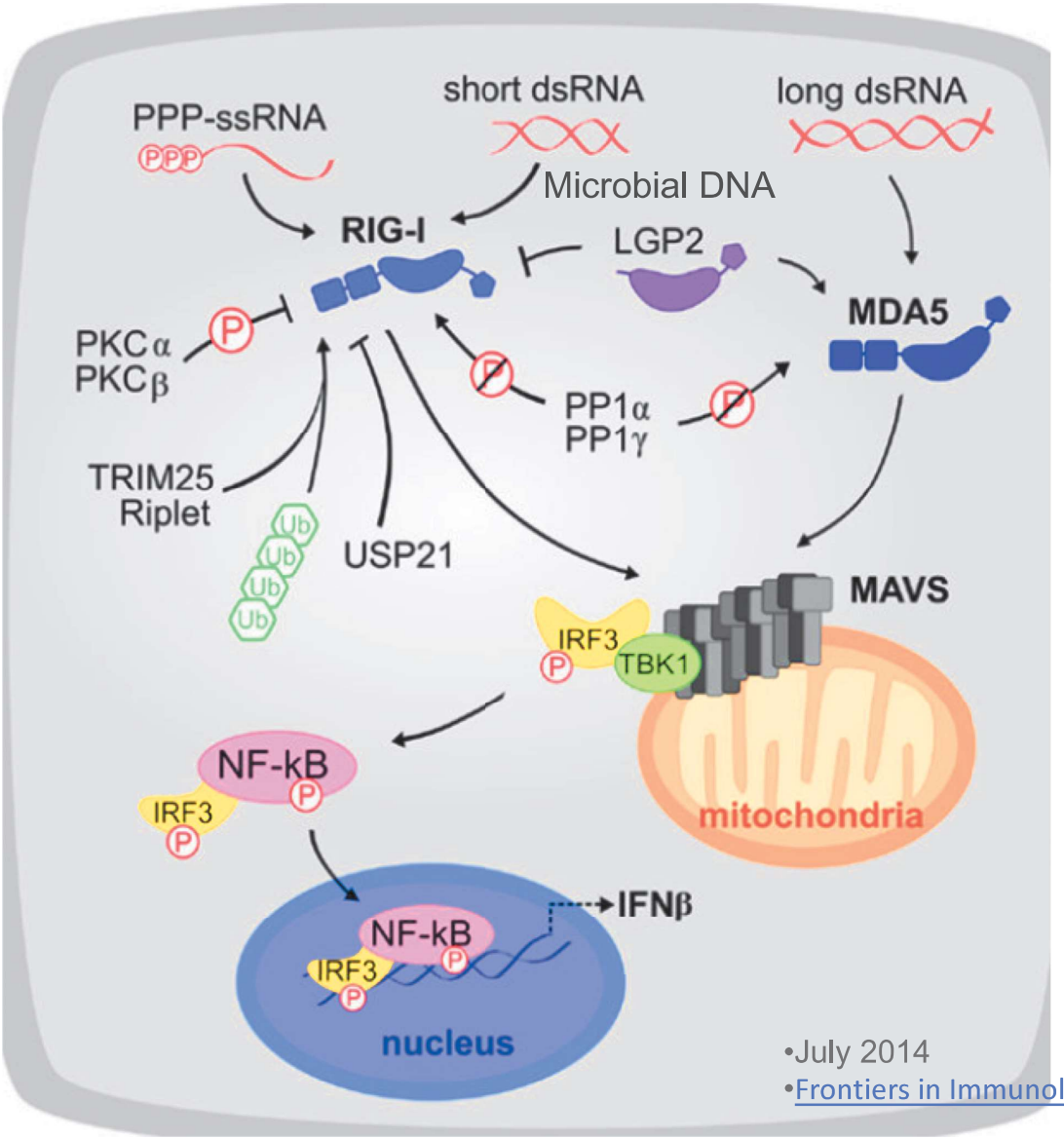
These viral RNAs often contain 5’-triphosphate (5’-ppp) and panhandle-like secondary structures composed of double-stranded segments

RNA polymerase III–mediated conversion of microbial DNA into 5′-triphosphate double-stranded RNA that activates the RNA helicase RIG-I.

RLRs are RNA sensors localized in the cytosol

RIG-I and MDA5 recognize a complementary set of cytosolic viral ss/dsRNA ligands. Their activation is tightly regulated by phosphorylation, ubiquitination, and host proteins such as LGP2.

RIG-I and MDA5 signal to MAVS (mitochondrial protein), which initiates the production of interferon signaling.



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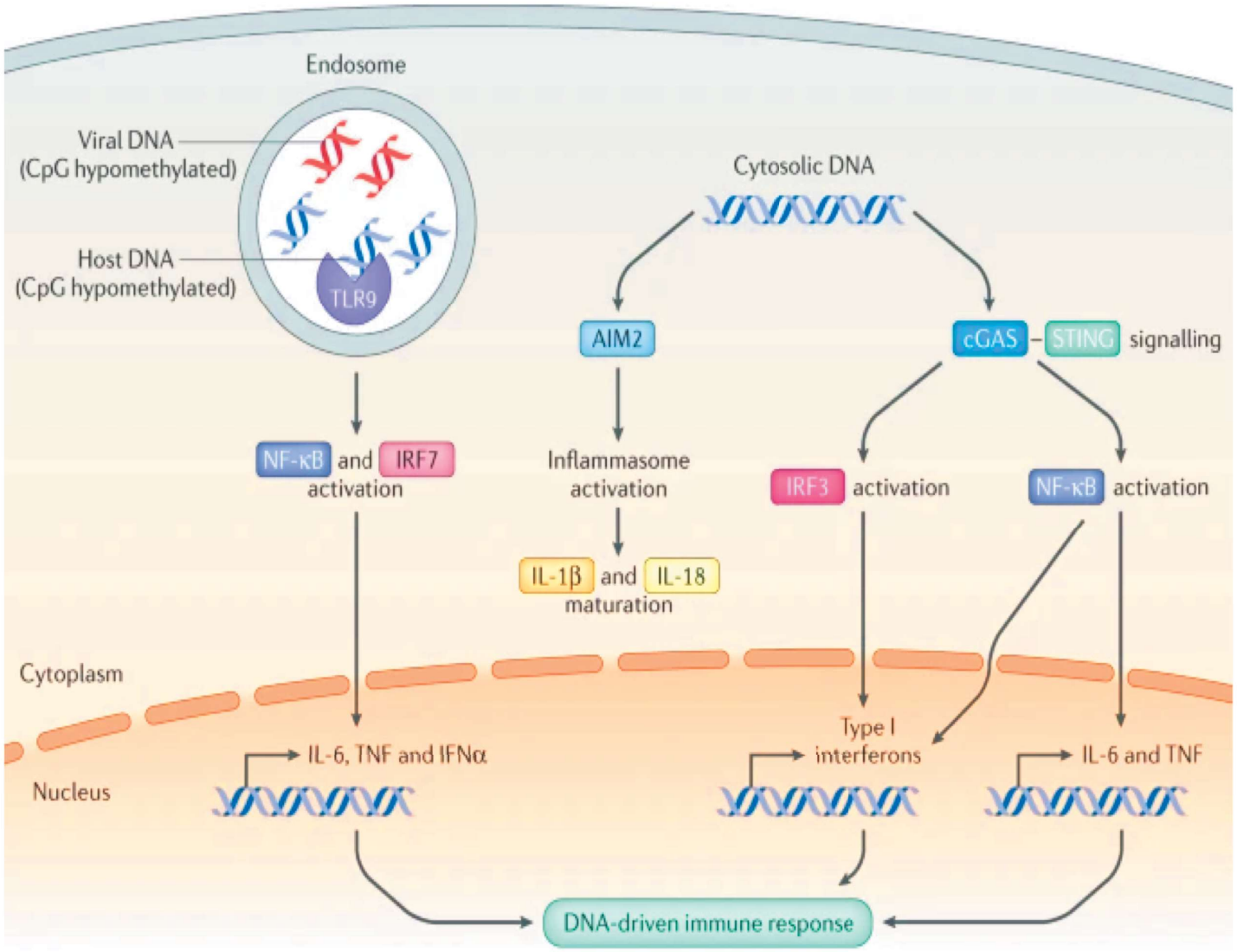
Baltimore classification	Virus family (examples)	RLR
I dsDNA	Herpesviridae (herpes simplex virus type 1; Kaposi's sarcoma-associated herpesvirus; Epstein–Barr virus)	RIG-I and MDA5
	Poxviridae (vaccinia virus)	RIG-I and MDA5
	Adenoviridae (adenovirus)	RIG-I
II ssDNA	No known examples that activate RLRs	NA
III dsRNA	Reoviridae (rotavirus)	RIG-I and MDA5
IV ssRNA (+)	Picornaviridae (encephalomyocarditis virus; rhinovirus; coxsackie B virus)	RIG-I and MDA5
	Flaviviridae (West Nile virus; hepatitis C virus; Zika virus)	RIG-I and MDA5
	Coronaviridae (SARS coronavirus)	RIG-I and MDA5
V ssRNA (–)	Orthomyxoviridae (influenza A virus)	RIG-I
	Paramyxoviridae (measles virus)	RIG-I and MDA5
	Filoviridae (Ebola virus, Marburg virus)	RIG-I and MDA5
VI ssRNA (RT)	Retroviridae (human immunodeficiency virus)	RIG-I and MDA5
VII dsDNA (RT)	Hepadnaviridae (hepatitis B virus)	RIG-I and MDA5

In mammalian cells, the three major DNA-sensing receptors that drive immune responses to foreign DNA are

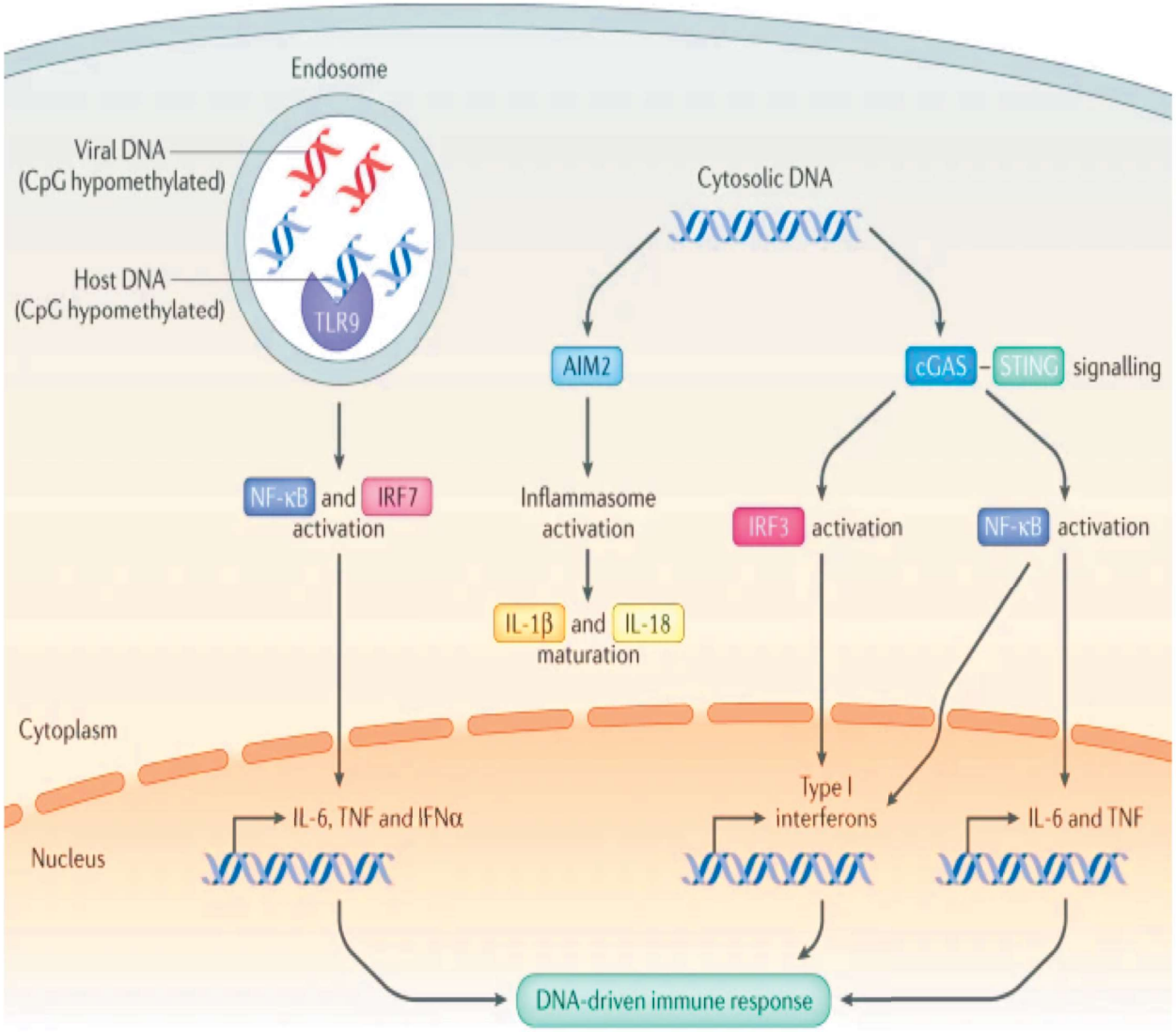
1. Toll-like receptor 9 (TLR9),
2. absent in melanoma 2 (AIM2)
3. cyclic GMP–AMP synthase (cGAS).

Fig. 1: DNA-sensing receptors.



TLR9, which is localized to the endosomal membrane, senses CpG hypomethylated DNA and, in turn, activates the transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor 3/7 (IRF3/7), leading to expression of genes encoding proinflammatory cytokines and interferons, respectively.

Fig. 1: DNA-sensing receptors.

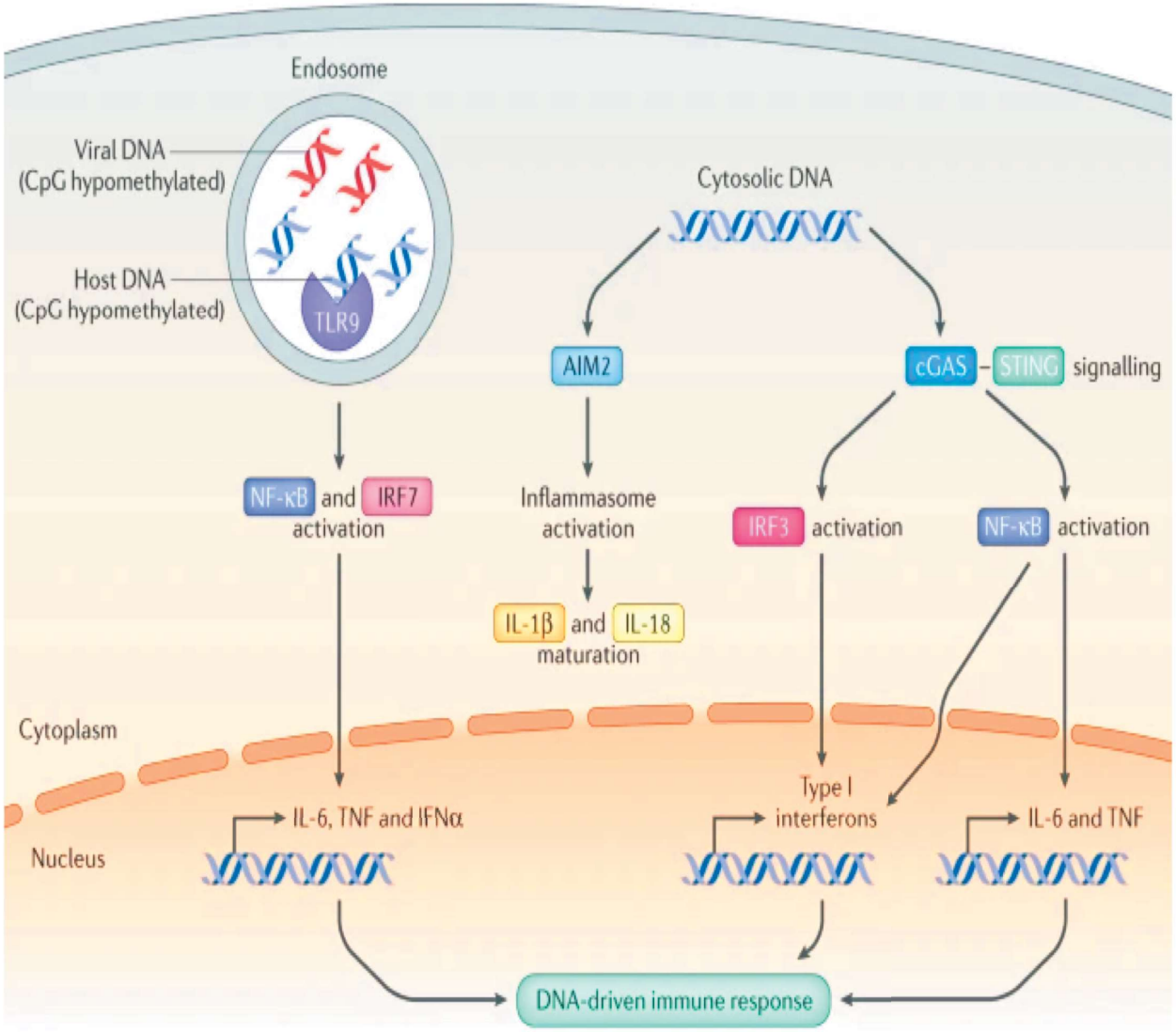


In the cytosol, AIM2 binds to double-stranded DNA, leading to the formation of a multimeric protein complex called the AIM2 inflammasome, which leads to the activation of caspase 1 and the maturation of the proinflammatory cytokines IL-1β and IL-18, and, ultimately, pyroptotic cell death.*

Biochemical studies have shown that 70-base pairs (bp) of dsDNA is the minimum length necessary to activate the AIM2 inflammasome, but 200-bp of dsDNA allows for optimal AIM2 activation.

- Pyroptosis is **an inflammatory cell death** usually caused by microbial infection, accompanied by activation of inflammasomes and maturation of pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18)
- *AIM2 sensing of cytosolic DNA is an important innate immune strategy since the presence of DNA in the cytosol can be indicative of pathogen invasion.

Fig. 1: DNA-sensing receptors.

