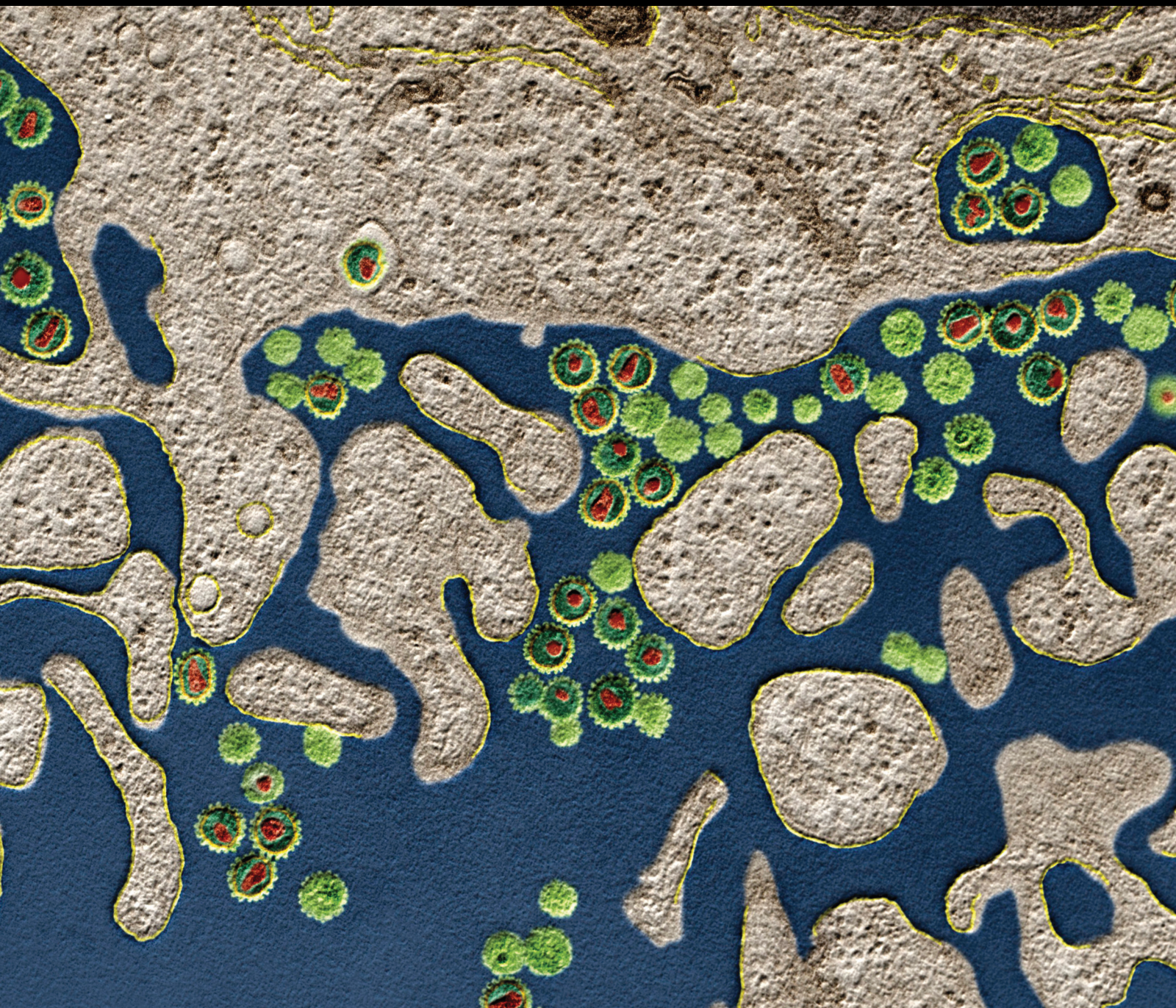# Innate Immune Responses against Human RNA Viruses

Lead Guest Editor: Laura B. Talarico
Guest Editors: Patricio L. Acosta and Diego R. Hijano

Innate Immune Responses against Human RNA Viruses

Innate Immune Responses against Human RNA Viruses

Lead Guest Editor: Laura B. Talarico
Guest Editors: Patricio L. Acosta and Diego R. Hijano

Contents

Review Article

Human Type I Interferon Antiviral Effects in Respiratory and Reemerging Viral Infections

Patricio L. Acosta [iD],[1,2] **Alana B. Byrne** [iD],[1,2] **Diego R. Hijano** [iD],[3] **and Laura B. Talarico** [iD][1,2]

[1]*Laboratorio de Investigaciones Infectológicas y Biología Molecular, Unidad de Infectología, Departamento de Medicina, Hospital de Niños Dr. Ricardo Gutiérrez, Buenos Aires (1425), Argentina*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires (1425), Argentina*
[3]*Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, USA*

Correspondence should be addressed to Laura B. Talarico; ltalarico@conicet.gov.ar

Type I interferons (IFN-I) are a group of related proteins that help regulate the activity of the immune system and play a key role in host defense against viral infections. Upon infection, the IFN-I are rapidly secreted and induce a wide range of effects that not only act upon innate immune cells but also modulate the adaptive immune system. While IFN-I and many IFN stimulated genes are well-known for their protective antiviral role, recent studies have associated them with potential pathogenic functions. In this review, we summarize the current knowledge regarding the complex effects of human IFN-I responses in respiratory as well as reemerging flavivirus infections of public health significance and the molecular mechanisms by which viral proteins antagonize the establishment of an antiviral host defense. Antiviral effects and immune modulation of IFN-stimulated genes is discussed in resisting and controlling pathogens. Understanding the mechanisms of these processes will be crucial in determining how viral replication can be effectively controlled and in developing safe and effective vaccines and novel therapeutic strategies.

1. Introduction

Type I interferons (IFN-I) are members from a large family of signaling proteins known for their potent antiviral activity. IFN-I were discovered in 1957 by Lindenmann and Isaacs and received their names based on the ability to interfere with influenza virus replication in chick cell culture [1–3]. In recent years, the knowledge about the mechanism of IFN-I production has quickly expanded.

IFN-I are produced by most cell types, the best known members of this group are IFN-α and IFN-β, and it also includes IFN-o, IFN-δ, IFN-κ, IFN-ε, IFN-τ, and IFN-ω [4, 5], which directly mediate a potent antiviral response. IFN-α includes 13 partially homologous members, encoded by chromosome 9, while IFN-β is composed by a single member and the gene is located on chromosome 12 [4].