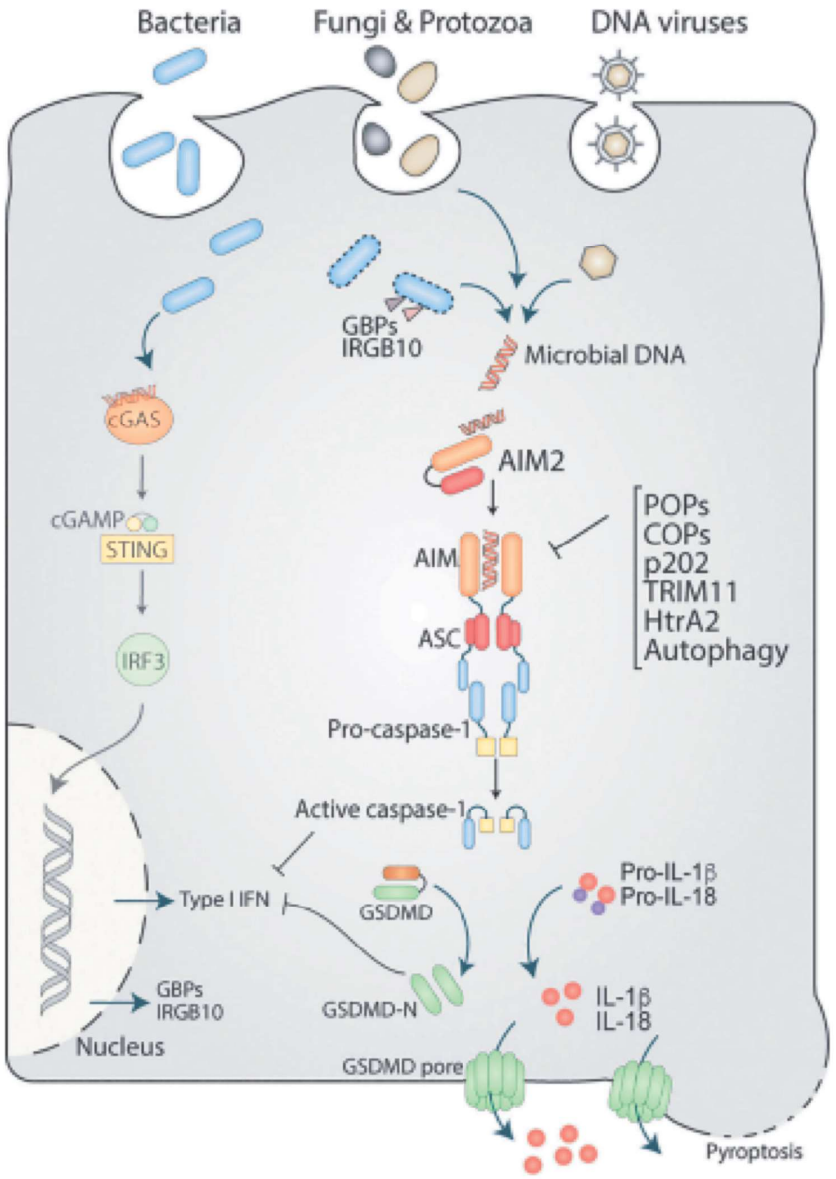


AIM2 (absent in melanoma 2) plays a protective role against various intracellular as well as extracellular microbial infections.

Listeria and *Francisella* are intracellular bacteria that successfully replicate in the cytosol of macrophages and dendritic cells (DCs), and AIM2 senses their DNAs released into the cytosol upon bacterial lysi

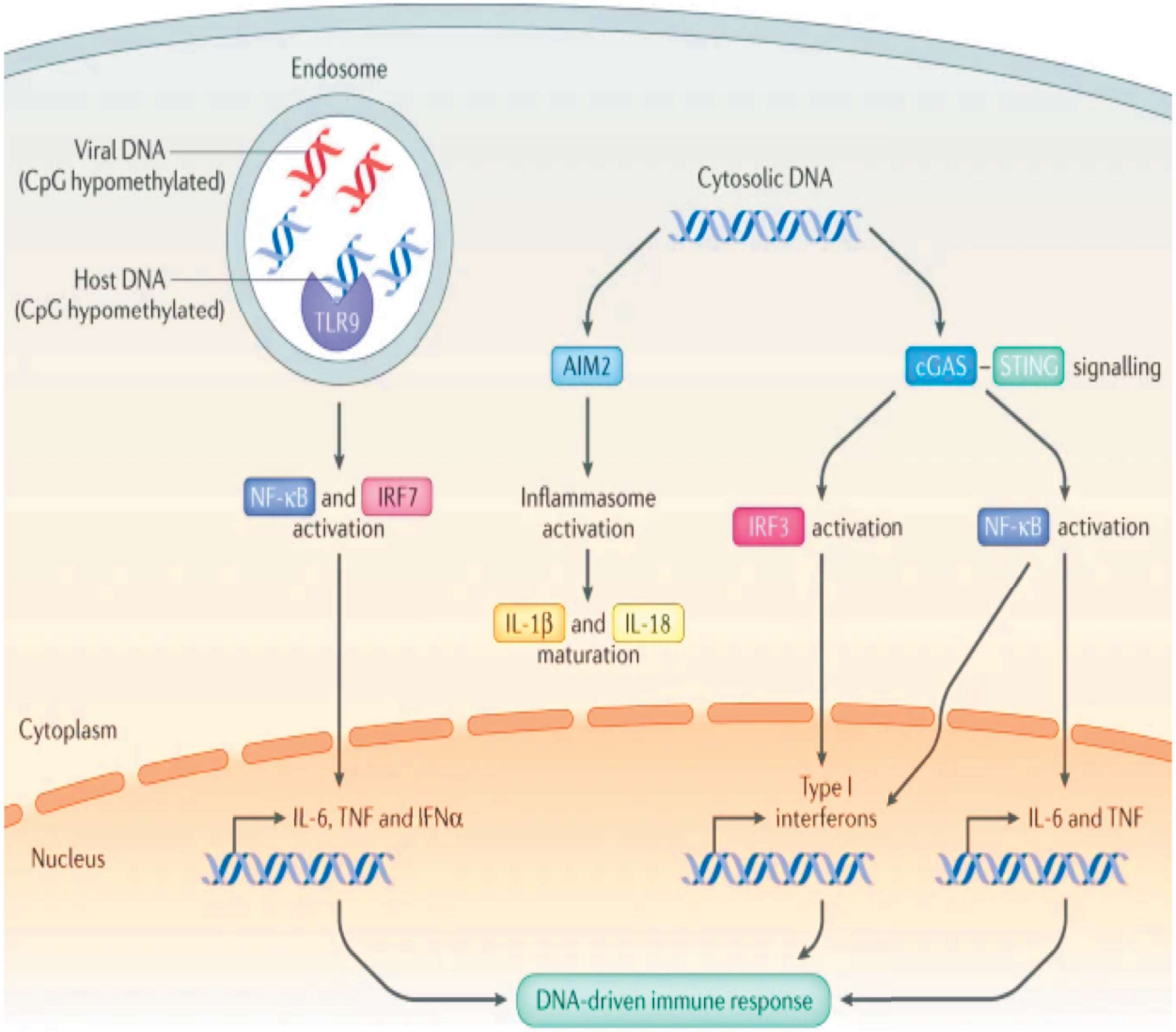
AIM2 inflammasome plays a vital role during DNA viral infections.

Murine cytomegalovirus (MCMV), a herpesvirus of the subfamily beta-Herpesviridae, is sensed by AIM2 in macrophages and DCs leading to caspase-1-dependent IL-1 responses



cGAS activation by cytosolic DNA leads to endogenous generation of **cyclic GMP–AMP**, a unique second messenger, **which binds to** stimulator of interferon genes (**STING**), leading to **activation** of TANK-binding kinase 1 (TBK1) **NF-kB** and **IRF3**, resulting in the transcription of genes encoding **interferons and cytokines**

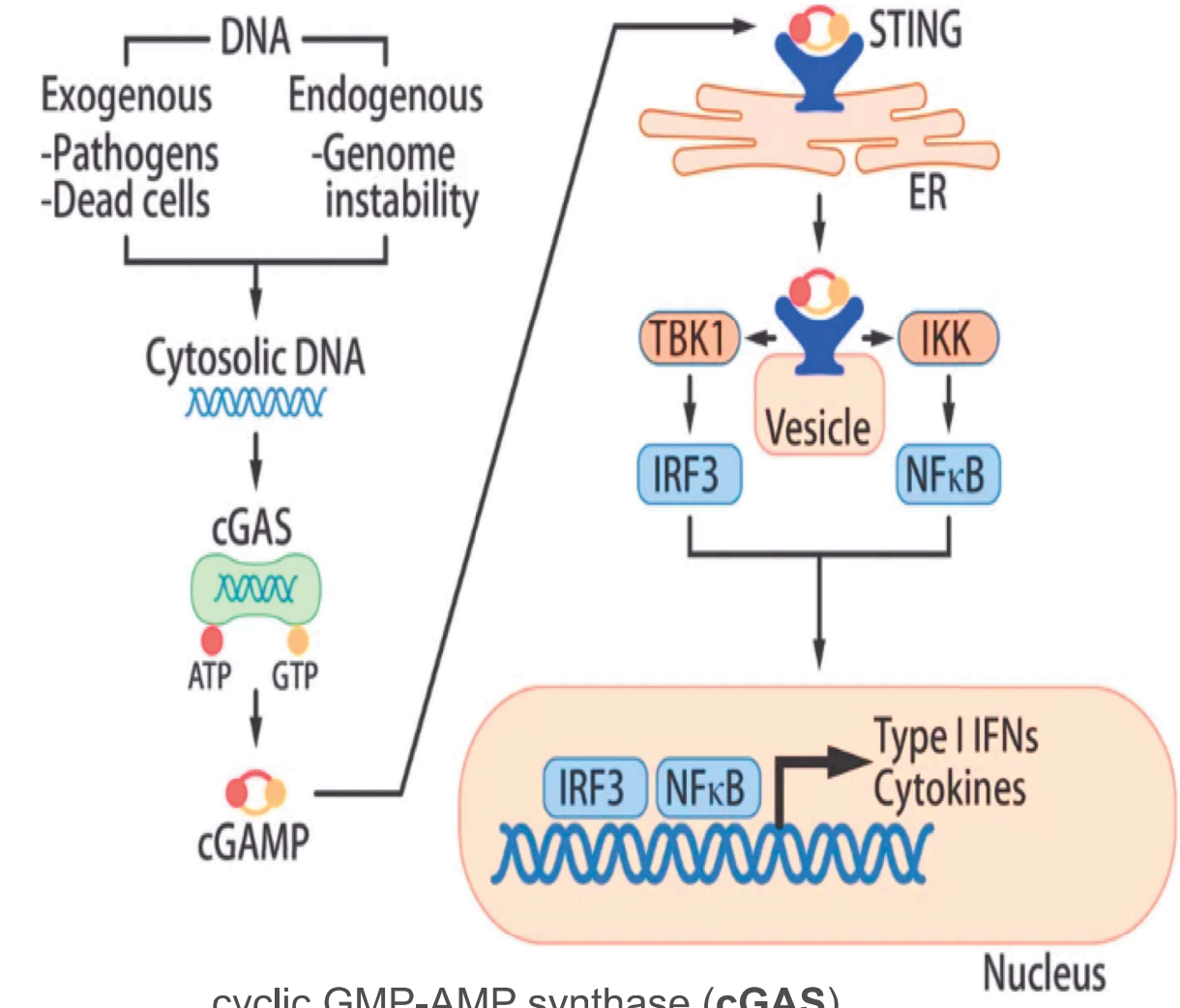
Fig. 1: DNA-sensing receptors.



The cGAS–STING signalling pathway has emerged as a key mediator of inflammation in the settings of infection, cellular stress and tissue damage.

- Abnormal localization of DNA in the cytosol elicits an immune response through the cGAS-STING pathway.
- Cytosolic DNA derives from exogenous (pathogens and dead cells) and endogenous (genome instability or mitochondrial damage) sources.
- cGAS binds to DNA in the cytosol and converts ATP and GTP into 2′3′-cGAMP.
- cGAMP then binds to STING on the ER to trigger STING trafficking to vesicles. cGAMP-bound STING activates the downstream kinases TBK1 and IKK to activate the transcription factors IRF3 and NF-κB, respectively.
- These transcription factors induce expression of type I IFNs and cytokines, which propagate the immune response in an autocrine and paracrine manner.

Fig. 1: The cGAS-STING pathway.



cyclic GMP-AMP synthase (**cGAS**)

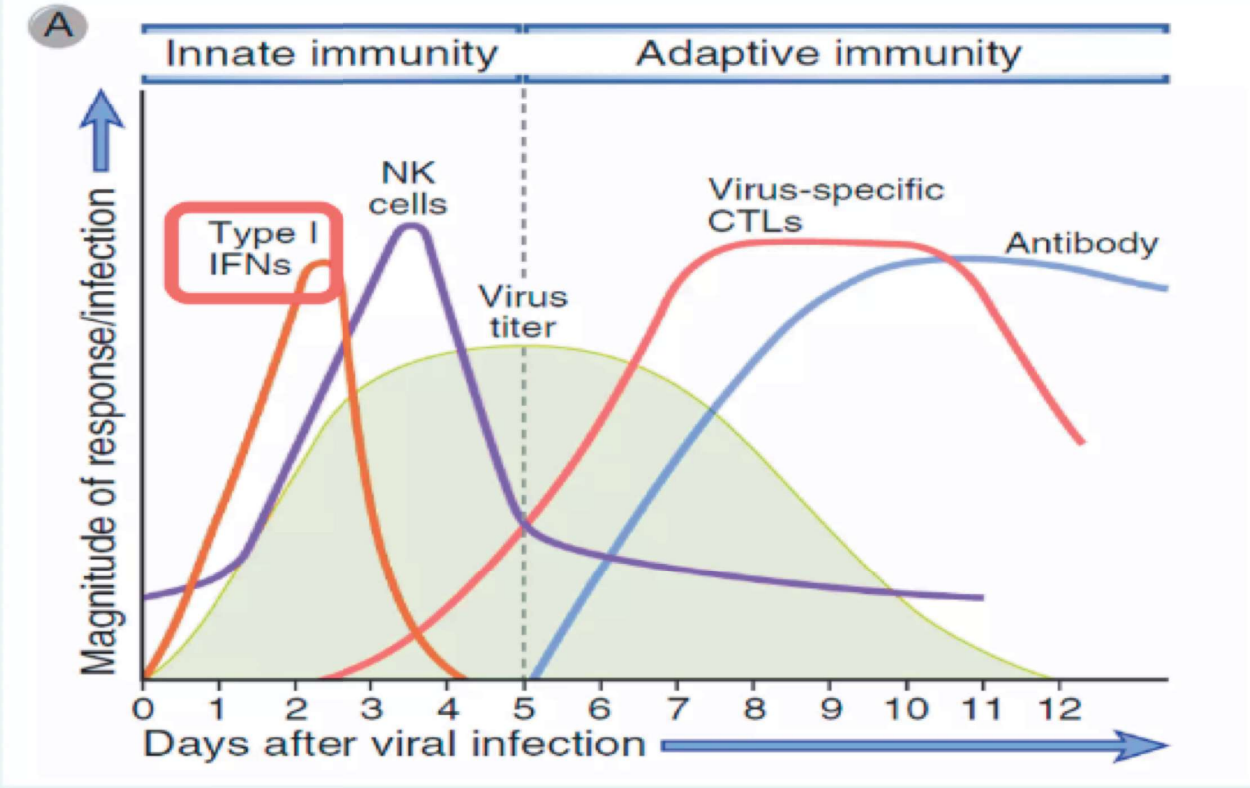
The innate immune response is the first line of defense against an invading pathogen

Two events required to trigger an effective anti-viral/microbial innate immune response are:

a) detection of the invading virus/bacteria by immune system receptors; and

b) initiation of protein signaling cascades that **regulate the synthesis of IFNs** and cytokines

FIGURE 16-7 Innate and adaptive immune responses against viruses.

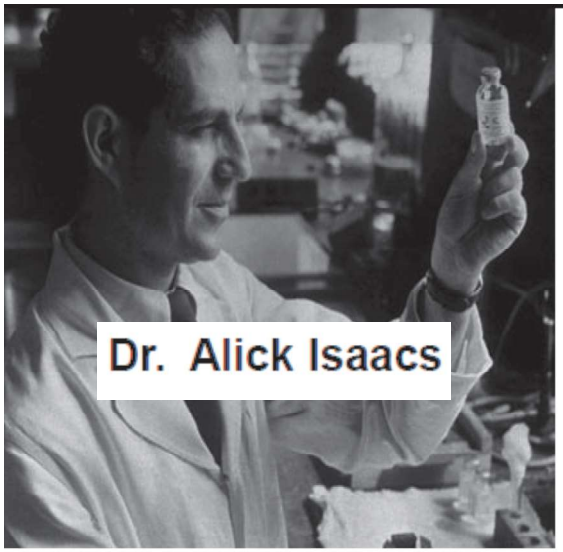


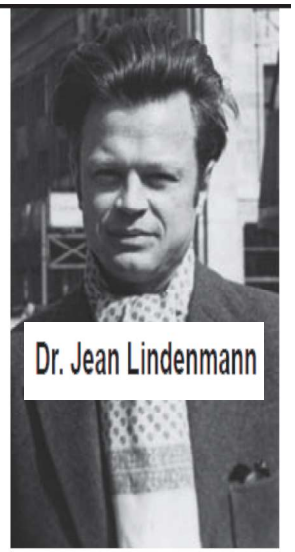
Abbas AK, Lichtman AH, Pillai S, editors. Cellular and molecular immunology. 8th ed. Philadelphia: Elsevier Saunders;2015

INTERFERON COMES ON THE SCENE (1)

Discovery of Interferons

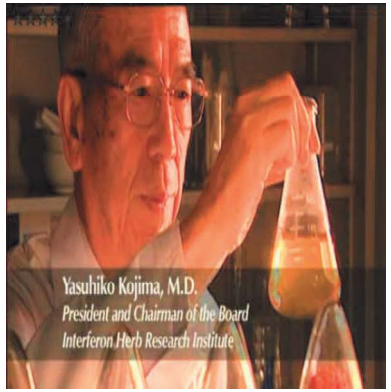
- 1957
- Isaacs and Lindenmann
- Did an experiment using chicken cell cultures
- Found a substance that interfered with viral replication and was therefore named interferon
- Nagano and Kojima also independently discovered this soluble antiviral protein





Proc. Royal Soc. London 147:258-267, 1957
Proc. Royal Soc. London 147:268-273, 1957

1957



C R Seances Soc Biol Fil. 152(11):1627-9, 1958
C R Seances Soc Biol Fil. 152(2):372-4,1958

1958

*National Institute for Medical Research (NIMR) in London

Type I IFN: the first cytokine

Alick Isaacs Jean Lindenmann

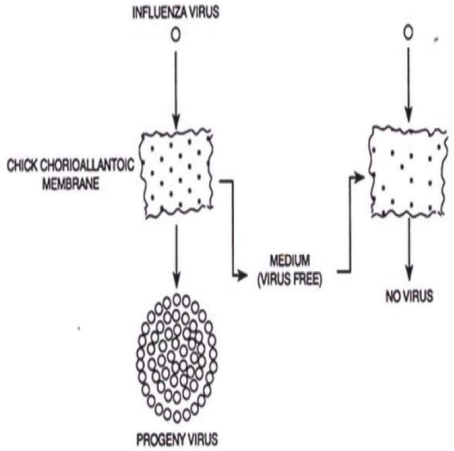


FIG. 11.14. Discovery experiement.

Isaacs, A., and Lindenmann, J., *Proc. Roy. Soc.*, B, **147**, 258 (1957)

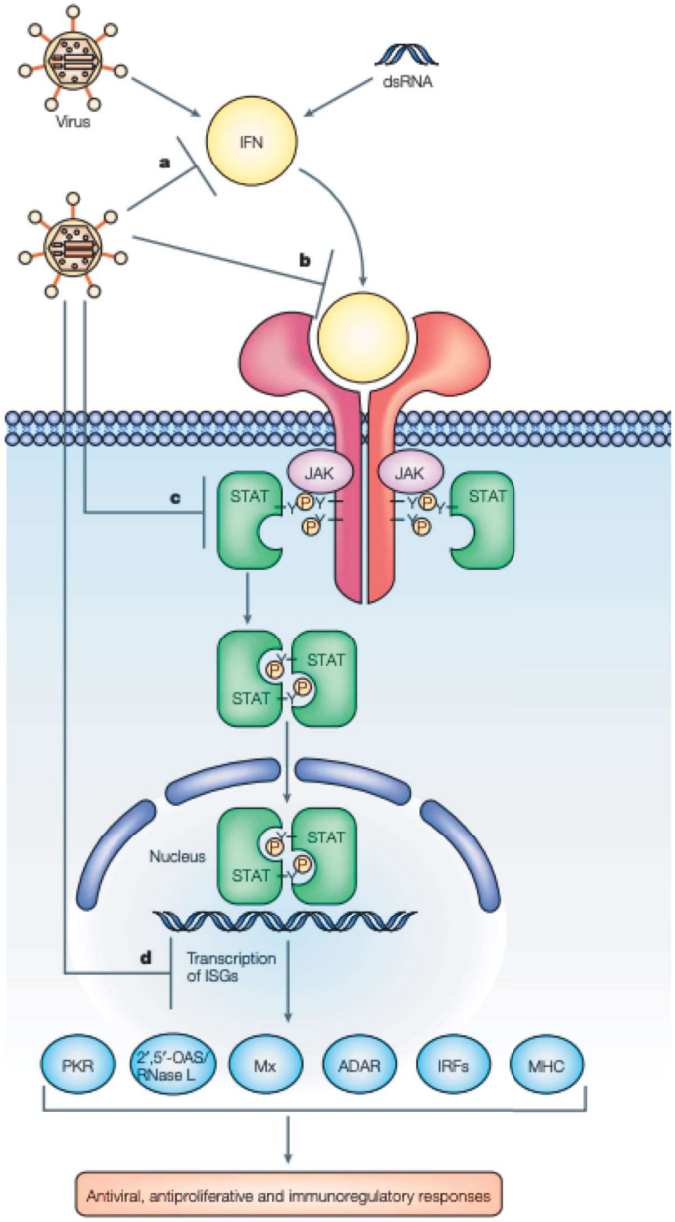
Memorial Sloan Kettering Cancer Center

Definition of Interferons

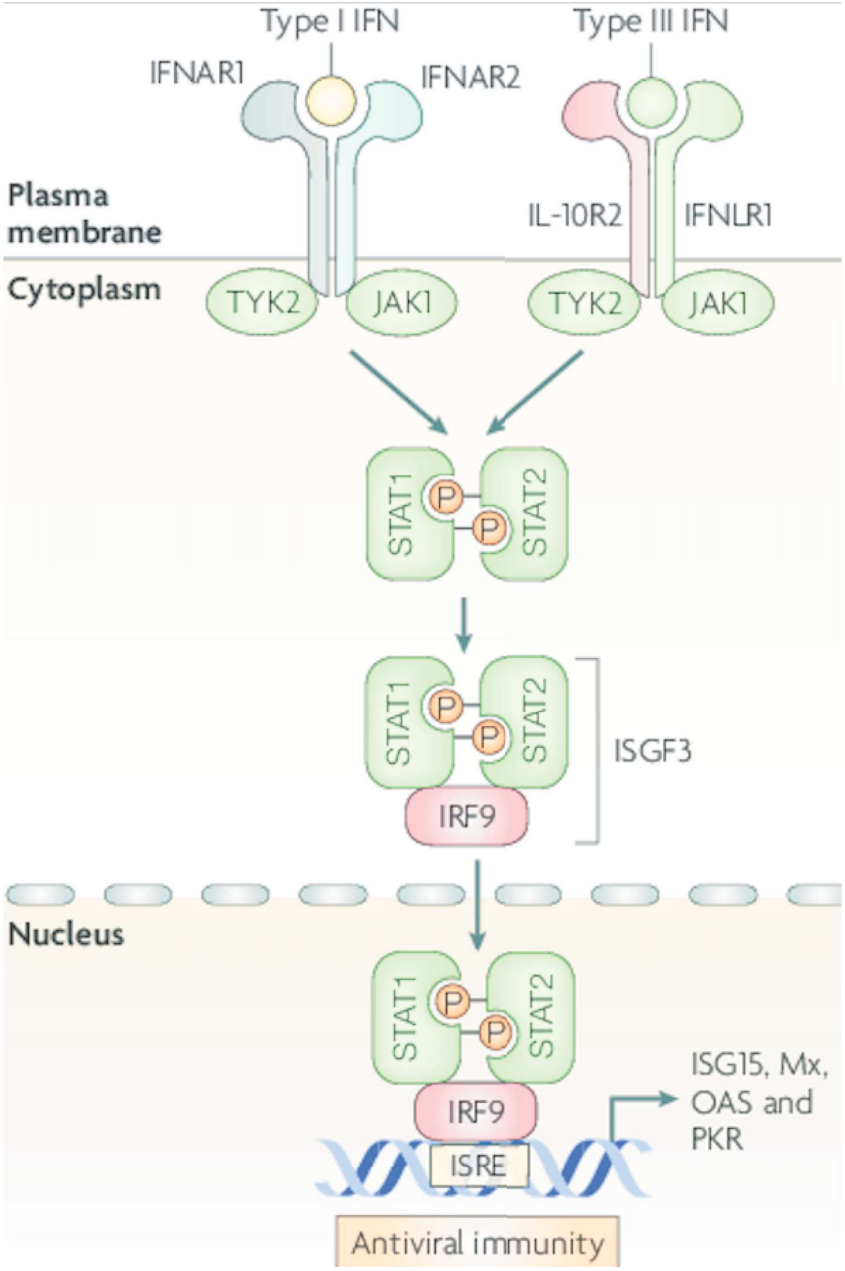
Interferons(INF) are set of proteins which are released by virus infected cells in vivo and which reacts with uninfected cells so as to render them resistant to infection to virus.

In 1957, Isaacs and Lindenmann reported a secreted factor termed “interferon” that could induce a virus-resistant state in chick cells after influenza virus infection

- Interferons (IFNs) are a group of antiviral cytokines that are induced during viral infection by viral replication products, such as double-stranded (ds)RNA.
- IFNs **exert their biological functions by binding to specific cell-surface receptors.**
- **In turn, this triggers the intracellular IFN signalling pathway — mainly the JAK–STAT pathway — which induces the expression of a large number of IFN-stimulated genes (ISGs)**.
- The ISGs, the workhorses of the IFN response, set up an antiviral, antiproliferative and immunoregulatory state in the host cells.

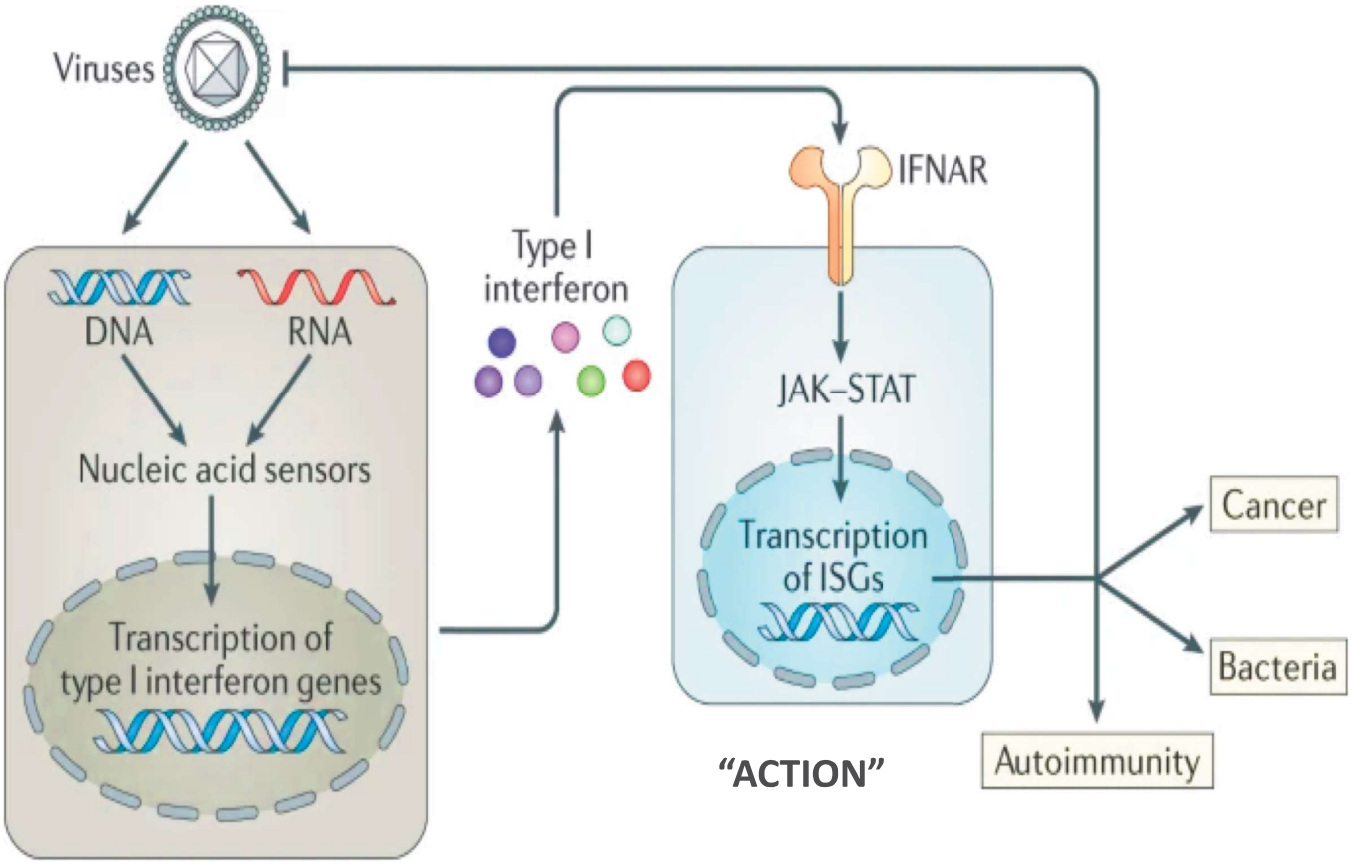


IFN binds to its receptor and thereby activates Tyk2 and Jak1, which then phosphorylate Stat1 and Stat2.
Together with IRF9, Stat1 and Stat2 form the ISGF3, which translocates into the nucleus and induces the expression of ISGs.



- Type I IFNs are primarily induced by the activation of pattern-recognition receptors, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and cytoplasmic DNA sensors
- Nucleic acid sensors of the innate immune system recognize unusual DNA and RNA molecules, for example, viral genomes
- This results in the triggering of an intracellular signalling cascade that transcriptionally induces the genes encoding type I interferons, which are subsequently secreted.
- Type I interferons act in an autocrine (not shown) and paracrine manner by binding to their receptor, interferon-α/β receptor (IFNAR), which activates the Janus kinases (JAKs).
- These in turn activate the transcription factors signal transducer and activator of transcription 1 (STAT1) and STAT2, leading to expression of interferon-stimulated genes (ISGs).
- Several ISGs have direct antiviral effects.
- Type I interferons and ISGs also play important roles in bacterial infections and cancer; these effects can be both beneficial and detrimental, depending on the setting.
- Production of type I interferons and ISGs over extended periods of time can lead to autoinflammatory and autoimmune diseases.

Fig. 1: The type I interferon system.



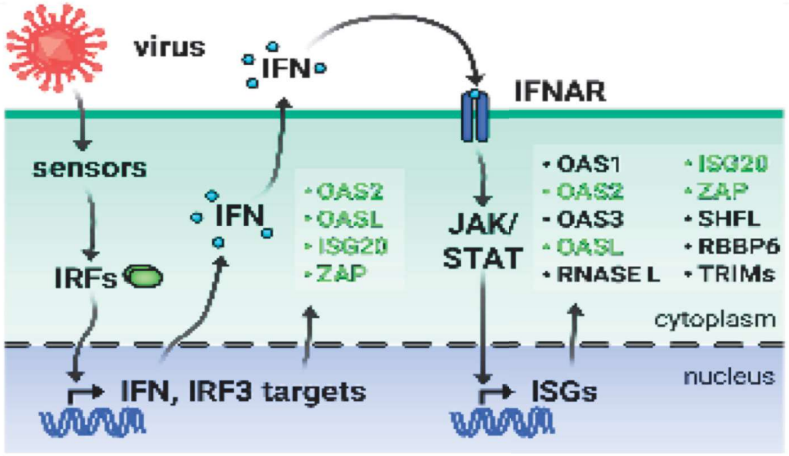
Nature Reviews Immunology **volume 20**, pages537–551 (2020)

Gene	Protein function	Mechanism of action	Refs
OAS, RNASEL	RNA cleavage	Degrades viral and cellular RNA, induces IFN-α/β	81–91
PKR	EIF2α phosphorylation	Blocks protein synthesis, transcriptional signalling	94–98
p56	Binds EIF3	Blocks protein synthesis	99,100
Mx1	Wraps around viral nucleocapsids	Interferes with intracellular trafficking	101–104
ISG15	ISGylation	Cytokine-like, protein modification	105–107
PLSCR1	Phospholipid migration, DNA binding	Enhances expression of some ISGs	110–112
TRAIL/APO2L	Ligand of death receptor	Apoptosis	113–115
XAF1	Blocks inhibitor of apoptosis (XIAP)	Apoptosis	247
G1P3 (6–16)	Inhibits caspase 3	Anti-apoptotic	116,117
ISG12	Not known	Antiviral	119,120
GBP1	GTPase	Antiviral, angiogenesis inhibitor	121,268
ISG20	3′-exonuclease for RNA and DNA	Antiviral	122
PML	Not known	Antiviral, antitumor	123
ADAR1	Adenosine deaminase for dsRNA	RNA editing, altered translation	124
Viperin (cig5)	Not known	Antiviral	125
iNOS	Nitric oxide synthase	Antiviral	126
Nup98/Nup96	Nucleoporin, RNA and protein transporters	Antiviral	127,270
IRF7, RIG-I, MDA5, STAT1	Signalling to IFN-α/β genes or to ISGs	Induction of type I IFNs	79,80

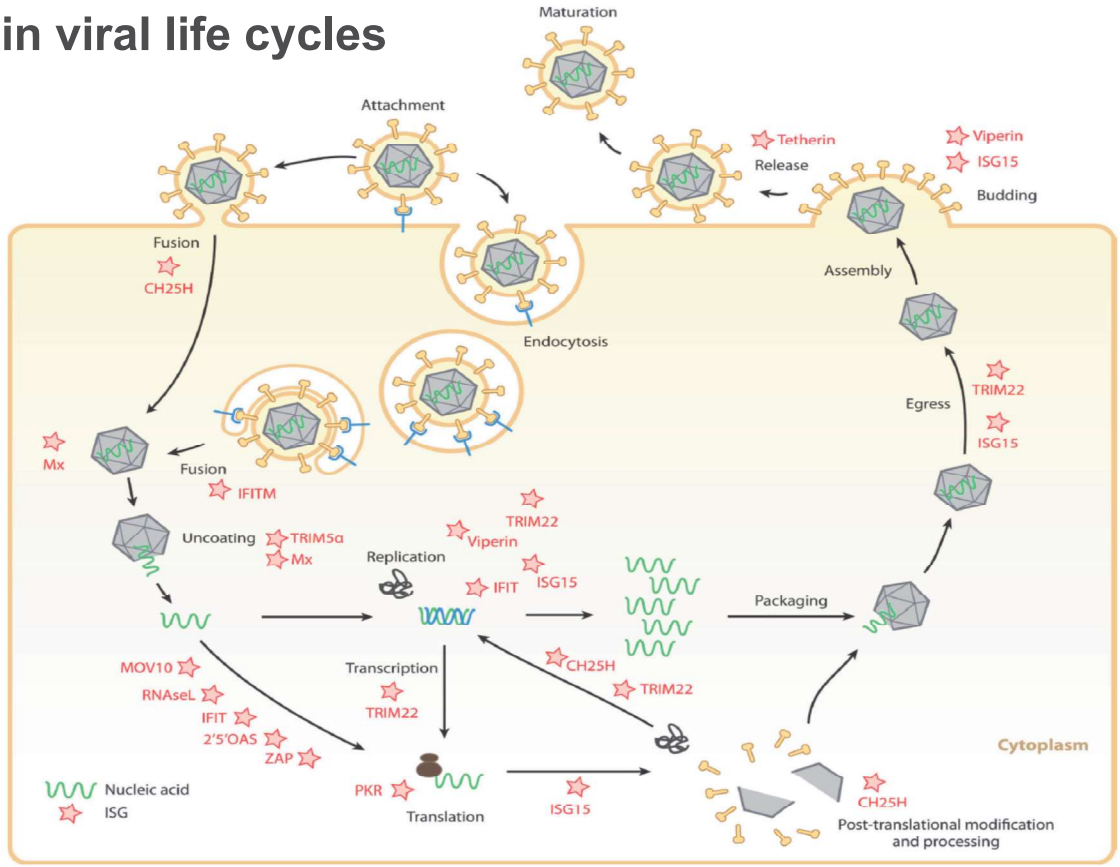
ADAR1, adenosine deaminase, RNA-specific; dsRNA, double-stranded RNA; EIF2α/3, eukaryotic initiation factor 2α/3; *G1P3*, interferon, α-inducible protein; *GPB1*, guanylate binding protein 1, interferon-inducible, 67kDa; *iNOS*, inducible nitric oxide synthase; *IRF7*, interferon regulatory factor 7; ISGs, interferon-stimulated genes; *MDA5*, melanoma differentiation associated protein 5 (also known as *IFIH1*); *Mx1*, myxovirus (influenza virus) resistance 1, interferon-inducible protein p78; *Nup98/Nup96*, nucleoporin 98/96 kDa; *OAS*, 2′-5′-oligoadenylate synthetase; *PKR*, protein kinase R; *PLSCR1*, phospholipid scramblase 1; *PML*, promyelocytic leukaemia; *RIG-I*, retinoic acid-inducible gene I (also known as *DDX58*); *RNASEL*, ribonuclease L; *STAT1*, signal transducer and activator of transcription 1, 91 kDa; *TRAIL/APO2L*, tumour necrosis factor-related apoptosis-inducing ligand (also known as *TNFSF10*); *Viperin* (cig5), also known as *RSAD2*; *XAF1*, X-linked inhibitor of apoptosis-associated factor 1; XIAP, X-linked inhibitor of apoptosis protein (also known as *BIRC4*).

ISGs are a diverse group of more than 300 genes that mediate the biological effects of IFN stimulation

Targets for ISGs within viral life cycles



- Many so-called ISGs are also direct targets of interferon regulatory factors (IRF1, IRF3, IRF7), NFκB, or IL-1 signaling. These ISGs can be induced even in the absence of IFN signaling.



- More than 300 ISGs genes have been identified, some were found to contribute to host defense, while other genes contributed to inflammation, signaling, transcription, and immunomodulation, among other activities

- Many ISGs were capable of inhibiting virus replication, with some acting on a wide range of viruses, while others were only effective against particular viruses.