IFN-I production occurs primarily when pattern recognition receptors (PRRs) present on the cell surface or in the cytosolic compartment of virtually all cell types are stimulated by pathogen-associated molecular patterns (PAMPs) [6–8]. The most widely studied PRRs are Toll-like receptors (TLRs). Ten different TLRs have been identified in humans, all of which detect PAMPs either on the cell surface or the lumen of intracellular vesicles, such as endosomes or lysosomes, and are involved in the recognition of a particular type of PAMP [6]. TLRs recognize different pathogen components, including double-stranded RNA (dsRNA) (TLR-3), single-stranded RNA (ssRNA) (TLR-7), or CpG DNA [6, 9, 10]. Other PPRs include retinoic acid-inducible gene-I- (RIG-I-) like receptors (RLRs) and nucleotide-binding oligomerization domain- (NOD-) like receptors (NLRs).

The recognition of TLR ligands leads to the recruitment of adaptor molecules that contain Toll interleukin-1 receptors (TIR) such as TIR-domain-containing adapter (TRIF), TRIF-related adaptor molecule (TRAM), Myeloid differentiation primary response gene 88 (MyD88), or TIR-domain-containing adaptor protein (TIRAP), leading the activation of molecular cascades that finally promote the activation of

nuclear transcription factors such as nuclear factor κB (NF-κB), IFN regulatory factor 3 (IRF-3), and 7 (IRF-7) [11, 12], which leads to the induction of genes encoding IFN-I (Figure 1(a)).

Both IFN-α and IFN-β use the same receptor, IFN-α/β receptor (IFNAR), which is expressed on a vast variety of cell types [5] (Figure 1(b)). This receptor is associated with Janus kinase 1 (JAK1) and Tyrosine kinase 2 (TYK2). IFN-α/IFN-β binding to IFNAR activates JAK1 and TYK2, which subsequently phosphorylate the transcription factor signal transducer and activator of transcription 1 (STAT1) and STAT2 [13], among other less characterized transcription factors such as STAT3, STAT4, and STAT5. Once phosphorylated, these factors associate with IRF-9 to form the IFN-stimulated gene factor 3 complex (ISGF3). In the nucleus, this complex binds specific DNA sequences containing IFN-stimulated response elements (ISRE) that promote the transcription of hundreds of IFN-stimulated genes (ISGs) including IRF-1, IRF-7, IRF-8, and IRF-9, whose function is to inhibit viral replication and induce an antiviral response in the area of the infected cell [13–15]. Some ISGs have been widely studied; the best known members include IFN dsRNA-dependent protein kinase R (PKR), $2'$-$5'$oligoadenilate synthetase (OAS), IFN-inducible transmembrane proteins (IFITM), dsRNA-specific adenosine deaminase (ADAR), and Myxovirus resistance protein A (MxA) and B (MxB) [16, 17].

The viral infectious cycle involves several steps, and ISGs target different stages of the viral cycle. For example, PKR, which was among the first ISG described, is a PRR that recognizes dsRNA, inducing IFN production and further ISG production [18]. PKR inactivates eIF-2α, leading to a global translation blockage of both viral and cellular mRNA [19]. OAS is also activated by cytoplasmic dsRNA and is subsequently involved in the degradation of RNA (through RNAse L), contributing to the inhibition of protein synthesis and therefore viral replication [20]. In the case of IFITM, the four members (IFITM-1, IFITM-2, IFITM-3, and IFITM-5) are present in endosomes and lysosomes and have a critical role in the inhibition of viruses that require vesicles for effective infection [21]. ADAR is a nucleic acid editing enzyme that disrupts base pairing. ADAR catalyzes the deamination of adenosine (A) to produce inosine (I) in dsRNA, inducing the replacement of the AU base pair by IU, which produces dsRNA destabilization [22]. On the other hand, MxA and MxB play a critical role during viral entry; while MxA acts in the early stages of infection once the virus has traversed the plasmatic membrane, MxB acts at the nuclear stage, inhibiting nuclear entrance [23, 24].

Dendritic cells (DCs) play a key role in the production of IFN-I. Although both myeloid DC (mDC) and plasmacytoid DC (pDC) produce IFN, the latter express specialized TLR-7 and TLR-9 and has a high constitutive expression of IRF-7 that allow them to respond to viruses with rapid and extremely robust IFN-α production [25–27]. Upon activation and production of IFN, pDCs mature into antigen-presenting cells (APC) serving as a key link between the innate and adaptive immune responses [28]. In addition, DCs produce IL-12, which drives naïve T cells into Th1-type responses, as well as IFN-α, which increases the frequency of IFN-γ producing

CD4$^+$ T cells [29]. Moreover, IFN-I also boost natural killer (NK) and CD8$^+$ T cell responses, promoting both survival and clonal expansion of the latter [30–32]. IFN-α and IFN-β lead to an immediate innate antiviral response and stimulate the adaptive immune system, affecting myeloid cells, NK cells, T cells, and B cells to mount an adequate Th1-biased immune response [16, 33].

Since its discovery, IFN's role as an important player of the immune system against viral infections has become evident. Although IFN is known for its innate beneficial response, recent studies have also linked these immune mediators to pathogenesis.

This review summarizes the current knowledge of the roles of IFN-I in respiratory virus and reemerging flavivirus infections (Table 1) and on the strategies that different viruses adapt to subvert IFN-I responses, mainly focusing on studies in human cells and subjects.

2. Human IFN-I Responses to Viral Pathogens

2.1. Human Respiratory Viruses. Respiratory viruses including influenza virus, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza virus (PIV), human rhinovirus (HRV), and human coronavirus (HCoV) are a major cause of respiratory disease and mortality in humans [34–36]. The disease can range in severity from mild or asymptomatic upper airway infections to severe wheezing, bronchiolitis, pneumonia, or death. Numerous factors can increase the risk of severe disease, including neurological conditions, chronic lung and/or heart disease, metabolic disorders, or a weakened immune system [37]. However, the majority of severe cases occur in previously healthy persons [34, 35].

When a cell is infected, the viral sensing activates pathways that trigger host immune responses. The viral presence is detected by several distinct PRRs including TLR-2, 3, 4, 6, 7, and 8, RLRs including RIG-I and MDA5 pathways among others [38–40]. These signaling pathways converge on IRF-3/IRF-7 and NF-κB that promote the induction of IFN-I that inhibit viral replication and contribute to the initiation of more specific adaptive immune responses [40]. IFN-α and IFN-β bind to receptor complexes, which activate STAT1 and STAT2 phosphorylation. IRF-9 binds to STAT1/STAT2 heterodimers forming the ISGF3 complex, which translocates to the nucleus to induce transcription of ISGs and to mount an antiviral state within the cell [39, 41].

Respiratory viruses, like other pathogens, target core molecules of the immune cascade to evade the host response. Like other different viruses, one major strategy used by respiratory viruses is to modulate, evade, or inhibit the IFN response that allows viral replication and transmission [41–43].

2.1.1. Influenza Virus. Influenza virus is a negative-sense, ssRNA virus that belongs to the *Orthomyxoviridae* family. This pathogen is a leading cause of respiratory illness in humans and causes annual epidemics and pandemics of differing severity [44]. The human subpopulations most vulnerable to influenza virus infections are children, pregnant women, and persons over 65 years old.

(a)

(b)
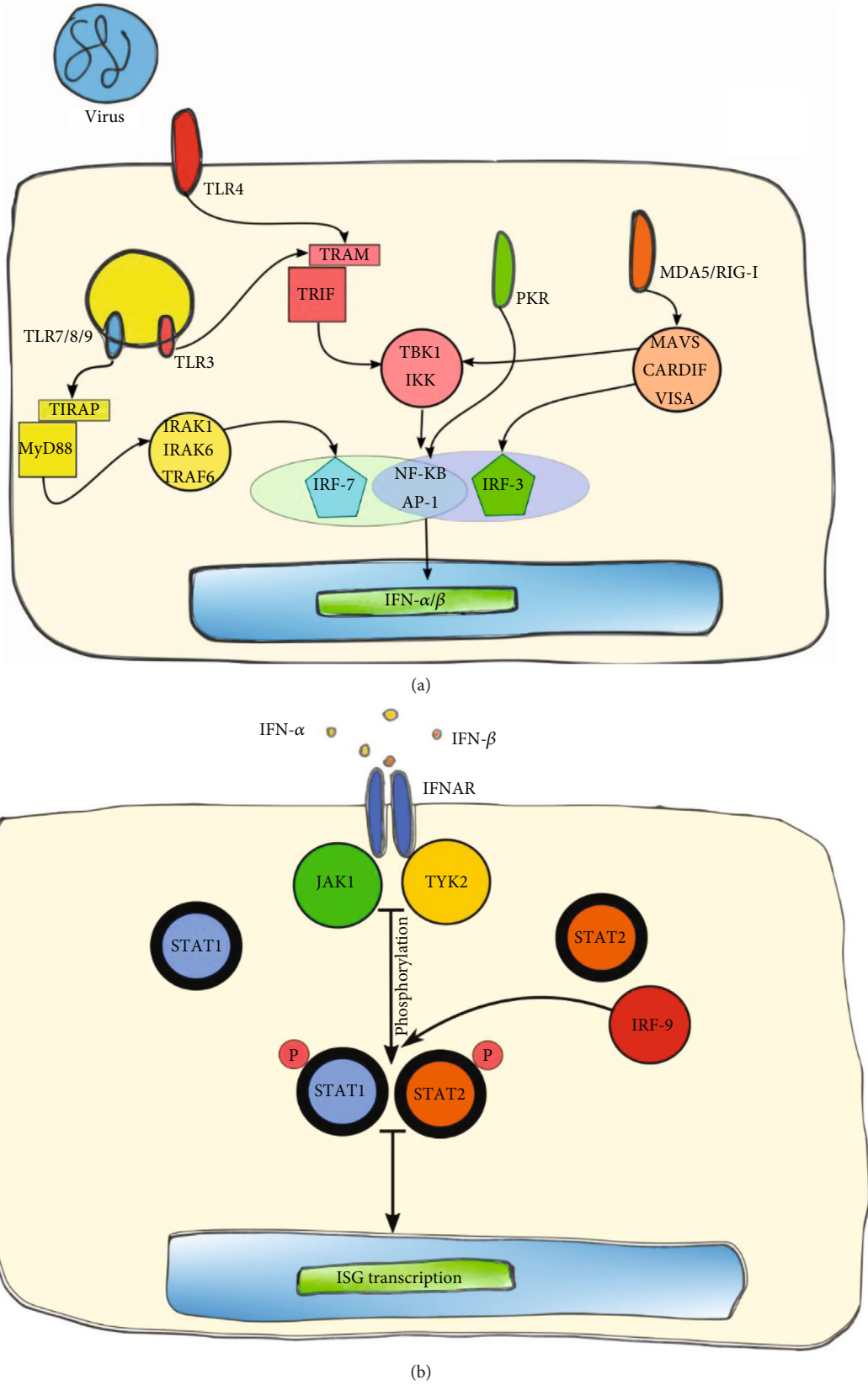
FIGURE 1: Type I interferon immune response. (a) Detection of viral infection by pathogen recognition receptors and signaling cascades resulting in the production of IFN-I. (b) IFN-I-α/β receptor (IFNAR) and activation of the JAK/STAT pathway leading to the induction of IFN-stimulated genes.

TABLE 1: Role of IFN-I in respiratory virus and flavivirus infections.