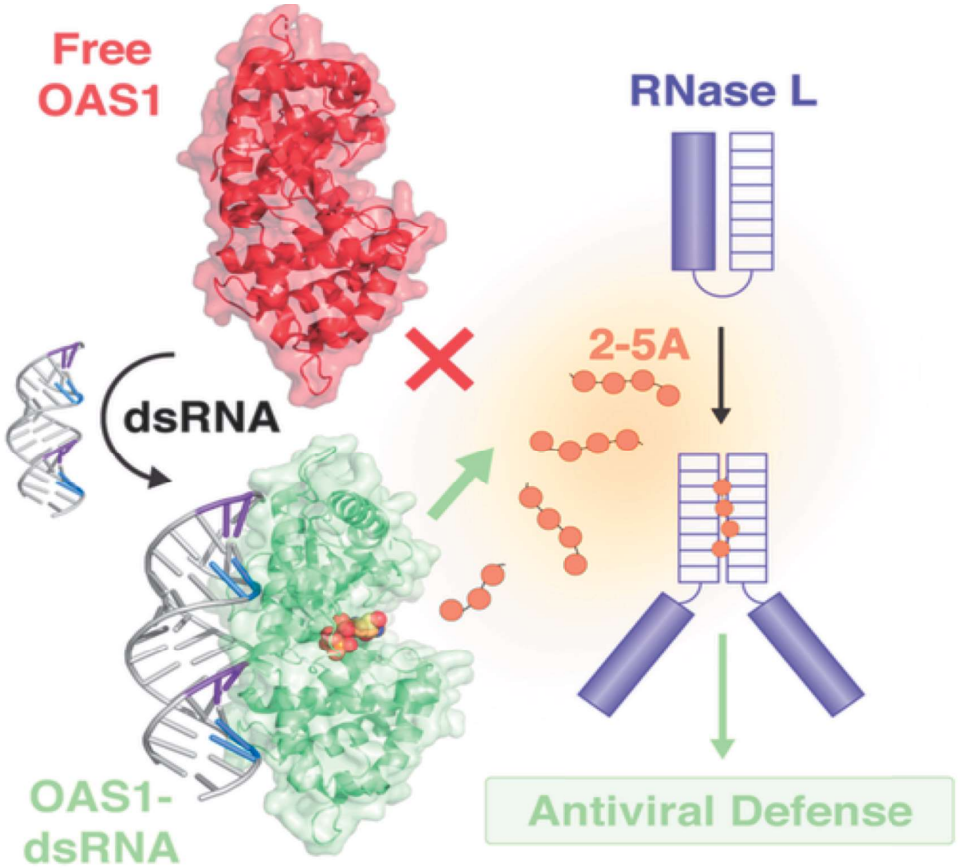
Annu Rev Immunol. 2014

The 2′-5′-oligoadenylate synthetase/ribonuclease L (OAS/RNase L) pathway is a critical component of innate immunity: double-stranded RNA (dsRNA) binding to OAS drives activation of 2′-5′-oligoadenylate (2-5A) synthesis to trigger the antiviral properties of RNase L.

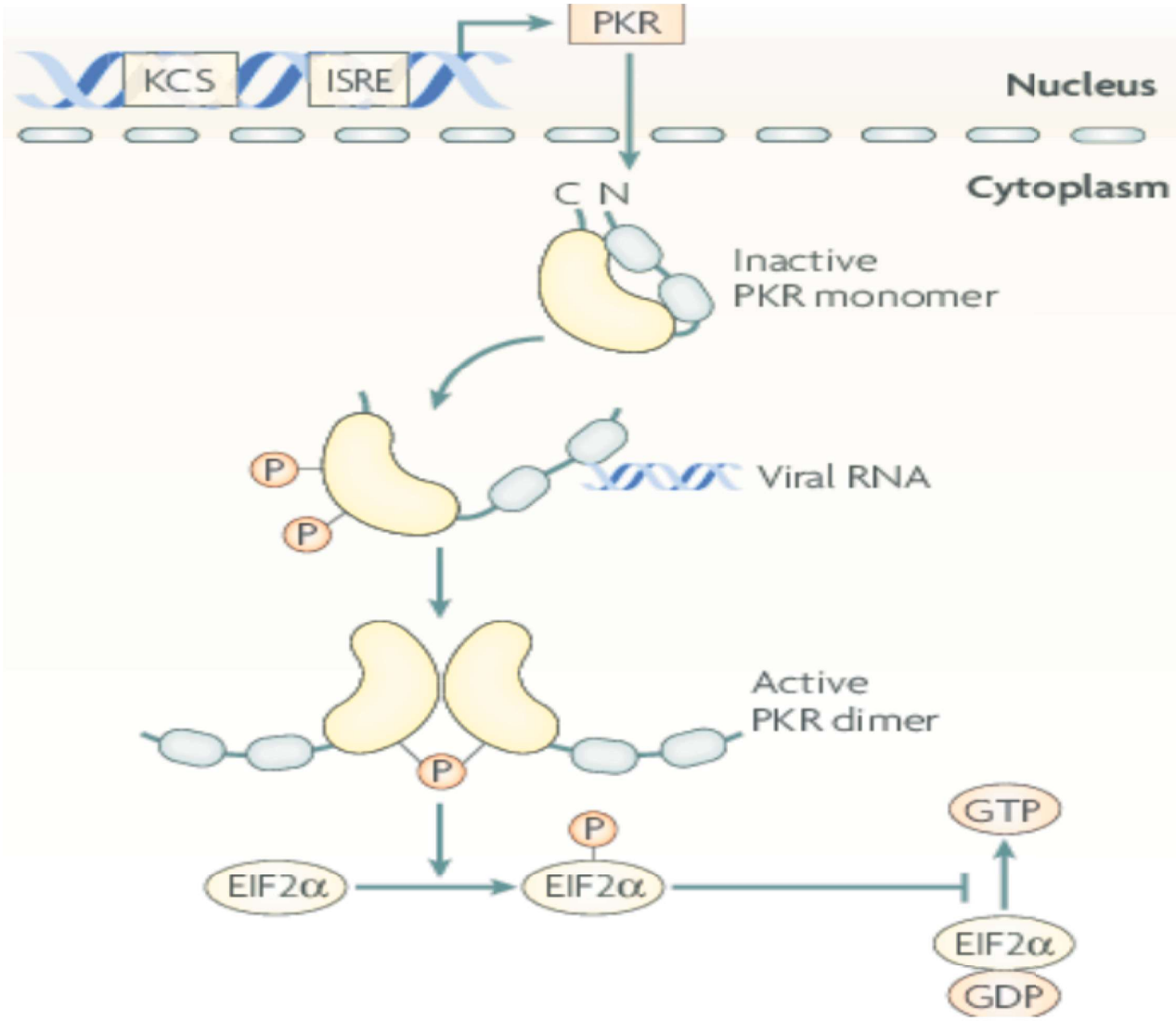
1. Viral dsRNA can directly activate one of several human OAS proteins to produce a unique 2′-to-5′ linked oligoadenylate of 3–6 bases (2–5A) from ATP.

2. The only well-established function of 2–5A is activation of the ubiquitous, latent enzyme, RNASEL.

3. 2–5A binding to RNASEL induces monomeric, inactive RNASEL to dimerize into a potent endoribonuclease that cleaves single-stranded regions of RNA



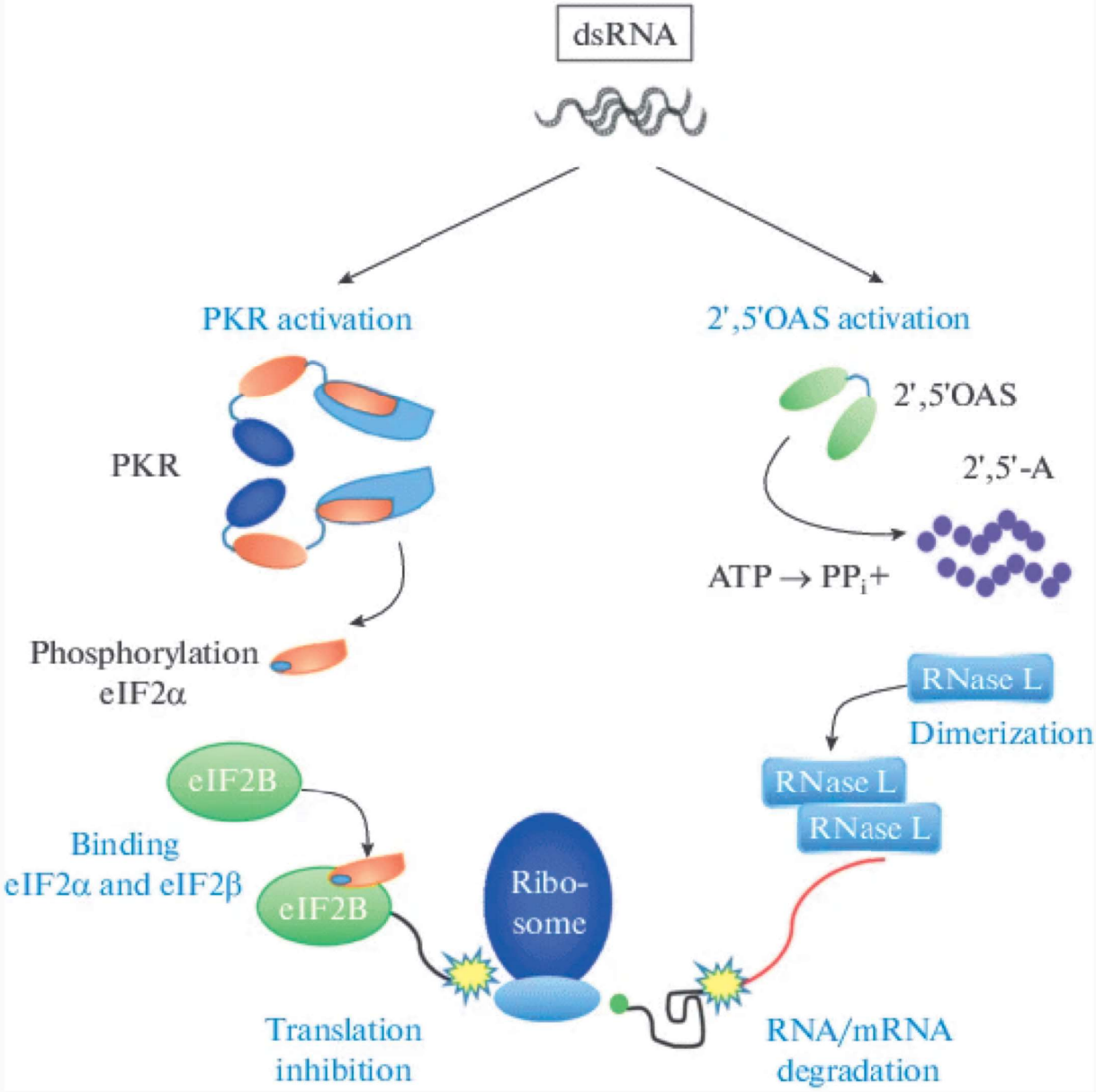
- PKR is a serine/threonine kinase that mediates translational control in response to dsRNA and other signals
- PKR mediates translational control by phosphorylating the protein synthesis initiation factor EIF2α, resulting in an inactive complex between EIF2–GDP and the recycling factor, EIF2B
- These events produce global inhibition of protein synthesis that blocks viral replication



Biochemical mechanisms of dsRNA-induced inhibition of translation and protein synthesis.

DsRNA activated, IFN-induced protein kinase R (PKR) inhibits mRNA translation via phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF-2α) and complex formation with eIF2B.

Initiation of 2′-5′-oligoadenylate synthesis from ATP by dsRNA-activated 2′-5′-oligoadenylate synthetase (2′-5′OAS) leads to the dimerization of RNase L and non-specific RNA degradation.



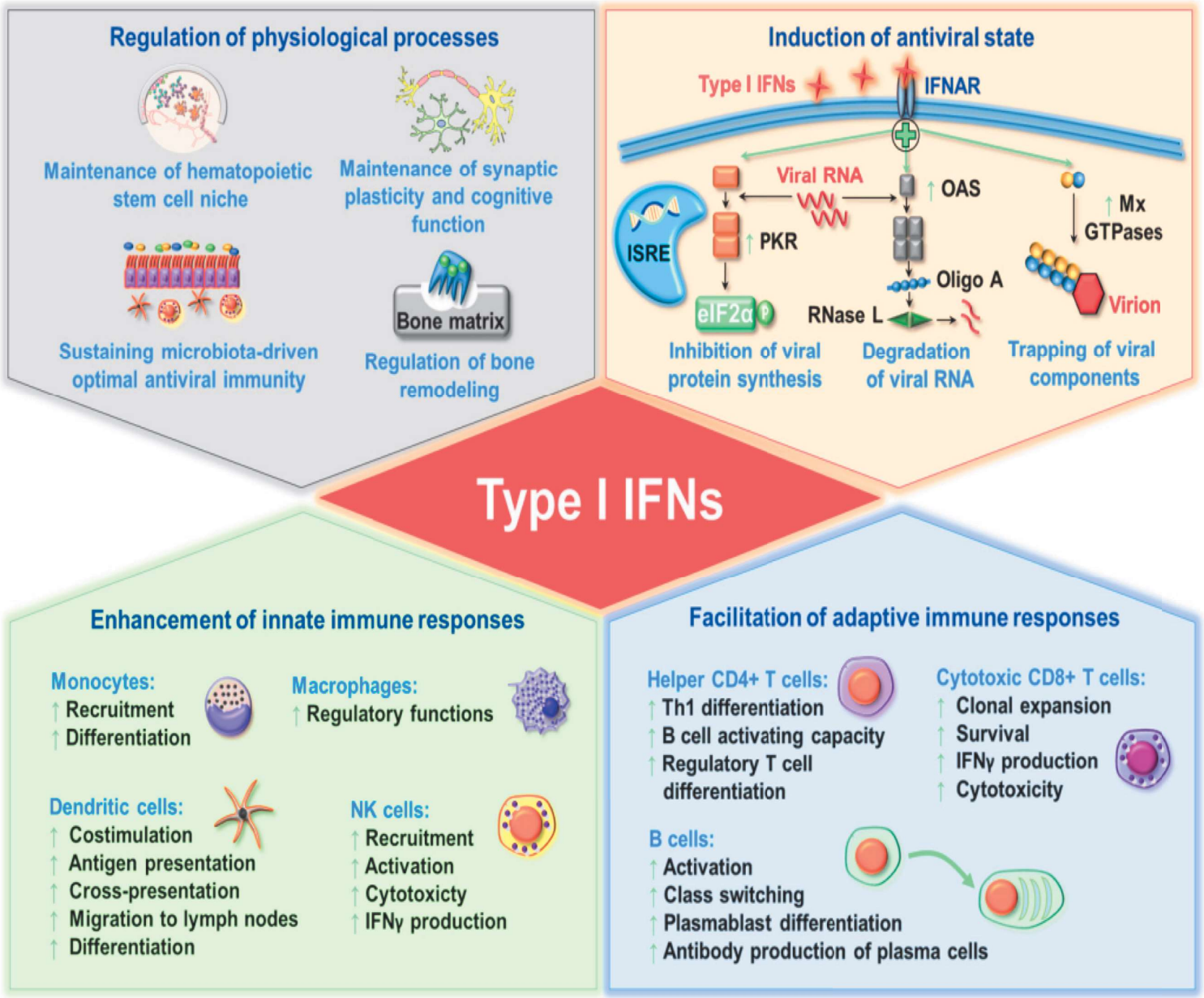


Figure 1. The pleiotropic effects of type I interferons (IFNs). Continuous baseline production of type I IFNs by various tissues and cells fine-tunes a wide variety of physiological processes including hematopoietic stem cell functions, synaptic plasticity, bone remodeling and immune homeostasis. In addition, the microbiota-induced basal IFN-signature prepares stromal and immune cells for upcoming infections (**upper left panel**). Upon viral infection, type I IFN signaling induces antiviral state in all nucleated cells via the upregulation of IFN-stimulated genes that inhibit the replication and spreading of viruses (**upper right panel**). Type I IFNs also control the cells of innate (**lower left panel**) as well as adaptive (**lower right panel**) immune system by shaping the activation, differentiation, effector functions and trafficking of these cells. *eIF2α: eukaryotic initiation factor 2α; IFN: interferon; IFNAR: interferon-alpha/beta receptor; ISRE: IFN-stimulated response element; Mx GTPase: myxovirus resistance guanosine triphosphatase; NK: natural killer; OAS: 2'-5' oligoadenylate synthetase; Oligo A: 2'-5'-oligoadenylate; PKR: protein kinase R; Rnase L: ribonuclease L; Th: T helper.*

Impact of Type I Interferons on Susceptibility to Bacterial Pathogens

- Type I interferon (IFN) signaling can be detrimental or beneficial to the host during bacterial infections and this varies between species and by infection site.

- Bacterial factors can directly modulate type I IFN signaling and its downstream effects.

- Significant diversity is seen between strains of the same species to activate this response.

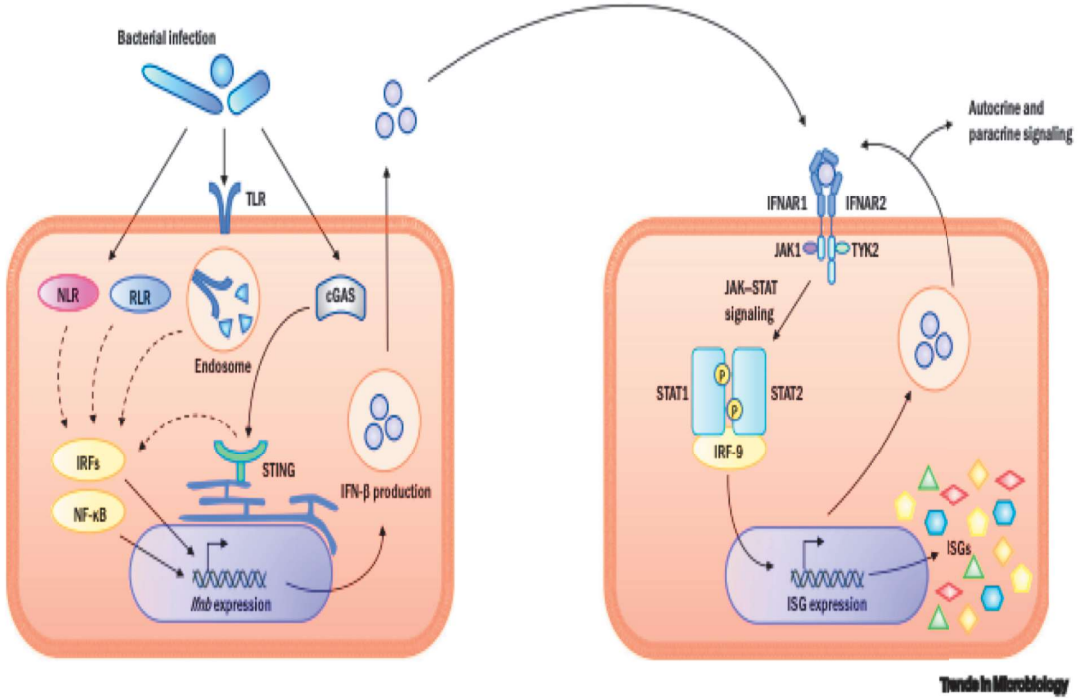


Figure 1. Type I Interferon (IFN) Signaling in the Context of Bacterial Infection. Type I IFNs are induced when bacteria are recognized by pattern-recognition receptors (PRRs), including nucleotide-binding leucine-rich repeat proteins such as NOD-1 and NOD-2 (NLRs), RIG-I like receptors (RLRs), and Toll-like receptors (TLRs) and cyclic GMP-AMP synthase (cGAS). PRR sensing activates the transcription factors of the interferon regulatory factor (IRF) family, which, along with NF-κB, stimulate the expression of type I IFNs, depicted here with IFN-β. IFN-β is then secreted and binds to the interferon alpha and beta receptor subunit (IFNAR) receptor which signals through the Janus kinase (JAK)–STAT (signal transducer and activator of transcription pathway. The phosphorylated forms of STAT-1 and STAT-2 and the interferon regulatory factors form a transcription factor complex that translocates to the nucleus where it induces the expression of hundreds of interferon-stimulated genes (ISGs). IFN-β is also produced, allowing a positive feedback loop and paracrine signaling.

The fact that most viruses devote part of their limited genome to mechanisms that perturb IFNα/β production and/or IFNα/β-mediated signalling, thereby preventing ISGs from being induced, illustrates the importance of this cytokine family in host cell protection against viral infection

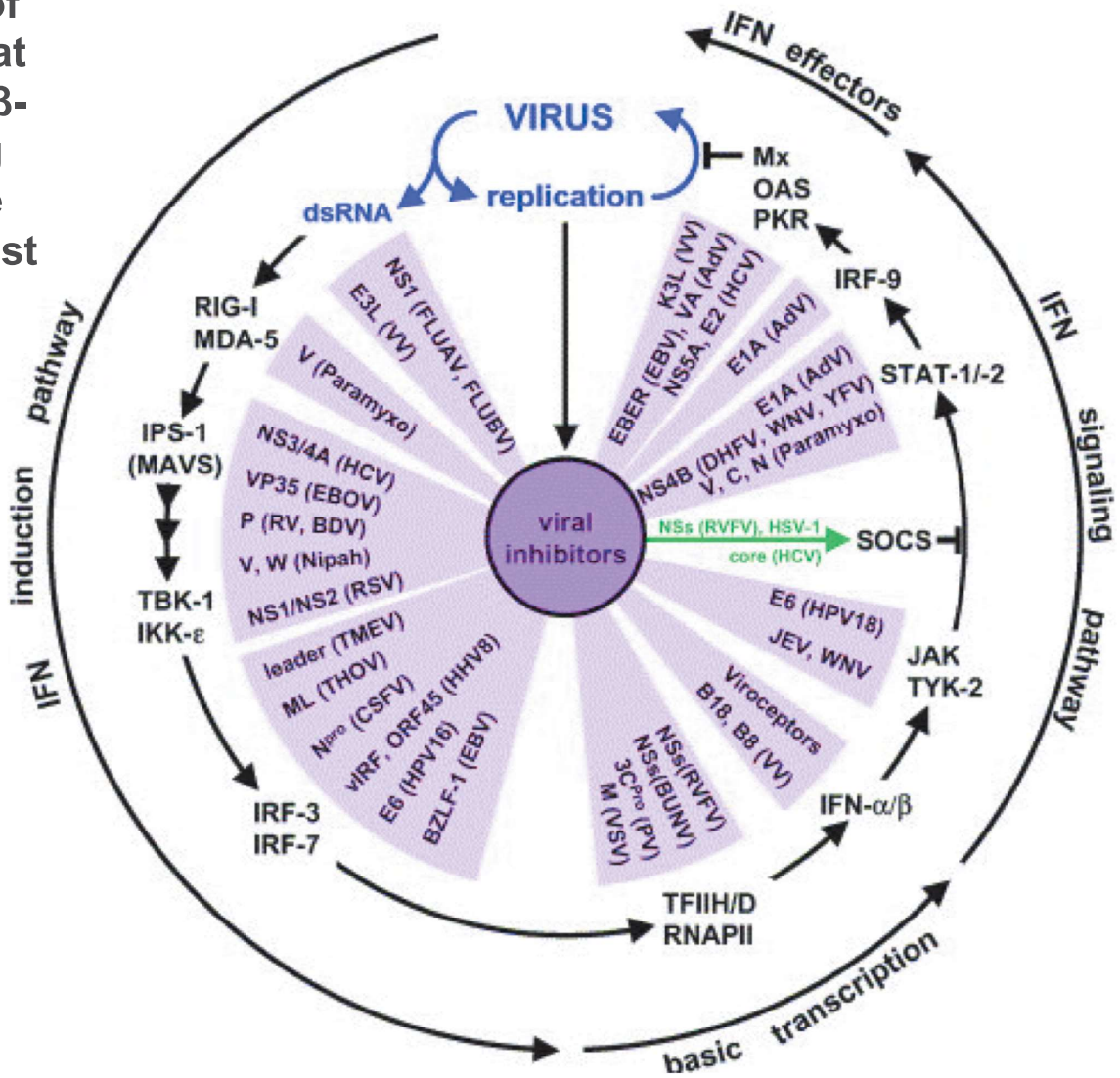
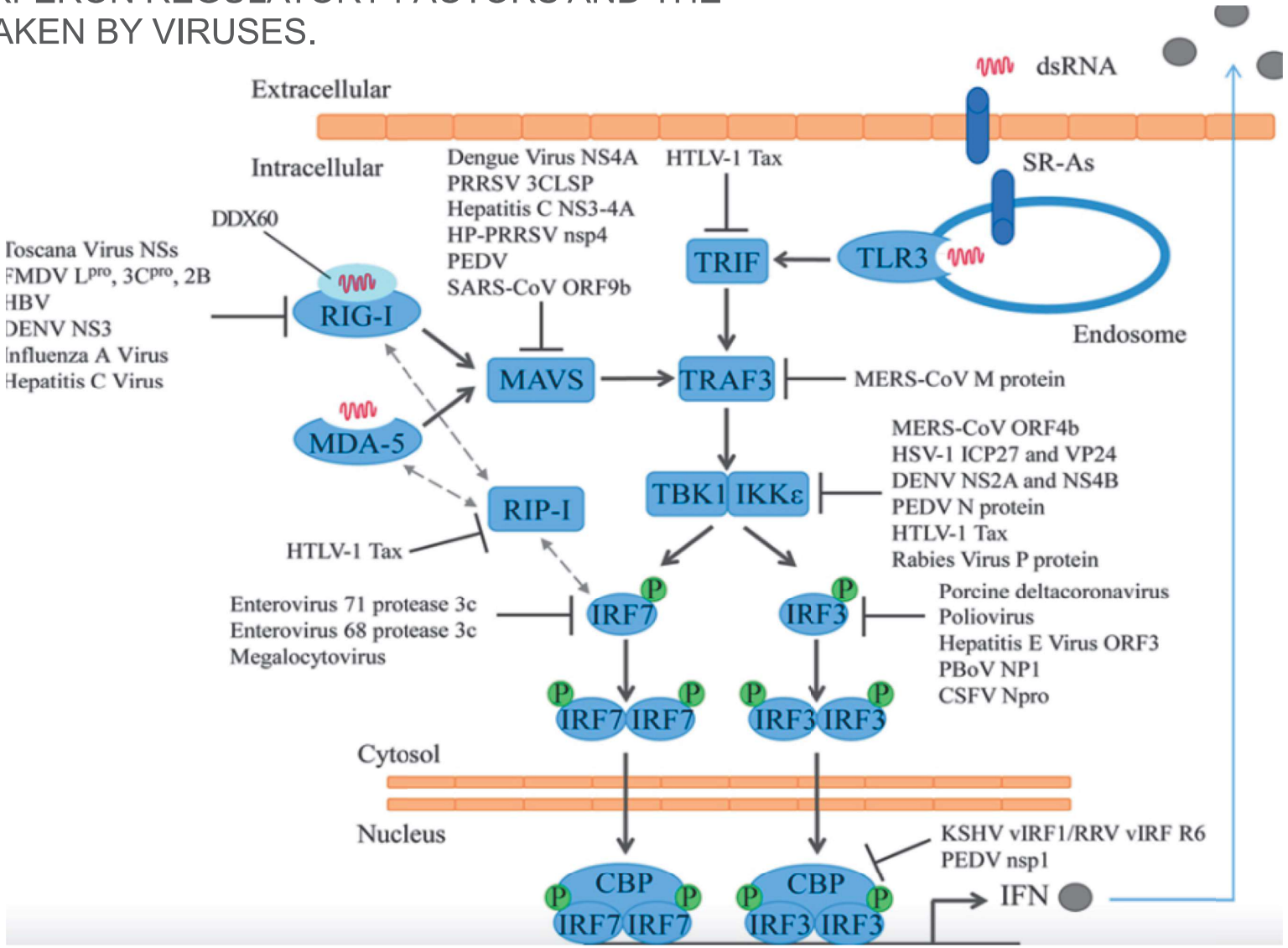


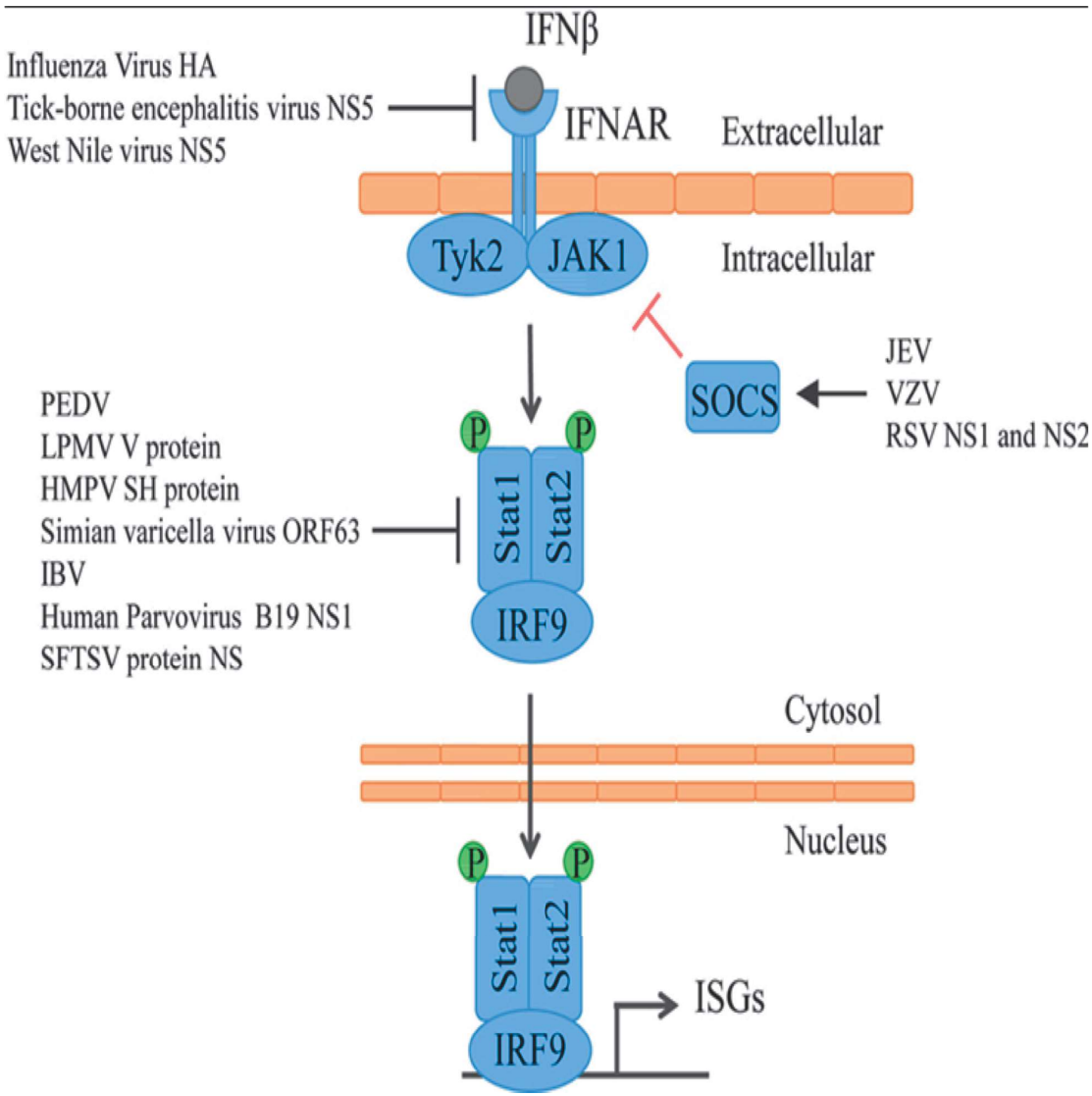
Fig. illustrates the range of activities mediated by IFN antagonists of various viruses. Only a few examples are listed but they demonstrate several important points.
First, viral proteins or functions have been identified that cover the whole spectrum of the IFN response in infected cells.
Second, a single viral protein may inhibit quite different components of the IFN induction and signaling cascade.
Third, a given virus may display more than one IFN-antagonistic activity targeting different pathways.

ACTIVATION OF INTERFERON REGULATORY FACTORS AND THE COUNTERACTIONS TAKEN BY VIRUSES.



CSFV, classical swine fever virus; DENV, dengue virus; FMDV, foot-and-mouth disease virus; HBV, hepatitis B virus; HP-PRRSV, highly pathogenic porcine reproductive and respiratory syndrome virus; HSV-1, herpes simplex virus type 1; HTLV-1, human T-cell lymphotropic virus type I; KSHV, Kaposi's sarcoma-associated herpesvirus; MERS-CoV, middle east respiratory syndrome coronavirus; PBoV, porcine bocavirus; PEDV, porcine epidemic diarrhea virus; PRRSV, porcine reproductive and respiratory syndrome virus; RRV, rhesus macaque rhadinovirus

FIGURE 3. TYPE I IFN SIGNALING AND THE COUNTERACTIONS TAKEN BY VIRUSES.

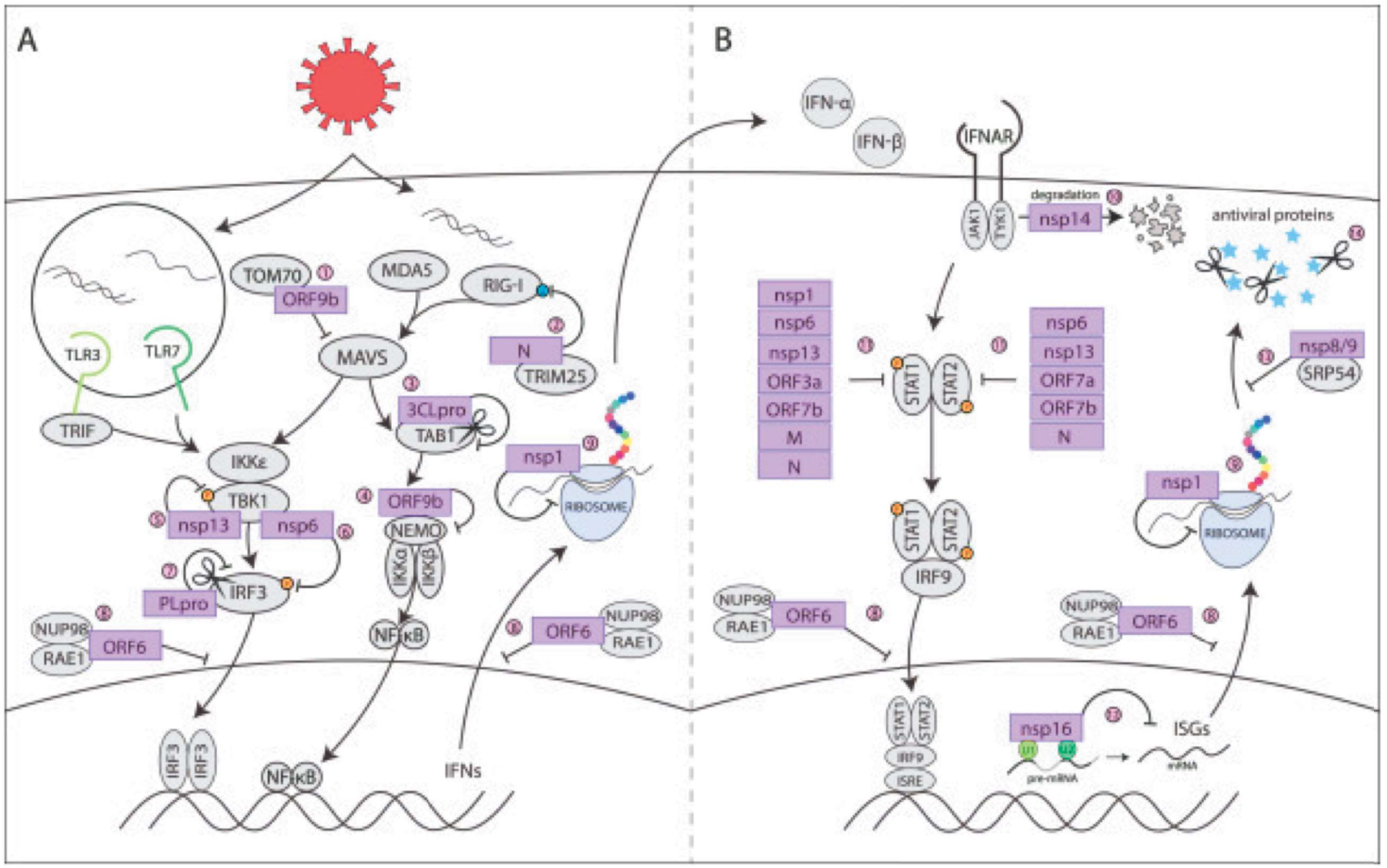


HMPV, human metapneumovirus; IBV, infectious bronchitis virus; JEV, Japanese encephalitic virus; LPMV, La Piedad Michoacán Mexico Virus; PEDV, porcine epidemic diarrhea virus; RSV, respiratory syncytial virus; SFTSV, severe fever with thrombocytopenia syndrome virus; VZV, varicella-zoster virus.

Viral proteins disrupt the interferon response at many different points

SARS-CoV-2 proteins antagonize interferon induction.

SARS-CoV-2 proteins inhibit interferon response and ISG production



[Current Research in Virological Science](#)
[Volume 2](#), 2021, 100015

IFN = Heterogeneity

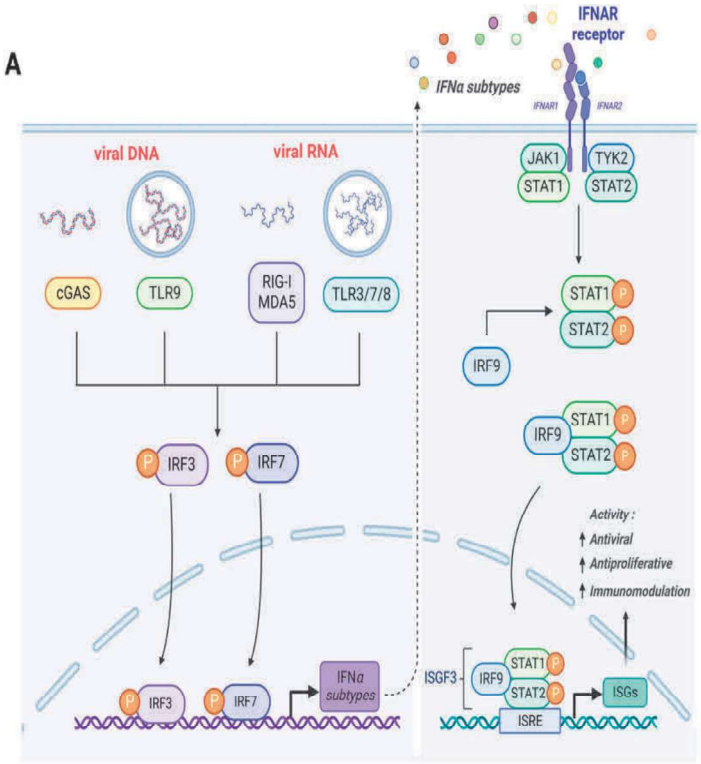
Heterogeneity and functions of the 13 IFN-α subtypes – lucky for some?