Virus	Model	Effect	Main finding	References
Human respiratory viruses				
Influenza virus	Human (*in vivo*)	Protective	Hospitalized subjects show enrichment for a minor *IFITM3* allele that leads to reduced influenza virus restriction	[70]
	Human (*in vivo*)	Pathogenic	High levels of IFN-α2 at enrollment predicted progression to severe disease	[278]
RSV	Human (*in vitro*)	Protective	Age and premature birth were independently associated with attenuated RIG-I-dependent IFN-α responses	[89]
	Human (*in vivo*)	Protective	Infants with severe RSV bronchiolitis have lower type-I IFN levels	[90]
hMPV	Mouse (*in vivo*)	Pathogenic	IFN-I contributed to disease pathogenesis due to increased inflammatory lung disease during infection	[102]
PIV	Human (*in vivo*)	Protective	Mean quantities of the virus in the secretions of those children with interferon was significantly lower compared to those without detectable IFN	[65, 117]
HRV	Human (*in vitro*) Human (*in vivo*)	Protective	Exogenous IFN-α, IFN-β significantly reduce HRV replication	[130, 131]
HCoV	Human (*in vivo*)	Pathogenic	High levels of IFN correlated with early sequelae	[148]
	Human (*in vivo*)	Protective	Patients treated with IFN show clinical response	[289, 290]
Emerging flaviviruses				
DENV	Human (*in vivo*)	Protective	Higher levels of IFN-α are observed in patients with milder dengue disease	[77, 190, 193–196]
	Human (*in vivo*)	Pathogenic	Higher levels of IFN-β in severe vs. nonsevere dengue children	[197]
	Human (*in vivo*)	Pathogenic	Higher levels of IFN-β in primary DHF patients compared to those with primary DF	[198]
ZIKV	Human (*in vivo*)	Protective	SNP at TLR-3 that decreased IFN-I response has been associated with microcephaly in newborns	[232]
	Human (*in vitro*)	Protective	Replication of Asian ZIKV strain Brazil 2015 (associated with neurodevelopmental disorders) was less sensitive to IFN-I, compared to other ZIKV strains	[233]
WNV	Human (*in vitro*)	Protective	PI3K that induces IFN-I controls WNV infection	[238]
	Human (*in vitro*)	Protective	Early activation of RLR or IFN-I signaling could block WNV infection	[239]
	Human (*in vivo*)	Inconclusive	IFN-α treatment was well tolerated and might have potential beneficial effects, due to treatment or chance	[293–296]
YFV	Human (*in vitro*)	Protective/pathogenic	ISGs shown to inhibit or enhance viral replication	[258]
JEV	Human (*in vitro*)	Protective	IFN-α at higher concentrations showed more efficacy in combating the replication of JEV	[297]
	Human (*in vivo*)	Inconclusive	No benefits from IFN-α2a treatment against children with JEV infection	[298]
SLEV	Human (*in vivo*)	Protective	IFN-α2b therapy for meningoencephalitis suggested a beneficial effect on the early neurologic course of the disease and clinical improvement	[299, 300]

FIGURE 2: Induction of IFN-I by influenza virus. PRRs involved in viral recognition include TLR-3, TLR-7/8, MDA5, and RIG-I that can detect viral products to signal IFN-α/β production in infected cells.
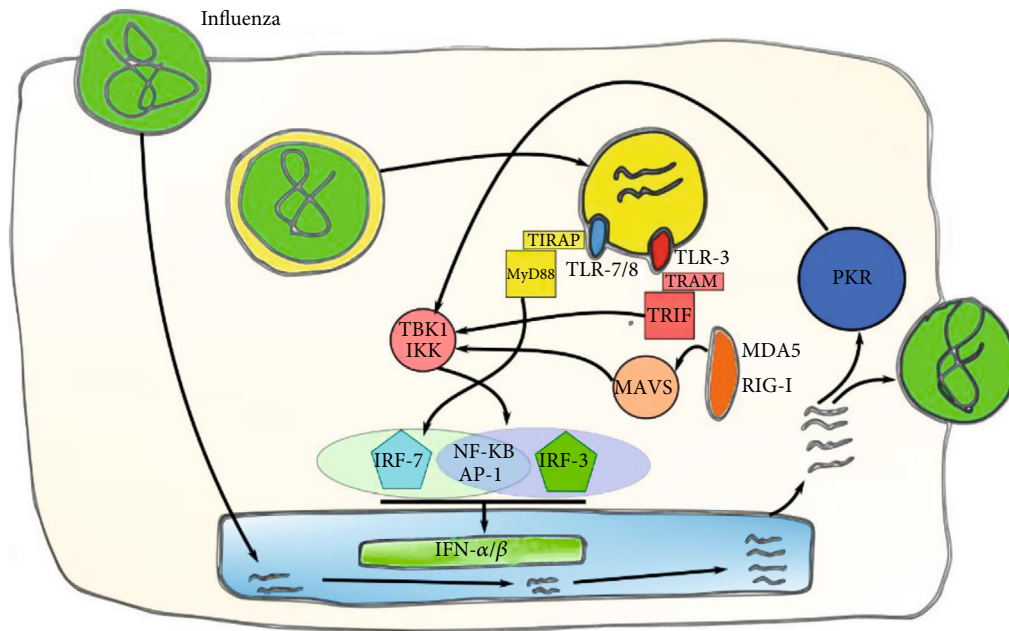
Influenza virus is primarily recognized by two different types of PRRs: RLRs and TLRs. Regarding the RLR family, RIG-I, LGP2, and MDA5 sense viral RNA during viral replication in the cytoplasm [45–47]. Once activated, RIG-I and MDA5 interact with the mitochondrial antiviral-signaling protein (MAVS), leading the activation of NF-κB and IRF-3 to stimulate IFN production (Figure 2). On the other hand, LGP2 has been related to the regulation of RIG and MDA5 activation [48]. Two different TLRs are activated by influenza virus, TLR-3 and TLR-7. The stimulation of TLR-3 in DCs and macrophages signal through TRIF, activating IRF-3/ NF-κB, leading to IFN-β secretion. On the other hand, TLR-7 in DCs activates MyD88, which activates IRF-7 and produces the secretion of both IFN-α and IFN-β [49, 50].

Influenza virus NS1 suppresses IFN synthesis through several different mechanisms [51–54] (Figure 3). First, it limits the pretranscriptional induction of IFN by forming a complex with RIG-I and limiting its signaling [55, 56]. Second, it directly limits the extent of the antiviral state by inhibiting PKR by sequestering dsRNA and by forming a complex with PKR avoiding its activation [57, 58]. Third, it inhibits mRNA maturation by interfering with the effector domain of the 3$'$-end processing [59]. Finally, NS1 activates phosphatidyl-inositol-3 kinases (PI3K) which can delay apoptosis of infected cells [60].

The evidence supporting the role of IFN-I during influenza infection in human populations is limited. Much of the available information focused on animal studies that have yielded mixed results. While some studies have found a protective role of IFN-I, others found no effect or even a pathogenic role [61–64]. Originally, Hall et al. detected that increased IFN production in nasal washes in children infected with influenza virus was associated with decreased shedding of virus [65]. Subsequent studies identified the high levels of IFN-I in breastfeeding children infected with influenza virus, providing support to the hypothesis that breastfeeding protects against respiratory viral infection [66, 67]. Additionally, a study of elderly subjects demonstrated that IFN-α production is decreased during influenza virus infection and suggested that this impairment can produce multiple defects in their innate and adaptive immune responses that could lead to increased severity [68]. Likewise, other studies have shown that pregnant women have an attenuated IFN-α response to influenza virus [69].