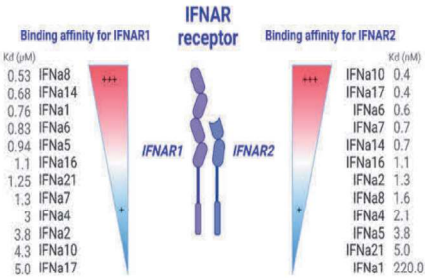
Multiple subtypes exist within the type I IFN family, in particular 13 distinct IFN-α genes, which signal through the same heterodimer receptor that is ubiquitously expressed by mammalian cells.

A number of 17 subtypes of IFN-I have been identified in humans, including 13 IFN-α, as well as IFN-β, IFN-ω, IFN-ε and IFN-κ.

The 13 subtypes of human IFN-α (IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α13, IFN-α14, IFN-α16, IFN-α17 and IFN-α21) are characterized by high sequence homology (76–96% shared amino acid sequences) that determines identical secondary and tertiary folding

The expression patterns and antiviral activities of IFN-α subtypes vary among different viruses in both acute and chronic infection.





Explanations for their distinct functions come from studies reporting that the various human IFN-α subtypes all bind with different affinities to the IFNAR receptor subunits 1 and 2.

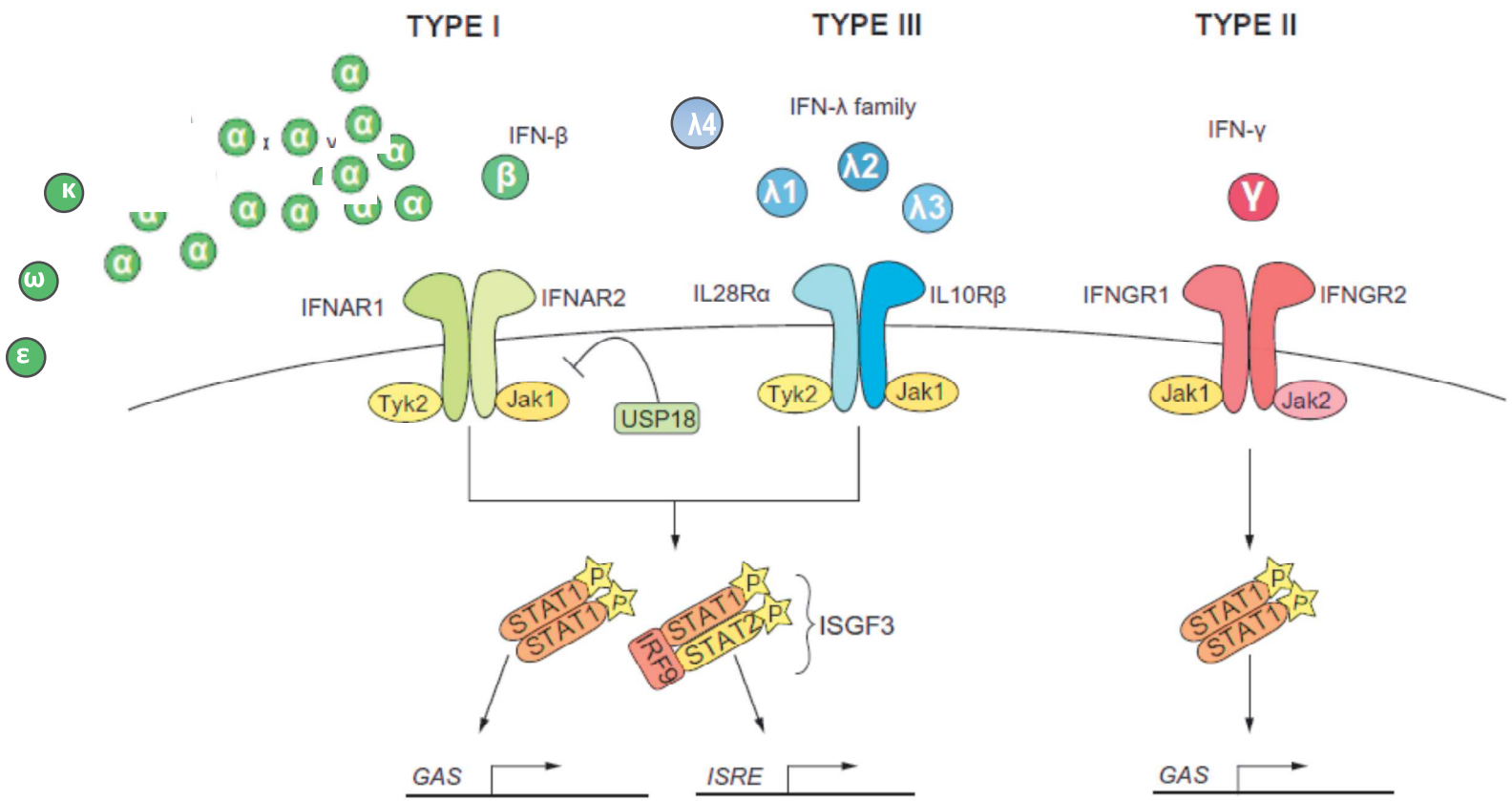
Eur J Immunol, Volume: 53, Issue: 8, First published: 27 June 2023, DOI: (10.1002/eji.202250307)

There are 22 human IFNs, classified in 3 types (16 Type-I: 13 IFNαs, IFNβ, IFNε, IFNκ, and IFNω; 1 Type-II, IFNγ; and 4 Type-III, IFNλ1, IFNλ2, IFNλ3, and IFNλ4)

Type I IFNs are produced by many cell types including lymphocytes (NK cells, B-cells and T-cells), macrophages, fibroblasts, endothelial cells, osteoblasts, leukocytes, plasmacytoid* and dendritic cells.

Type II IFN is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4+ and CD8+ T cells of the adaptive immune cells.

The main producer of Type III IFNs are type 2 myeloid dendritic cells, epithelial cells and hepatocytes



*Although, each cell is capable of producing type I IFNs, plasmacytoid dendritic cells (pDCs) possess a unique ability to rapidly produce large amounts of them

Journal of Hepatology **2013** vol. 58 | 564–574

INTERFERON COMES ON THE SCENE (1)

Discovery of Interferons

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- Isaacs and Lindenmann
- Did an experiment using chicken cell cultures
- Found a substance that interfered with viral replication and was therefore named interferon
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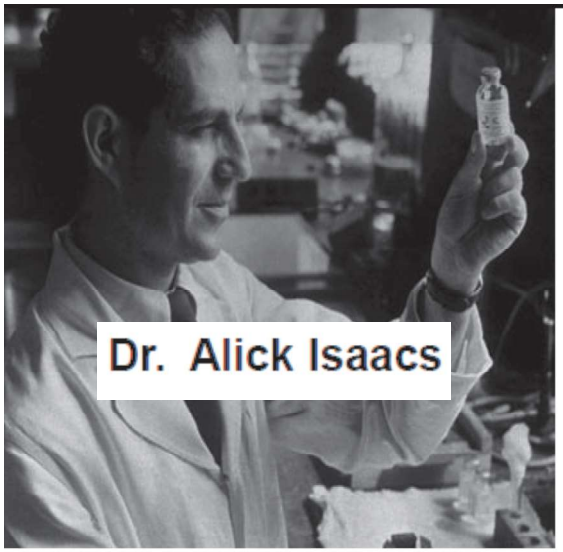
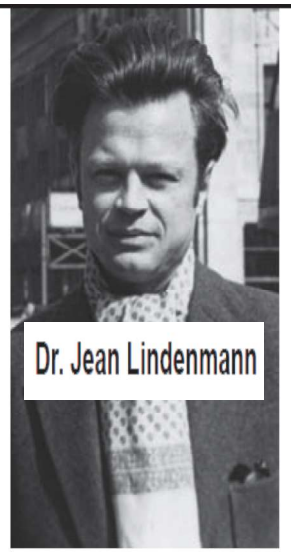
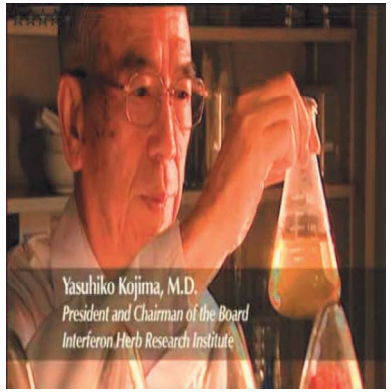




Proc. Royal Soc. London 147:258-267, 1957
Proc. Royal Soc. London 147:268-273, 1957

1957



C R Seances Soc Biol Fil. 152(11):1627-9, 1958
C R Seances Soc Biol Fil. 152(2):372-4,1958

1958

*National Institute for Medical Research (NIMR) in London

in the absence of any other salts. Riklis reported a small amount of a thymine photoproduct with similar chromatographic properties in lyophilized DNA which had been irradiated in the dry state (7). One of the photoproducts (*a*), appearing in small amount, chromatographs in the region of the uracil-thymine dimer (11), but other evidence indicates that the photoproduct may not be this dimer. Smith (12) reported finding small amounts of thymine-containing photoproducts chromatographing in the region of our photoproducts (*b* and *c*) in DNA from *E. coli* irradiated in vivo and in an irradiated solution of polydeoxyadenylate-thymidylate.

The absence of thymine dimers in the DNA of irradiated spores is sufficient to explain their resistance to ultraviolet irradiation. However, the appearance of large amounts of unidentified photoproducts implies either that such products do not interfere with DNA synthesis or that the cells have a very efficient repair mechanism for dealing with the photoproducts. Certain data (13) indicate that the photoproducts do not remain in the DNA of spores during differentiation into vegetative cells. Our data show that during sporulation the physical state of the DNA within the cell is changed from that found either in vegetative cells or in solution, because normal thymine dimers are not found in irradiated spores.

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References and Notes

1. A. Wacker, in *Progress in Nucleic Acid Research*, J. N. Davidson and W. E. Cohn, Eds. (Academic Press, New York, 1963), vol. 1, p. 369; K. C. Smith, in *Photoimmunology*, A. C. Giese, Ed. (Academic Press, New York, 1964), vol. 2, p. 229; J. E. Setlow and D. E. Duggan, *Biochim. Biophys. Acta* 87, 664 (1964); I. E. Troshin, E. H. Y. Chu, W. E. Carrier, *Radiation Res.* 24, 667 (1965).
2. P. A. Benson and R. B. Setlow, *Biochim. Biophys. Acta* 68, 598 (1963).
3. R. B. Setlow and J. K. Setlow, *Proc. Nat. Acad. Sci. U.S.* 48, 1250 (1962).
4. R. B. Setlow, *J. Cellular Comp. Physiol.* 64 (Suppl. 1), 51 (1964).
5. J. C. Lewis, N. S. Snell, H. K. Burr, *Science* 132, 544 (1960).
6. E. Riklis and E. Simon, *Abstr. Annual Meeting 1964, Biophys. Soc.* (1964).
7. E. Riklis, in preparation.
8. H. S. Taylorson and M. G. Sevag, *J. Gen. Physiol.* 36, 617 (1953).
9. L. E. Sacks, P. B. Pepeil, R. S. Thomas, G. F. Bailey, *J. Bacteriol.* 87, 932 (1964). A "Wig-L-Bug" dental amalgamator was obtained from Crescent Dental Mfg. Co., Chicago, Ill.
10. J. E. Donnellan, Jr., K. H. Nega, H. S. Levinson, in *Spores III*, L. L. Campbell and H. O. Halvorson, Eds. (Amer. Soc. Microbiol., Ann Arbor, 1965), p. 122.
11. K. C. Smith, *Photochem. Photobiol.* **2**, 503 (1963).
12. ———, *ibid.* **3**, 1 (1964).
13. J. E. Donnellan, Jr., R. S. Stafford, R. B. Setlow, in preparation.
14. Research sponsored by AEC under contract with Union Carbide Corporation. We thank Dr. Jane K. Setlow for many helpful suggestions.

12 April 1965

Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin

Abstract. *Phytohemagglutinin, an extract of the kidney bean, Phaseolus vulgaris, induces in human leukocyte cultures an inhibitor of the cytopathic effects of Sindbis virus. The physicochemical and biological properties of this virus-inhibitor are similar to those of interferon induced by Newcastle disease virus, except for an instability at pH 2 and 10 and at 56°C.*

During attempts to culture peripheral leukocytes from the blood of persons with and without viral infections, phytohemagglutinin (PHA), an extract of the kidney bean (*Phaseolus vulgaris*) which agglutinates red blood cells in the preparation of cultures, induced synthesis, in leukocytes cultured from normal individuals, of a virus-inhibitor with interferon-like properties. Interferon production in virus-infected cultures of human leukocytes has been previously reported (*1*, *2*).

In this report the properties of the phytohemagglutinin-induced virus-inhibitor are described and compared with interferon induced in white blood cells by infection with Newcastle disease virus (NDV).

Cells were grown in Eagle's minimum essential medium supplemented with tryptose phosphate broth (4 percent) and fetal calf serum (10 percent). The concentration of sodium bicarbonate was 1.75 g/liter, and all cell-culture vessels were gassed with 5 percent CO_2 in air before incubation at 37°C. White blood cells were obtained from the venous blood of normal adults; the blood was placed in tubes containing phenol-free heparin (0.5 ml for each 15 ml blood). The tubes were incubated at 37°C for 50 to 60 minutes and then centrifuged at room temperature at 800 rev/min for 2 minutes. The supernatant leukocyte-rich plasma was then recentrifuged at 1600 rev/min for 8 minutes, and the pellet was suspended in growth medium by gentle pipetting. The leukocytes were counted in a hemocytometer, and the cells were diluted in growth medium to a final concentration of 2×10^6 cells per milliliter. Screw-cap culture tubes were each planted with 2 ml of the cell suspension, placed in a roller drum, and incubated at 37°C.

Three types of cell cultures were used for the interferon assay: (i) cells derived from a human fetal lung, grown in continuous culture, and used between the 10th and 25th passage; (ii) the BHK 21 clone 13 (C13) an established cell line derived from baby hamster kidneys (*3*); (iii) the 'L' cell strain of mouse fibroblasts derived from normal mouse skin (*4*).

Phytohemagglutinin P (5-ml bottles, Difco Co.) was dissolved in phosphate-buffered saline (100 ml) at room temperature and passed through a filter with a pore size of 600 mμ. The filtrate was stored at 4°C.

The specimens to be tested for interferon were diluted in growth medium, and 1 ml of each dilution was added to 1-day-old cultures of human fetal lung cells grown in incomplete monolayers in screw-cap tubes. After 20-hour incubation at 37°C, the cultures were washed once with 4 ml of phosphate-buffered saline. One milliliter of warm Eagle's medium was added to each tube, and then 1 ml of cold growth medium containing 5000 tissue culture infective doses ($TCID_{50}$) of Sindbis virus (Egypt AR 339 strain) was inoculated. This amount of virus produces gross cytopathic effects in cultures in 24 to 30 hours. Cultures were considered to be protected when there was less than 10-percent cytopathic effect at a time when control cultures exhibited more than 75-percent cytopathic effect.

Interferon titers are expressed as reciprocals of the highest dilution of the specimen, 1 ml of which protected cultures against challenge with Sindbis virus. No specimen was tested at less than 1:10 dilution.

To prepare Newcastle disease virus interferon white blood cell cultures were inoculated with 10^6 EID_{50} (egg infective doses) of virus (Hickman strain) and incubated for 48 hours. The media were then collected, and all infective virus was completely neutralized with hyperimmune guinea pig antiserum to the virus (*5*). The inhibitory property of this preparation could be attributed to interferon on the basis of (i) no reduction in the virus inhibitory titer of the supernatant after centrifugation at 105,000*g* for 3 hours; (ii) no reduction in inhibitory titer on acidification to *p*H 2 for 24 hours; and (iii) complete loss of inhibitory activity on treatment with crystalline trypsin (0.2 mg/ml) for 1 hour at 37°C. The titers of Newcastle disease virus–induced interferon were approximately 10^4 culture-protecting units per milliliter.

Phytohemagglutinin (0.1 ml) was added to each 2 ml of white-blood-cell cultures in suspension and incubated at 37°C; at hourly intervals thereafter media and cells were collected and assayed for virus-inhibitory activity. An inhibitor of cytopathic effects of Sindbis virus in lung cells of the human fetus appeared in the white-cell cultures 2 hours after addition of phytohemagglutinin, increased in titer until approximately the 20th hour, and remained at a constant titer thereafter. The titers of the virus inhibitor produced in leukocyte cultures obtained from different individuals varied from 10 to 80 culture-protecting units per milliliter with an occasional culture producing no detectable inhibitor. The virus-inhibitor was present in the media; it was not detected in cells disrupted by high-frequency sound. In white-cell cultures incubated without phytohemagglutinin, virus-inhibitor could not be detected in either the media or cell-disrupted fraction.

The 2-hour incubation period between the addition of phytohemagglutinin and the appearance of virus-inhibitor suggests a succession of intracellular events in the course of inhibitor production. To provide evidence that the virus-inhibitor was synthesized in white blood cells, the following experiments were performed. The white cells were treated with phytohemagglutinin for 30 minutes and then washed five times in growth media to remove all phytohemagglutinin not associated with cells. On incubation at 37°C these cells synthesized the virus-inhibitor, but at 4°C failed to produce the inhibitor. When phytohemagglutinin was added to cell-free growth media and incubated for 24 hours at 37°C, no virus-inhibitor was produced. Finally, lung cell cultures treated with phytohemagglutinin were as susceptible to the cytopathic effects of Sindbis virus as were untreated cultures. These experiments suggest the intracellular synthesis of virus-inhibitor but do not rule out the possibility that phytohemagglutinin is degraded within white cells into a protein with virus-inhibitory properties.

The possibility of a virus contaminant in the media in which the white cells were cultured was eliminated by the demonstration that heparinized whole blood treated with phytohemagglutinin and incubated at 37°C produced a virus-inhibitor detectable in the plasma. Attempts to isolate a virus from phytohemagglutinin or the phytohemagglutinin-induced virus-inhibitor were unsuccessful, an indication that a virus was probably not introduced from without nor activated from white cells in the course of the experiments.

Table 1. Comparison between properties of phytohemagglutinin-induced and Newcastle disease virus–induced virus-inhibitors. Plus (+) indicates greater than a fourfold reduction in titer of inhibitor after treatment. Minus (−) indicates no reduction in titer of inhibitor after treatment.

Treatment	Effect on virus-inhibitor	
	PHA	NDV
Crystalline trypsin (0.1 mg/ml; 1 hr; 37°C)	+	+
Ribo- or deoxyribonuclease (0.5 mg/ml; 1 hr; 37°C)	−	−
Centrifugation 105,000*g*, 3 hr (supernatant assay)	−	−
46°C, 1 hr	−	−
56°C, 1 hr	+	−
70°C, 1 hr	+	+
*p*H 1; 24 hr	+	−
*p*H 2; 24 hr	+	−
*p*H 3–9; 24 hr	−	−
*p*H 10; 24 hr	+	−
*p*H 11; 24 hr	+	−
Dialyzable	No	No

The properties of the phytohemagglutinin-induced virus-inhibitor were determined and compared (at comparable concentration) with interferon induced by Newcastle disease virus in leukocyte cultures as described above. Both virus-inhibitors are nondialyzable, nonsedimentable proteins (Table 1) which have no direct virus-neutralizing properties but which can exert their virus-inhibitory effects by incubation with cells before inoculation of challenge virus. Further, both proteins inhibit virus multiplication in human cells and not in mouse or hamster kidney cells. In contrast to the virus-induced interferon, however, the phytohemagglutinin-induced inhibitor is unstable at *p*H 2 and 10 and at 56°C. Thus the phytohemagglutinin-induced virus-inhibitor is an interferon-like substance labile to heat and to extremes of pH.

Phytohemagglutinin stimulates RNA and DNA synthesis in human leukocytes, with subsequent transformation of lymphocytes into blastoid cells, and increases the mitotic rate (*6*). I now report a further and perhaps related effect by phytohemagglutinin.

Interferon-like substances have been produced in animals and cell cultures exposed to a variety of other nonviral agents: bacteria and endotoxin (*7*), an anionic polysaccharide (*8*) and nucleic acids from animal cells (*9*), yeast (*10*), and bacteria (*11*). The interferon-like substance produced in rabbits in response to endotoxin has properties similar to the phytohemagglutinin-induced virus-inhibitor (*12*).

The production of different interferons in human leukocyte cultures by phytohemagglutinin and Newcastle disease virus may either reflect the heterogeneous nature of the cell population or be the result of different mechanisms of interferon synthesis in the same cell type. In support of the latter hypothesis are the findings that phytohemagglutinin acts on lymphocytes (*6*) and that mononuclear cells do produce interferon in response to viral infection (*2*). Also, Ho has recently reported that the production of virus-induced interferon can be inhibited by actinomycin, whereas production of endotoxin-induced virus-inhibitor cannot; he attributes this phenomenon to different mechanisms of interferon production (*13*). The phytohemagglutinin-induced virus-inhibitor may, however, be produced in white cells in response to a stimulation of cellular RNA synthesis and may be a feedback mechanism for control of RNA synthesis.

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References and Notes

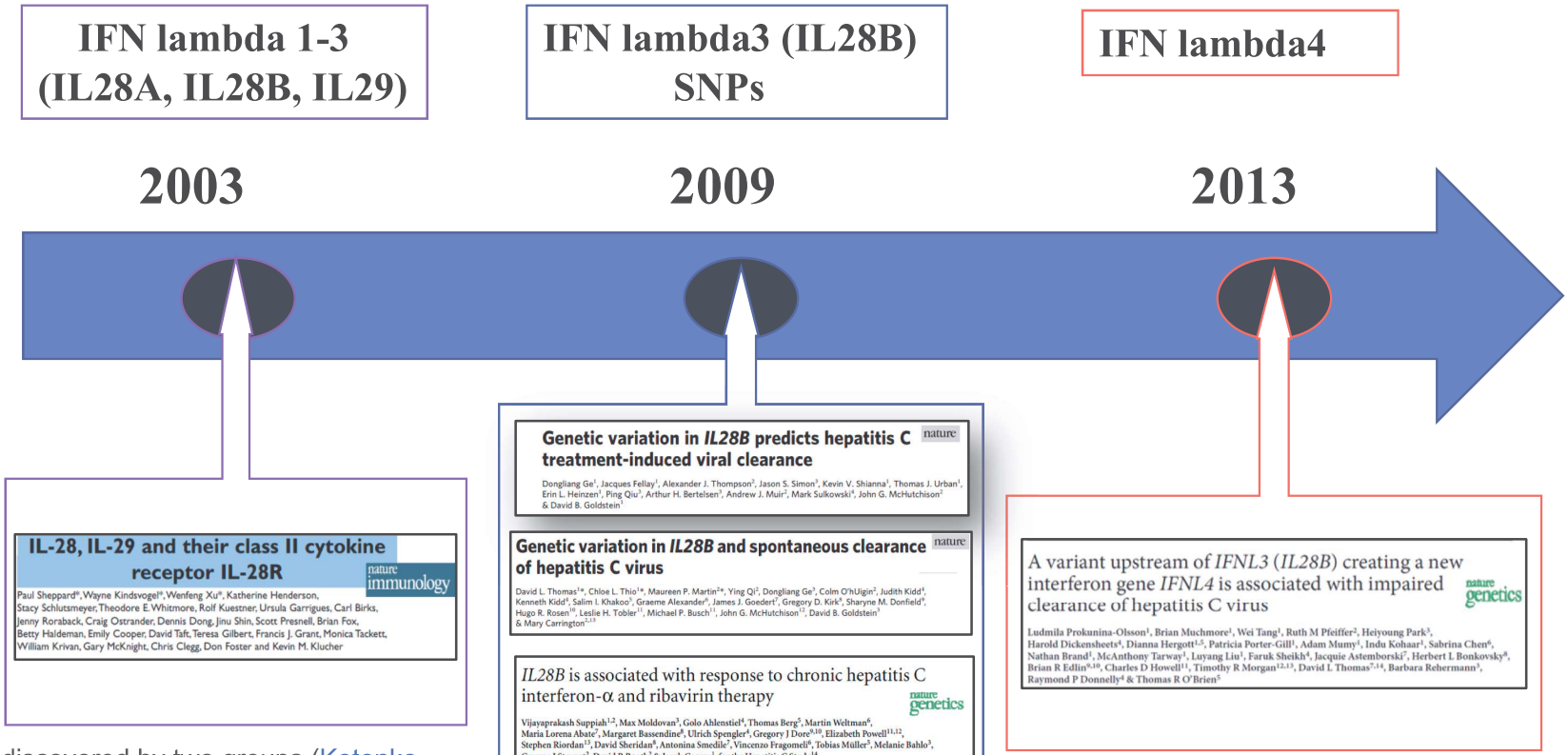
1. I. Gresser, *Proc. Soc. Exptl. Biol. Med.* **108**, 795 (1961).
2. S. H. S. Lee and R. L. Crowe, *ibid.* **118**, 190 (1965).
3. L. MacPherson and M. Stoker, *Virology* **16**, 147 (1962).
4. K. K. Stanford, W. R. Earle, G. D. Likely, *J. Natl. Cancer Inst.* **9**, 229 (1948).
5. E. F. Wheelock and J. Tamm, *J. Exptl. Med.* **113**, 301 (1961).
6. J. H. Robbins, *Science* **148**, 1648 (1965).
7. W. R. Stinebring and J. S. Younger, *Nature* **204**, 712 (1964).
8. W. J. Kleinschmidt, J. C. Cline, E. B. Murphy, *Proc. Nat. Acad. Sci. U.S.* **52**, 343 (1964).
9. I. Zetter, R. A. Cox, A. Isaacs, *Nature* **197**, 564 (1963).
10. K. E. Jensen, A. L. Neal, R. E. Owens, J. Warren, *ibid.* **200**, 433 (1963).
11. K. Takano, J. Watson, K. Jensen, A. Neal, personal communication.
12. M. Ho, *Science* **146**, 1472 (1964).
13. M. Ho and Y. Kono, *Proc. Natl. Acad. Sci. U.S.* **52**, 220 (1964).
14. The technical assistance of Mrs. Juanita Ruffner is gratefully acknowledged. Sponsored by the Committee on Acute Respiratory Diseases, Armed Forces Epidemiological Board; supported in part by the Office of The Surgeon General, Department of the Army, and in part by a grant from the Robert Hamilton Bishop, Jr., endowment fund.

18 May 1965

Paper Reporting the Discovery of IFN-γ

Wheelock, EF. *Science* 149:310-311, 1965

INTERFERON (lambdas) COMES ON THE SCENE (2)



Type III IFNs were discovered by two groups (Kotenko et al., 2003, Sheppard et al., 2003), and in humans include four subtypes, IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4. IFN-λ4 was the last to be discovered, and is a pseudogene in many human populations (Prokunina-Olsson et al., 2013).

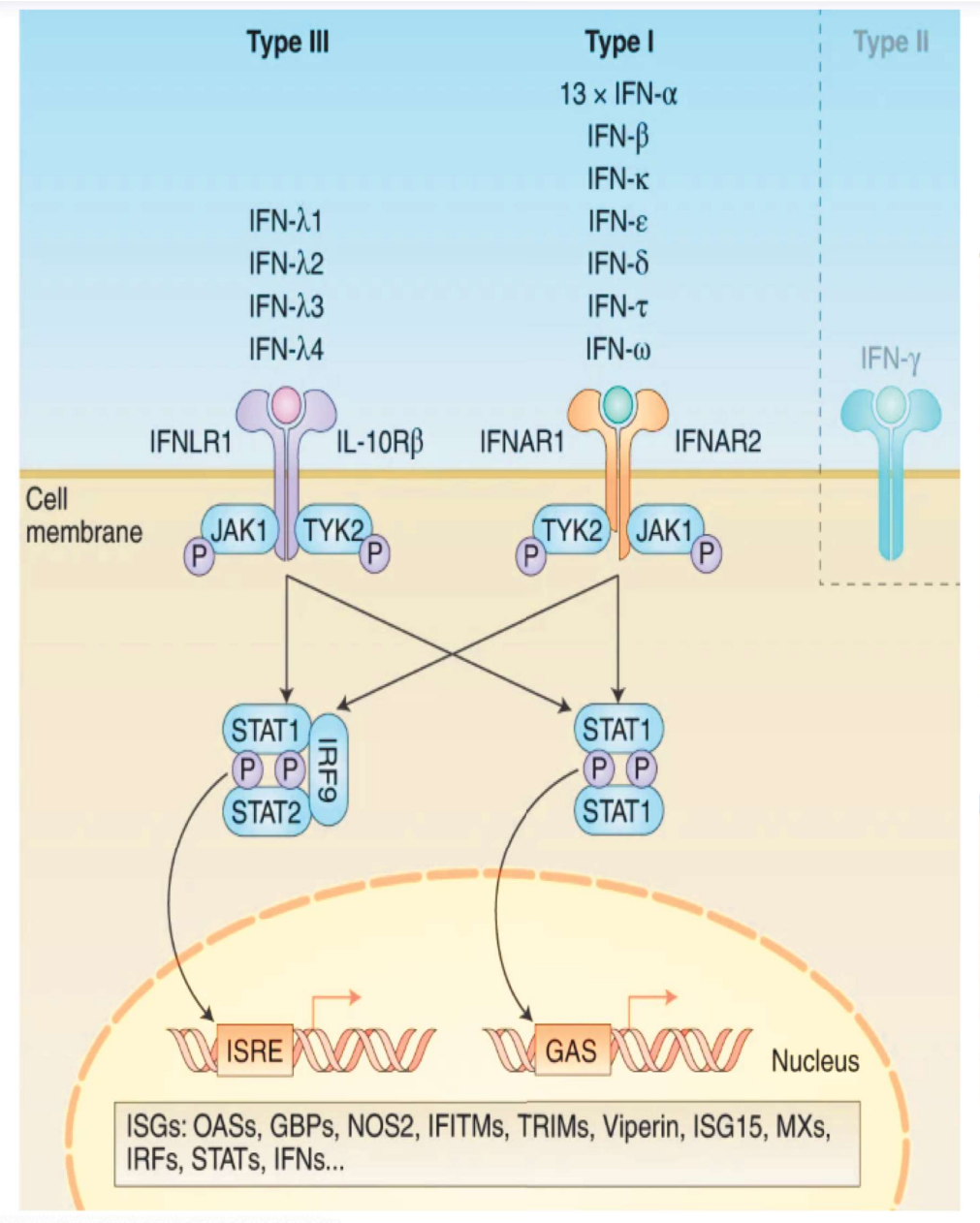
Within type I and III, there are multiple IFNs; within type II, there is only a single IFN.

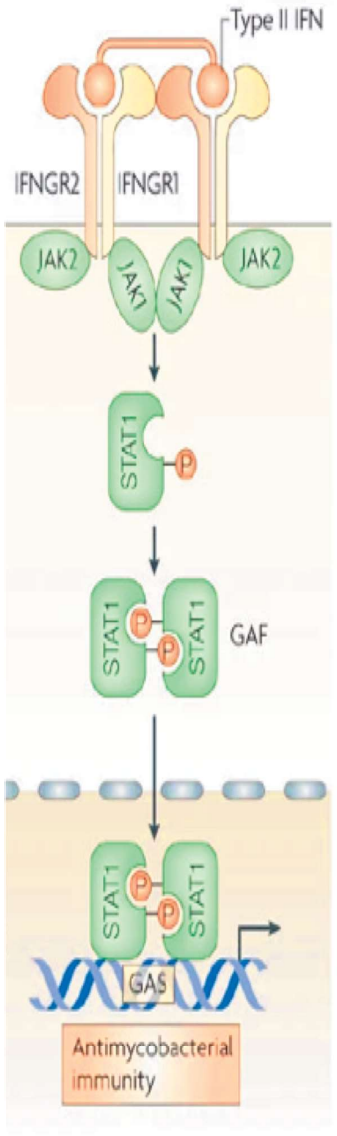
The type II class of IFN comprises the single IFNγ gene product that binds the IFNγ receptor (IFNGR) complex, and mediates broad immune responses to pathogens other than viruses.

The more recently described type III IFNs include four IFNλ gene products that signal through receptors containing IFNLR1 (IFNλ receptor 1; also known as IL-28Ra) and IL-10R2 (also known as IL-10Rβ).

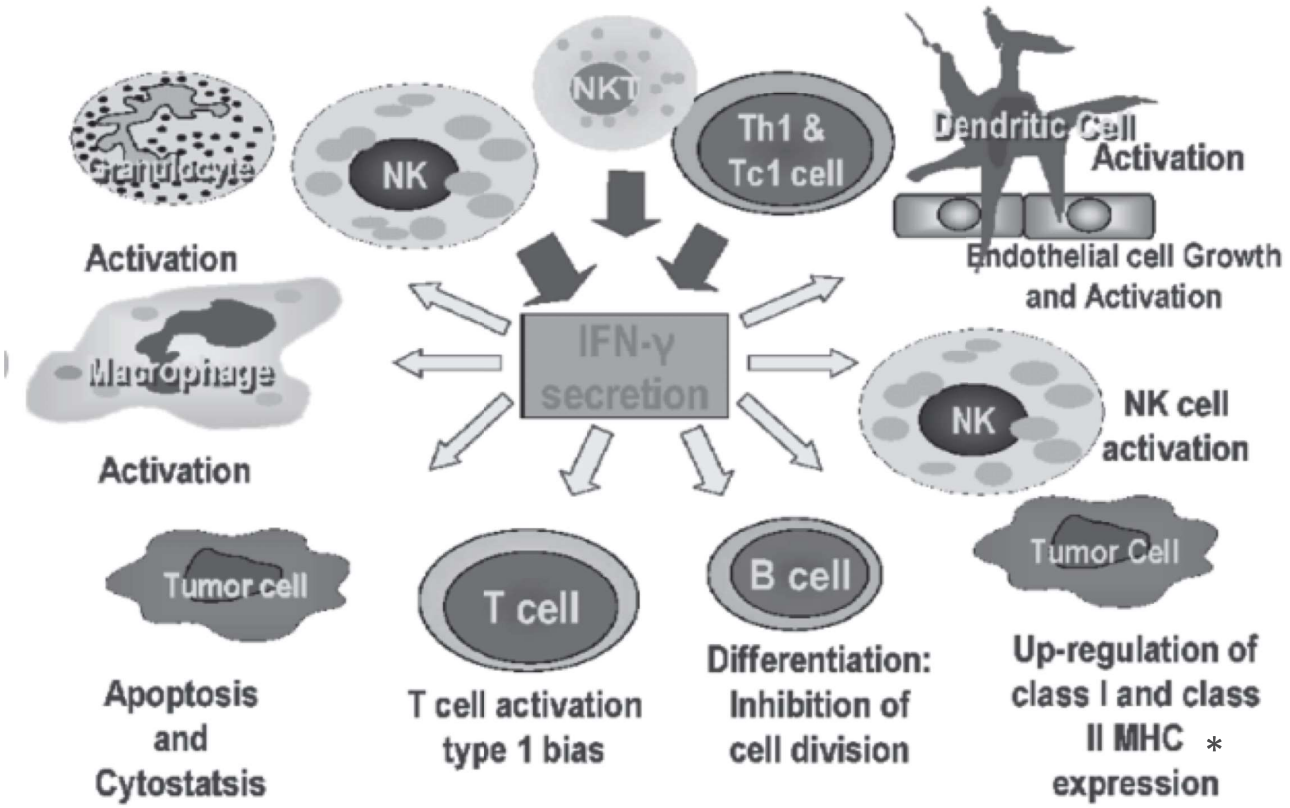
Type I IFNs, which in humans comprise 13 IFNα subtypes, IFNβ, IFNκ, IFNε, IFNo, engage the ubiquitously expressed IFNAR (IFNα receptor) complex that is composed of IFNAR1 and IFNAR2. The function of type I IFNs is well characterized and they are known to be essential for mounting a robust host response against viral infection.

Nature Microbiology **volume 4**, pages914–924 (2019)





Interferon-gamma (IFN-γ)



1 Regulation of Immune Responses by Interferon-Gamma (IFN-γ). IFN-γ is produced predominately by T H 1, T c 1, NKT and NK cells, resulting in the activation of T cells, NK cells, macrophages, dendritic cells (DCs) and granulocytes. In addition, it upregulates class I and class II MHC expression on tumor cells as well as the expression of a wide variety of receptors on both tumor cells and epithelial cells. IFN-γ: interferon-gamma; NK: Natural killer cell; T H 1: T-helper cell type 1; T c 1: T cytotoxic cell type 1; MHC: major histocompatibility complex.

Major histocompatibility complex (MHC) class I and class II

I) **Type I and type III IFNs bind to distinct receptors but activate similar signaling pathways and transcriptional responses.**

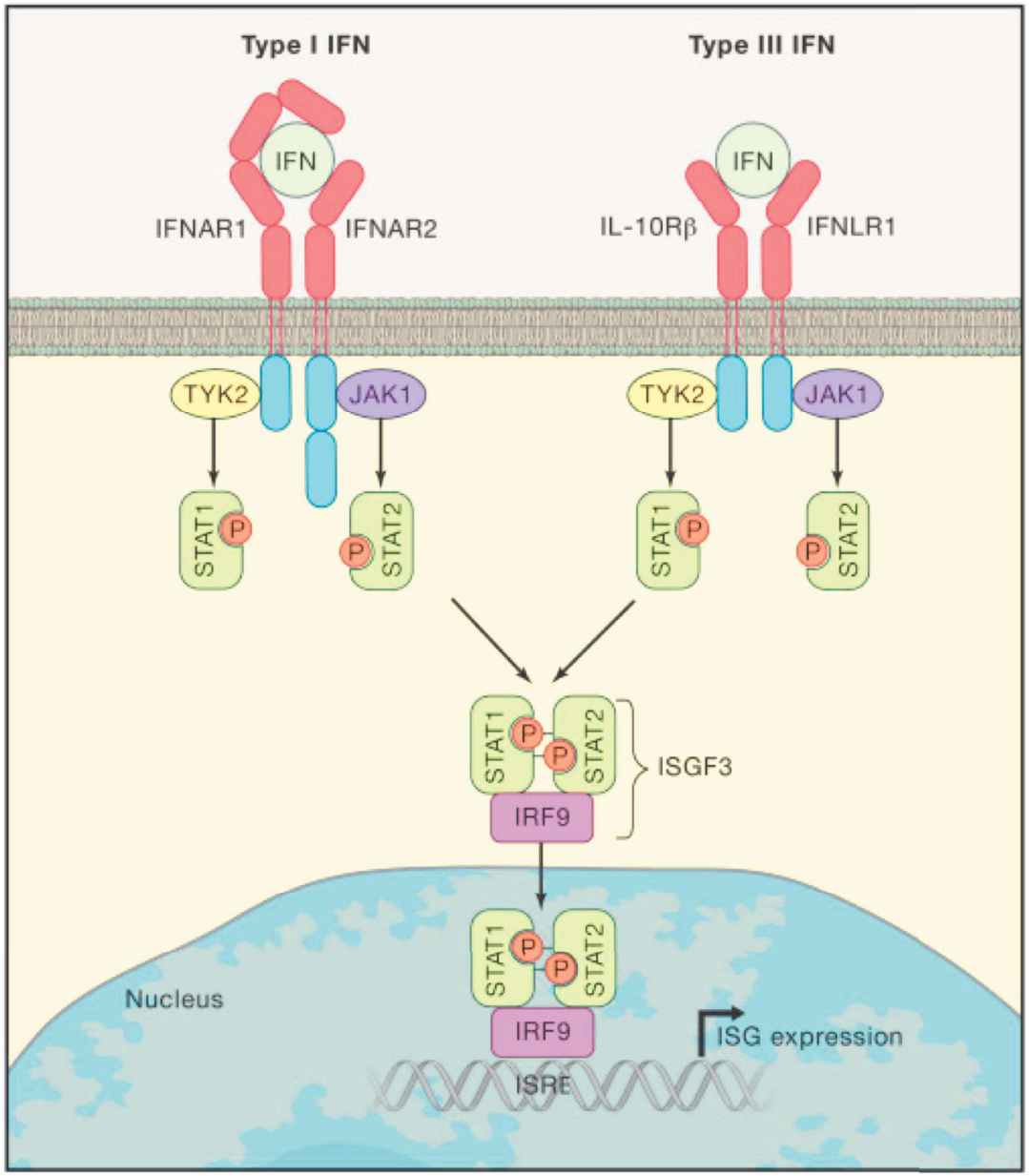
II) The type I and type III IFN receptors are heterodimers comprised of IFNAR1 and IFNAR2 subunits or IFNLR1 and IL10Rβ subunits, respectively.