As mentioned previously, IFITM are a family of interferon-induced antiviral restriction factors with constitutive expressions in different cell types that are known to be induced by IFN-I. Recent evidence has shown that ISG IFITM3 is a potent antiviral factor in restricting influenza virus infection and a decrease in its expression results in a higher risk of hospitalization [70, 71].

2.1.2. Respiratory Syncytial Virus (RSV). RSV is a common virus that belongs to the *Paramyxoviridae* family. RSV is a negative-sense ssRNA virus that causes a wide range of symptoms and the severity can vary from mild to lethal disease [59]. RSV is especially severe in infants, the elderly, and immunocompromised individuals [72, 73]. Despite years of research, RSV is the only major etiological agent of acute lower respiratory tract infection (ALRI) for which no vaccine or specific treatments are available.

Several PRRs are involved in RSV recognition, including TLRs (TLR-2, TLR-3, TLR-4, and TLR-7), RLRs (RIG-I and MDA5), and NLRs (NOD-2). IFN-I production during RSV infection is activated by different mechanisms (Figure 4). TLR-3 detects dsRNA during viral replication
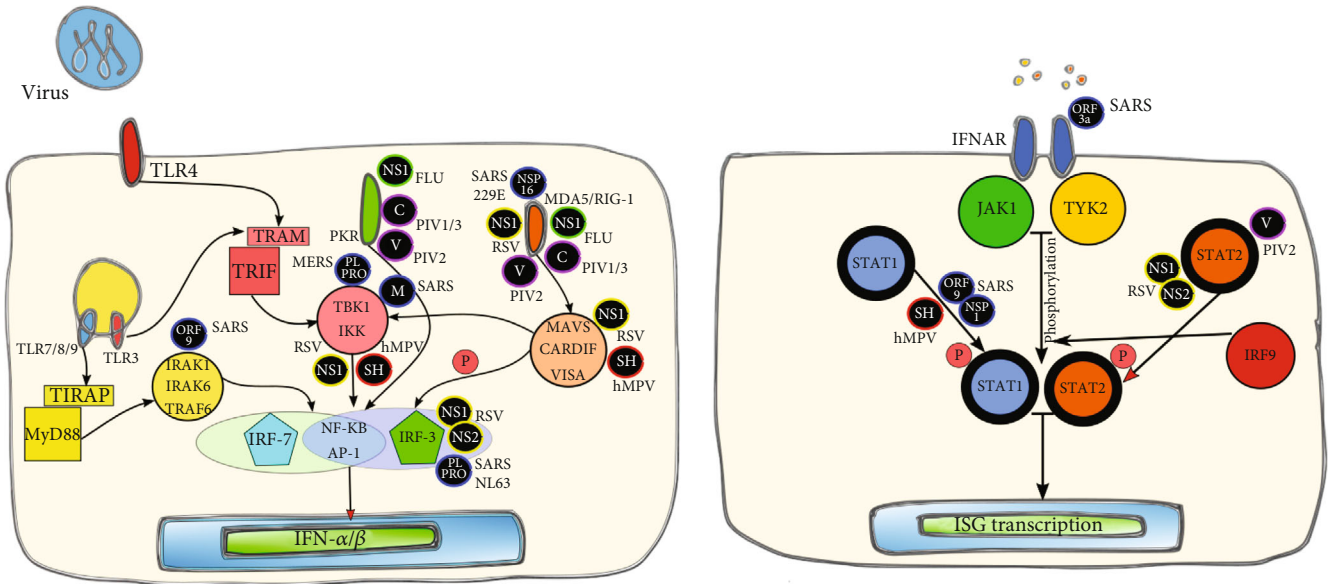
FIGURE 3: Evasion and inhibition of respiratory virus-induced IFN-I signaling. Many respiratory viruses can inhibit and modulate their detection using diverse strategies, thereby inhibiting the IFN-I production. Circular boxes in black background represent the viral protein involved in the pathway inhibition. Abbreviations: NS: nonstructural protein; V: V protein; C: C protein; SH: small hydrophobic protein.
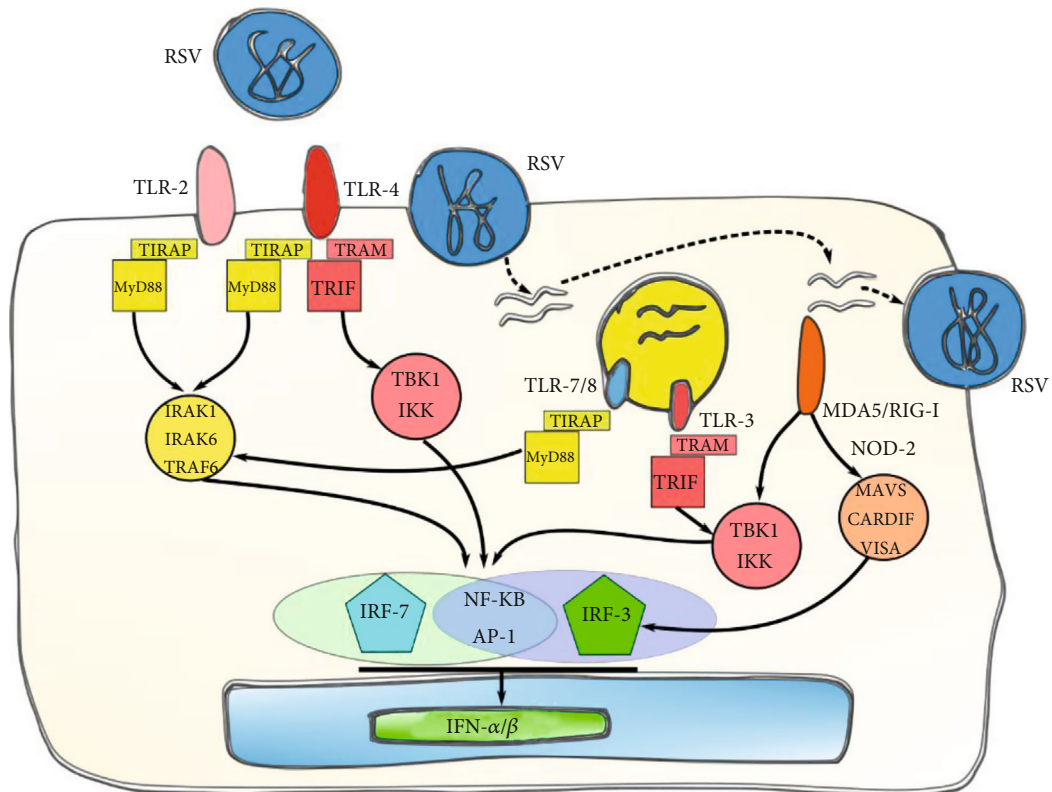
FIGURE 4: Induction of IFN-I by RSV. RSV is detected by different PRRs that include TLR-2, TLR-4, TLR-3, TLR-7/8, MDA5, and RIG-I and leads to the IFN-I pathway activation.

and signals through TRIF, which activates IFN-α production through NF-κB and IRF-3 pathway [74]. Other important PRRs for IFN-I production are RIG-I and MDA5 which subsequently link MAVS. This recruits TRAF2/6 and TRAF3 which leads to the activation of IRF-7 and IRF-3 through NF-κB [75]. Together, these IRFs stimulate ISRE sequences that promote IFN-I production [76, 77]. NLRs also play a role in activating the IFN-I pathway. NOD-2 can recognize

RSV ssRNA, thus, mediating IRF-3 and NF-κB activation pathways and leading to the IFN-β production [78].

Although RSV can lead to IFN-I production through multiple pathways, it has been established that this virus in particular is a poor stimulator of IFN-I. Unlike other respiratory viruses, studies have shown a limited role for IFNs in response to RSV infection [65, 79, 80]. Thereby, NS1 and NS2 (2 proteins encoded by RSV) appear to interact (and interfere) with multiple molecules of the IFN pathway (RIG-I, TRAF3, and IRF-3), which then interfere with IFN synthesis [81–84] (Figure 3). Studies of RSV NS1 and NS2 proteins have demonstrated that they are crucial for virus replication *in vitro*, however, through IFN-I antagonism, they contribute to adequate RSV replication *in vivo* (and *in vitro*) [85]. Using interference RNA that allowed the inhibition of NS1, the expression of IFN-α and IFN-β was augmented and this increase was associated with a reduction in viral titer [86]. On the other hand, NS2 has been shown to be the main IFN-I antagonist related to the STAT-2 pathway [82, 87], thus, inhibiting IFN-α and IFN-β responses through JAK/STAT signaling [82, 88].

One interesting study by Marr et al. showed that RSV-induced IFN-α expression by primary pDCs exposed to RSV is strongly correlated with age, observing higher IFN-α expression in healthy adults, followed by preschool children and little to no expression in healthy full-term infants [89]. This analysis of developmental innate immunity associated with poor IFN-I production during the first year of life suggests a role for IFN-I in RSV pathogenesis. Deficits in MAVS or RIG-I signaling events could explain this deficit. Furthermore, infants with severe bronchiolitis have less IFN-I levels when compared to those with moderate disease [90]. Taken together, these results suggest a critical role for IFN-I in RSV infection.

2.1.3. Human Metapneumovirus (hMPV). hMPV is a member of the *Paramyxoviridae* family, genus *Metapneumovirus* [91]. Isolated in 2001 [91], hMPV is a nonsegmented negative-strand RNA virus [92] and has been associated with the upper and lower respiratory tract infections with symptoms ranging from colds to pneumonia. hMPV particularly affects children, the elderly, and immunocompromised individuals [93]. Worldwide, hMPV is recognized as the second most common cause of bronchiolitis and pneumonia in children under 5 years old [94]. Despite the fact that hMPV is a clinically relevant pathogen, no vaccine is currently available.

Similar to RSV, two major pathways for the secretion of IFN-I during hMPV infection have been described. Each pathway involves different types of PRRs, one utilizes RLRs and the other TLRs. The former is primarily activated by RIG-I and MDA5, whereas the latter involves TLR-3 and TLR-7. RIG-I- and MDA5-mediated signal transduction begins when viral RNA is sensed in the cytosol. This recognition leads to the activation of IRF-3 and IRF-7 through the MAVS pathway [75, 95, 96]. TLR-3 activates the IFN pathway through IRF-3 and TLR-7 via IRF-7.

It is worth noting that the vast majority of cells express IRF-7 only after the activation of IRF-3 (which is expressed constitutively and is activated essentially by the RIG-I/MAVS pathway) or in response to IFNs. Importantly, TLR-7 is the main PRR that participates in sensing hMPV by pDCs, whereas MDA5-MAVS is the major pathway mediating hMPV sensing in conventional DCs [75].

Similar to other respiratory viruses, evidence suggests that hMPV has mechanisms for evading IFN-I production through interference in TLR- and RLR-dependent surveillance pathways. Early studies published by Dinwiddie et al. showed that hMPV can inhibit the IFN-α pathway in A549 cells. Therefore, this virus abolished the IFN-α pathway and downstream ISG signaling through mechanisms that regulate STAT1 activation [97]. One study supporting this information found that hMPV suppressed IFN-I responses through mechanisms involving the regulation of STAT1, STAT2, JAK1, TYK2, and the surface expression of IFNAR1 [98]. hMPV proteins impair the activation of PPRs through several different mechanisms. hMPV M2-2 protein interferes with MyD88 adaptor, a critical component for the activation of proinflammatory genes. This protein also interacts with MAVS, altering the production of IFN-β and also prevents IRF-3 phosphorylation [99]. In addition, hMPV G protein alters the recognition by RIG-I thereby influencing the secretion of IFNs, and the small hydrophobic protein can inhibit NF-κB, which is an important component in the IFN pathway [100]. Finally, the hMPV phosphoprotein B1 interferes with RIG-I recognition [75].

Studies describing the role of IFN-I in hMPV infections in humans are very limited. One study has shown that IFN-β was induced after hMPV infection in children [101]. In addition, a deleterious effect of IFN-I was reported in a murine model, in which IFN-I contributed to disease pathogenesis due to increased inflammatory lung disease during infection [102].

2.1.4. Parainfluenza Viruses (PIVs). PIVs are a group of nonenveloped, negative-sense, ssRNA viruses that belong to the *Paramyxoviridae* family. First isolated in the 1950s, PIVs are composed of five different (antigenic and genetic) types, PIV-1, PIV-2, PIV-3, and PIV-4 with two subtypes PIV-4a and PIV-4b [59, 103]. The virus was named *Parainfluenza* because it produces influenza-like disease and has a lipid envelope and hemagglutination and neuraminidase activities [59]. Worldwide, PIVs are important causes of upper and lower respiratory tract illnesses. Although PIV infections are generally self-limiting, some patients require hospitalization and the disease may lead to mortality, especially in children under the age of five years [104], the elderly, and the immunocompromised individuals.