III) IFNs first bind one receptor chain with high affinity (IFNAR2 or IFNLR1), then recruit a low-affinity receptor chain (IFNAR1 or IL10Rβ) to create a signaling-competent ternary complex.

IV) Receptor dimerization activates TYK2 and JAK1 kinases, which phosphorylate STAT1 and STAT2.

V) Phosphorylated STAT1 and STAT2 heterodimers complex with IRF9 to produce the transcription factor ISGF3.

VI) ISGF3 binds to ISREs and promotes expression of hundreds of ISGs.



Although their signaling pathways and transcriptional responses have many similarities, some features distinguish type I and type III IFNs:

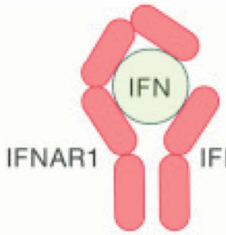
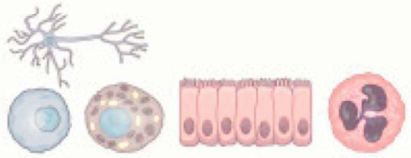
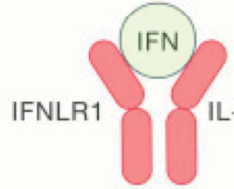
(1) most type I IFN genes lack introns, whereas type III IFN genes have 5 or 6 exons;

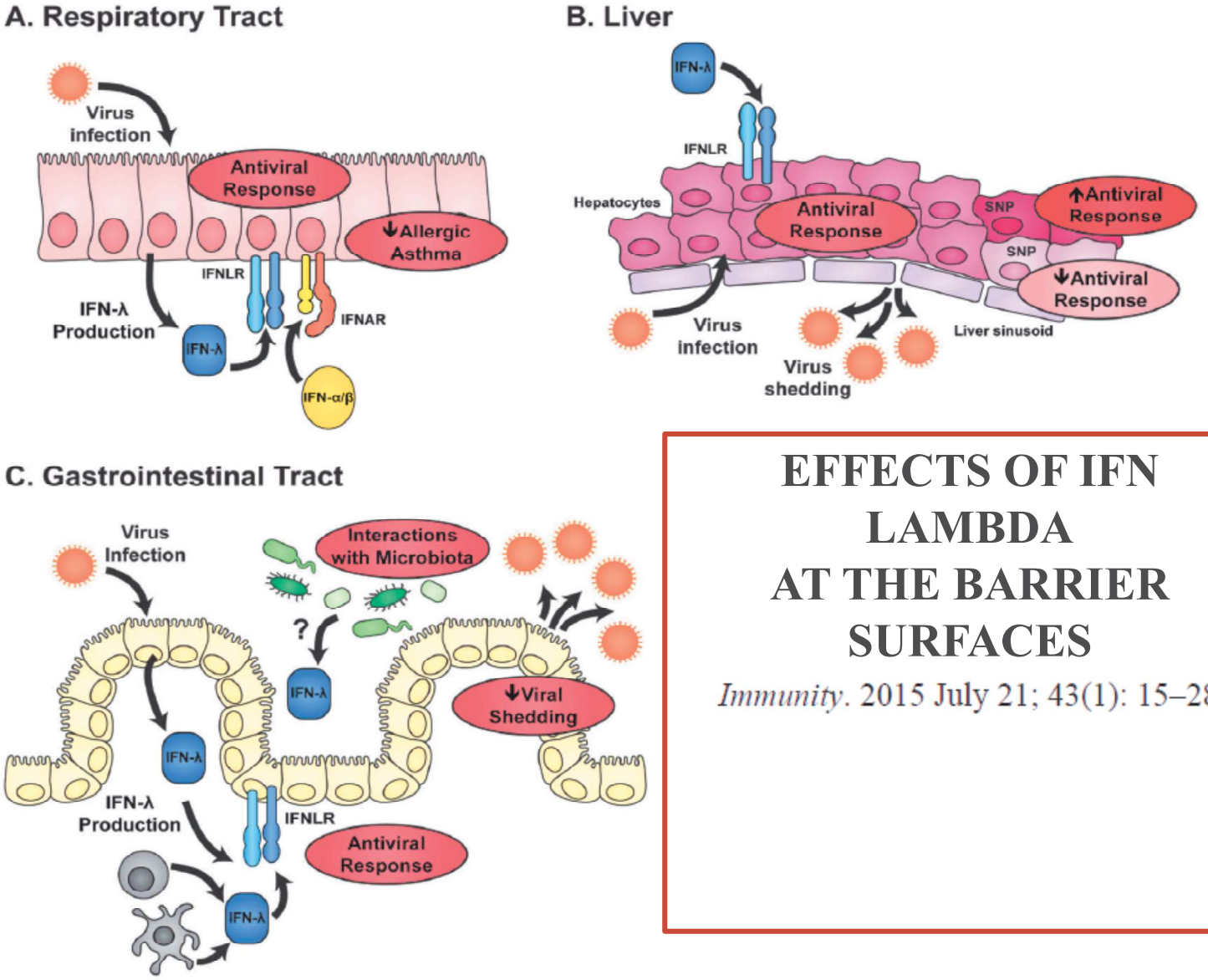
(2) the type I IFN family is larger, comprising 16 members in humans and 18 in mice, compared with 4 type III IFN members in humans and 2 in mice;

(3) **type I and type III IFNs bind distinct receptors. The type I IFN receptor (IFNAR) is ubiquitously expressed, whereas the type III IFN receptor (IFNLR) is expressed preferentially on epithelial cells, as well as neutrophils;**

(4) although the genes activated by type I and type III IFN signaling are similar, differences in cell type specificity and signaling kinetics result in distinct responses. The type I IFN response is more potent, rapid, and transient, whereas the type III IFN response is less potent, slower, and sustained.

(5) Many cell types respond to type I IFNs, resulting in a systemic response that is more inflammatory. In contrast, the type III IFN response is less inflammatory and concentrated at epithelial and barrier surfaces.

	Type I IFN	Type III IFN
Gene	 Single exon	 Multiple exons
Members	Human α1, α2, α4, α5, α6, α7, α8, α10, α13, α14, α16, α17, α21, β, ε, κ, ω Mouse α1, α2, α4, α5, α6, α7, α9, α11, α12, α13, α14, α15, α16, αBβ, ε, κ, ζ	Human λ1, λ2, λ3, λ4 Mouse λ2, λ3
Receptor binding	 • High-affinity binding to IFNAR2, then recruits low-affinity IFNAR1 to form signaling competent ternary complex • Receptor subunits bind on opposite sides of cytokine, no stem/stem contacts • Receptor is ubiquitously expressed 	 • High-affinity binding to IFNLR1, then recruits low-affinity IL-10Rβ to form signaling competent ternary complex • Less cytokine surface exposed, more stem-stem contacts in recpetor • Receptor preferentially expressed on epithelial cells (and some immune cells, e.g., neutrophils) 
Response	• High potency • Rapid kinetics • Systemic • Inflammatory	• Lower potency • Slower kinetics • Anatomic barriers • Less inflammatory



EFFECTS OF IFN LAMBDA AT THE BARRIER SURFACES

Immunity. 2015 July 21; 43(1): 15–28.

An Interferon Paradox

•Most, if not all, cells in humans and mice express the receptor for type I interferons (IFNs). Therefore, these cytokines have a range of direct and indirect effects on various cell types during infection with viruses, bacteria, parasites and fungi.

•Type I IFNs are important for host defence against viruses, through the induction of antiviral effector molecules that are encoded by IFN-stimulated genes. These IFNs can, however, cause immunopathology in acute viral infections. Conversely, they can lead to immunosuppression and loss of virus control during chronic viral infections.

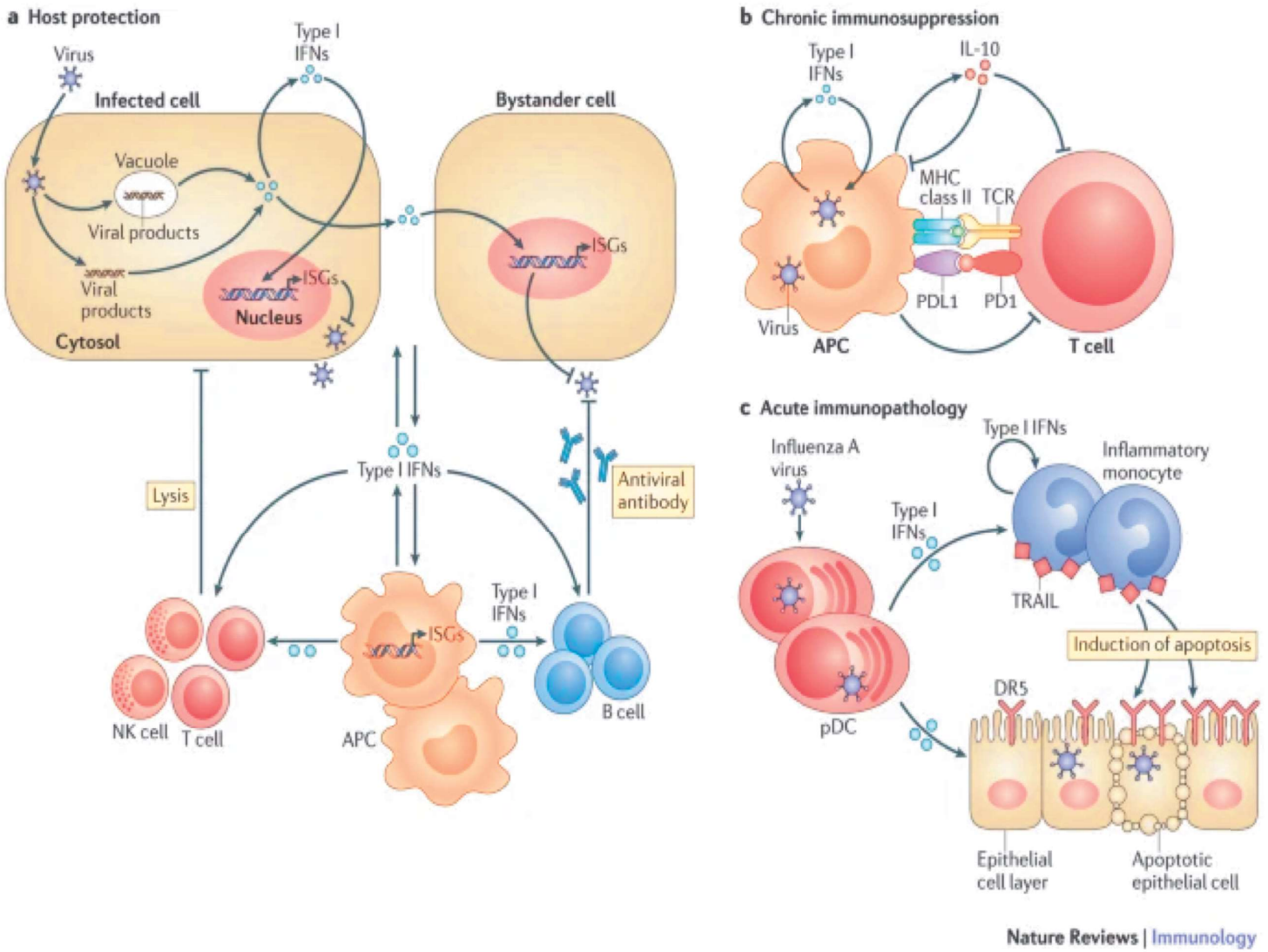
•Type I IFNs are part of a complex cross-regulatory network, which leads mostly, but not always, to protection of the host against infectious diseases with minimum damage to the host.

Nature Reviews Immunology **volume 15**, pages87–103 (2015)

During chronic viral infection, type I IFNs can induce the production of immunosuppressive cytokines such as interleukin-10 (IL-10).
They can also induce APCs to express ligands (such as programmed cell death 1 ligand 1 (PDL1)) for T cell-inhibitory receptors (such as PD1, the PDL1 receptor). These factors lead to the suppression of T cell function and failure to clear infection.

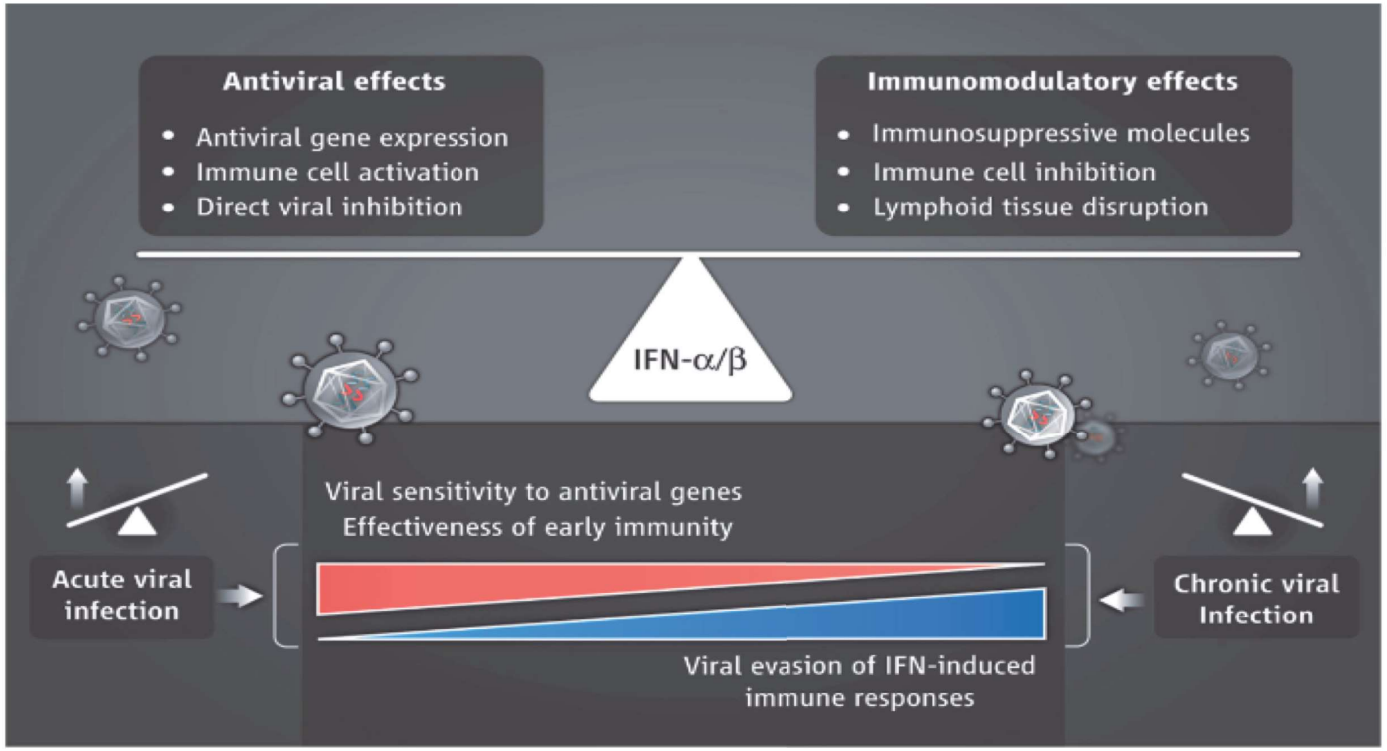
c | During acute viral infections such as with influenza virus, type I IFN production by myeloid cells, such as plasmacytoid dendritic cells (pDCs) and inflammatory monocytes, leads to the upregulation of expression of both the death ligand TNF-related apoptosis-inducing ligand (TRAIL) on inflammatory monocytes and the TRAIL receptor death receptor 5 (DR5) on epithelial cells. TRAIL-expressing inflammatory monocytes then induce immunopathology and host morbidity and/or mortality through killing epithelial cells.

Figure 2: Type I interferons during viral infection.



An Interferon Paradox

Interferons must balance antiviral actions against immunosuppressive effects during acute and chronic infections



Balancing dual roles.Type 1 interferons (IFN-α/β) may control viral replication and spread through two mechanisms. Antiviral responses include the expression of antiviral genes and the activation of specific im mune cells. Immunomodulatory responses include the expression of immunosuppressive molecules, im- mune cell inhibition, and cell death. The balance of these responses may shift, with enhanced antiviral ac- tions during acute infections and greater immunomodulatory effects during chronic infections. CREDIT: Y. HAMMOND/*SCIENCE*

Science

Interferons (IFNs) are glycoproteins belonging to the family of cytokines and have antiviral, antitumor, and immunomodulatory activities.

Table 1 Interferons and their uses

Generic names	Brand names	Uses
Interferon alfa* (IFN-α)		
Human natural leukocyte IFN-α	Alferon N	Malignant diseases: hairy cell leukaemia, chronic myelogenous leukaemia, cutaneous T cell lymphoma, follicular lymphoma, multiple myeloma, Kaposi's sarcoma, diffuse melanoma, renal cell carcinoma, carcinoid tumours Viral diseases: condylomata acuminata, chronic active hepatitis B and C
Natural fibroblast interferon	Fiblaferon	
Recombinant interferon IFN-α2a	Wellferon, Roferon	
Recombinant interferon IFN-α2b	Intron A	
Recombinant interferon IFN-α2c	Berofor	
Pegylated IFN-α2a	Pegasys	
Interferon beta (IFN-β)		
Recombinant interferon IFN-β1a	Avonex, Rebif	Multiple sclerosis
Recombinant interferon IFN-β1b	Betaferon, Betaseron, Extavia	
Pegylated IFN-β1a	Plegridy	
Interferon gamma (IFN-γ)		
Recombinant interferon IFN-γ1b	Immukin Actimmun	Serious infections in chronic granulomatous disease