All PIVs encode 6 universal proteins, N, P, M, F, HM, and L, and at least one additional protein from the P gene (C, V, D, W, and I), which is not essential for viral replication [59].

The innate immune response to PIV is not well-characterized, and little is known about the signaling pathways. Unlike other respiratory viruses, TLR signaling is not described but is thought to play a role in the IFN pathway activation during PIV infection. Regarding RLRs, a study showed that RIG-I is involved in IFN-I induction via IRF-3 after PIV-3 infection [105]. In addition, MDA5 has also been

found to be activated by degraded products of RNase L from PIV [106].

This group of viruses is known to encode proteins that block innate immune responses to viral infections, allowing PIV replication. As mentioned above, pathogen RNA synthesis provides strong stimuli to mount an IFN immune response. PIV-1 and PIV-3 encode C proteins, while PIV-2 encodes a V protein. Both proteins, C and V, are involved in the blockade of IFN-I induction by preventing PKR activation [107–110]. The viral strategy in the case of PIV-1 and PIV-3 is to avoid the IFN production. PIV-1 does not inhibit the IFN pathway, given that the synthesis of the viral RNA can activate IFN production. Rather, the viral C protein modulates its RNA production, preventing MDA5 activation [109, 111]. This protein also interferes with STAT1, avoiding signaling through this pathway and thus IFN production [112]. The V protein of PIV-2 interferes with the IFN production in different ways. One of the mechanisms is the inhibition of MDA5 activation [113]. In addition, the highly conserved V protein Cys-rich domain has been shown to be both necessary and sufficient to limit the activation of the IFN-β promoter [114]. Finally, the V protein interferes with the IFN pathway by abolishing STAT2 signaling [115]. Interestingly, there is evidence that shows that this protein also regulates viral RNA production (as PIV C protein) [116].

Overall, little is known about IFN-I response after the PIV infection in human population studies. An early work detected IFN-I in patients with primary PIV infection [65]. *In vitro* experiments have shown that PIV-2 induced IFN-α by day 2 postinfection, PIV-3 by day 3, and that PIV-1 did not produce this molecule. IFN-β production was shown to be poor in all serotypes [117].

2.1.5. Human Rhinovirus (HRV). HRV is a small, nonenveloped, positive-sense, ssRNA virus that belongs to the *Picornaviridae* family. The family is divided into three species: rhinovirus A, B, and C [59]. HRV is a ubiquitous seasonal microorganism and is the most frequent cause of common cold (causing more than 50% of upper respiratory tract infections in humans worldwide). Given that the viral genetic diversity is huge (>160 serotypes), recurrent infections with this virus are frequent. Although HRV infections are not life-threatening, they can be also detected in the lower airways where they can cause severe exacerbations in patients with asthma and chronic obstructive pulmonary disease [118, 119].

Different PRRs are involved in HRV recognition, belonging to two different families of receptors: TLRs (TLR-2, 3, 7, and 8) and RLRs (RIG-I and MDA5). TLR-2 on the cell surface recognizes the virus capsid, even without viral replication. In addition, TLR-3, TLR-7, and TLR-8 localized in intracellular compartments are stimulated once the viral particle is internalized and the dsRNA/ssRNA is sensed [120]. The TLR stimulation leads to the activation of downstream signaling molecules that activate IRF-3, IRF-7, and NF-κB, triggering IFN-I secretion. The evidence regarding TLR-3 is somewhat unclear; while one study described an important role for this receptor in the host response against the HRV infection [121], others found no function for this PRR

[120]. The HRV genome is also recognized by RIG-I and MDA5 (both localized in the cytosol) which can recognize ssRNA and dsRNA, respectively. The RLR stimulation leads to the activation of MAVS and the consequent activation of NF-κB and IRF, triggering the production of IFN-I in the airway cells [120, 122, 123]. Responses mediated by IFN-I *in vivo* are critical for the antiviral effects that limit HRV through the activation of NK cells [124].

Similar to other respiratory viruses, HRV seems to suppress IFN synthesis using multiple strategies. Even though IFN evasion mechanisms have been well-studied in other enteroviruses, the information for HRV is scarce. A study showed that the MAVS adaptor is cleaved by 2A and 3C proteases of HRV to stop IFN-I signal transduction [125]. Furthermore, the 3C proteases have been implicated in the cleavage of RIG-I altering IFN-I production [126]. Regarding TLRs, despite their crucial role in viral sensing, there is no evidence of TLR signaling evasion by HRV.

Different studies have attributed susceptibility to HRV infection to impaired IFN-I production that leads to a higher viral load [127, 128]. Abnormal HRV-induced IFN in asthmatic patients was shown in peripheral blood mononuclear cells (PBMCs) for IFN-α and in primary human bronchial epithelial cells for IFN-β [129]. In addition, *in vitro* studies on epithelial cells showed that exogenous IFN-I led to reduced viral load [130]. Furthermore, this has been confirmed in epithelial cells from asthmatic patients, suggesting a role for IFN-I in limiting induced viral exacerbations [131]. However, other groups have found no differences between viral loads in asthmatic and control subjects during HRV infections [132]. Further investigation regarding the role of IFN-I during HRV infections needs to be completed.

2.1.6. Human Coronavirus (HCoV). The family *Coronaviridae* contains two subfamilies, the *Coronavirinae* and the *Torovirinae*. They are a large group of positive-sense ssRNA genome viruses that can infect mammals and birds, causing a wide variety of diseases which can lead to frequent mutations and infections of new species. They have been divided into four genera, two of which, alphacoronavirus and betacoronavirus contain viruses infecting humans [133]. To date, four HCoVs (HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) circulate globally in the human population and contribute to approximately one-third of common cold infections in humans [134]. HCoVs had been regarded as mildly pathogenic until the early 2000s. At that time, a new disease appeared in China, severe acute respiratory syndrome (SARS), which was quickly attributed to a new HCoV, the SARS-CoV. Following that outbreak, a related but different HCoV producing severe respiratory disease emerged, the Middle East respiratory syndrome coronavirus (MERS-CoV) [135]. Recently, a novel coronavirus (2019-nCoV) has emerged. The 2019-nCoV is causing an outbreak of unusual viral pneumonia in patients. It initially began in Wuhan, China and has spread to many countries worldwide [136].

Similar to previously described respiratory viruses, HCoVs are detected by TLR-3, TLR-7, RIG-I, and MDA5 [137]. TLR-3 activated by dsRNA stimulates TRIF that activates AP-1, NF-κB, IRF-3, and IRF-7 leading to the IFN

production [138]. TLR-7 senses the ssRNA, leading to the activation of IRF-7 that also stimulates IFN secretion. RIG-I and MDA5 sense viral molecules in the cytoplasm, and their activation stimulates MAVS that induces the activation of IRF-3 and NF-κB to finally induce the IFN production [139].

Like other viruses, HCoVs use multiple mechanisms to evade IFN-I immune response. Evidence shows that different viral structures can inhibit or modulate the IFN production or activity. Thereby, NS protein-16 codified by SARS-CoV and HCoV-229E induces the methylation of viral mRNA cap structures that inhibit recognition by MDA5 [140, 141]. The papain-like protease (PLPRO) domain of NS protein-3 of SARS-CoV and HCoV-NL63 inhibits the activation of IRF-3 [142, 143]. Moreover, it was demonstrated that PLPRO encoded by MERS-CoV suppresses the induction of IFN-β through its deubiquitinating activity [144]. Protein M of SARS-CoV inhibits IFN-I production by impeding the formation of the TRAF3-TANK-TBK1/IKKϵ Complex [145]. ORF9b protein of SARS-CoV was shown to stimulate the degradation of MAVS and TRAF6, inhibiting the IFN-I pathway [146].

The IFN response seems to be contributing simultaneously to the protection against viruses and to the pathology induced by the same viral infections. Human pDCs demonstrate a robust IFN-I production after MERS-CoV infection, especially IFN-α, and this response is greater than that elicited by SARS-CoV [147]. High IFN-α and IFN-γ have been associated with early SARS-CoV sequelae suggesting that unregulated IFN responses during acute-phase SARS-CoV may be deleterious for this infection [148].

2.2. *Reemerging Flaviviruses.* The interplay of climatic and environmental changes as well as the growth of the human population and increased urbanization has triggered the reemergence and rapid spread of arthropod-borne viruses significant to public health. Yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), and Zika virus (ZIKV) are mosquito-borne flaviviruses that have reemerged in both hemispheres during recent decades. Other flaviviruses have emerged in specific regions of the world, including the Japanese Encephalitis virus (JEV) and Saint Louis encephalitis virus (SLEV), among others [149].

DENV, ZIKV, WNV, YFV, JEV, and SLEV belong to the flavivirus genus of the *Flaviviridae* family, which comprises a diverse group of enveloped, positive-sense, ssRNA viruses transmitted by blood-feeding mosquitoes, causing disease in humans [150]. The flavivirus RNA genome (11 kb), which encodes a single open reading frame flanked by highly structured 5$'$ and 3$'$ untranslated regions (UTRs), is transcribed as a single polyprotein that is proteolytically processed by host and viral proteases to yield three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), the latter of which regulate viral translation, transcription, and replication as well as attenuate host antiviral responses [150].

In human cells, the host responds to flavivirus infection by recognizing viral nucleic acids through several distinct PRRs including RLRs, TLR-3, 7, and 8, NLRs, and the cyclic GMP-AMP synthase/stimulator of IFN genes- (cGAS-STING-) dependent sensing pathway [151, 152] (Figure 5). Among RLRs, RIG-I and MDA5 are involved in the detection of cytoplasmic dsRNA produced during viral replication [153]. The TLRs of importance during flavivirus infections are TLR-7 and TLR-8, which detect ssRNA as well as TLR-3, which identifies dsRNA produced during viral replication [152, 153]. Recently, it has been reported that DENV infection activates TLR-9 signaling, which is known to recognize bacterial or viral DNA, by inducing mitochondrial DNA (mtDNA) release in human DCs [154]. The cGAS-STING pathway, which is known to sense DNA viruses, has also been recently involved in restricting flavivirus infections [151]. It has been reported that during DENV infection, mtDNA is spilled into the cytoplasm and this subsequently activates the cGAS/STING signaling pathway to stimulate the production of IFN-I [155]. pDCs are a predominant source of IFN-I during viral infection and TLR7 signaling in pDCs has been reported to promote the contact of these cells with infected cells in a specialized platform that enables viral RNA transfer and antiviral responses [156]. The binding of viral ssRNA and/or dsRNA to PRRs activates downstream signaling cascades, such as the activation of transcription factors IRF-3 and IRF-7 and NF-κB, that result in the induction of IFN-α and -β. Subsequent secretion of IFN-I and binding to IFNAR activates JAK/STAT dependent- and independent-signaling cascades that result in the transcription of hundreds of ISGs, which encode proteins that inhibit flavivirus replication and spread [151, 153, 157].

To facilitate propagation, flaviviruses, like other viruses, have evolved specific strategies involving one or more viral nonstructural proteins to either prevent IFN induction or to inhibit IFN signaling. The inhibition of IFN-I induction is achieved by either sequestration or modification of viral RNA and inhibition of PRRs [151]. In addition, several flavivirus nonstructural proteins such as NS2A, NS2B-NS3, NS4B, and NS5 have been shown to interfere with IFN signaling pathways through different mechanisms depending on the virus [152, 158, 159] (Figure 6). Recently, subgenomic flavivirus RNA (sfRNA), a nongene product encoded in 3$'$UTR generated by incomplete degradation of viral RNA by a cellular 5$'$-3$'$ exoribonuclease, has been proposed to play a modulatory role in the host antiviral response in mammalian cells by antagonizing IFN-I, as well as displaying viral interference in insect cells [24, 160–162].

The protective role of IFN-I against flaviviruses has been extensively demonstrated in mice since IFN-I signaling-deficient mice are highly susceptible to infection by DENV, ZIKV, and WNV [163–166]. The reported effects of IFN-I in flavivirus infections in human cells and subjects are discussed in the following sections.

2.2.1. *Dengue Virus (DENV).* DENV is an acute febrile disease caused by four distinct antigenically related DENV serotypes (DENV-1, -2, -3, and -4) that are transmitted to humans by the bite of *Aedes* spp. mosquitoes, mainly *Aedes aegypti* [167]. DENV infects an estimated 390 million people every year of which 96 million have apparent DENV infections, with different levels of disease severity [168]. The clinical manifestations of dengue can range from mild febrile
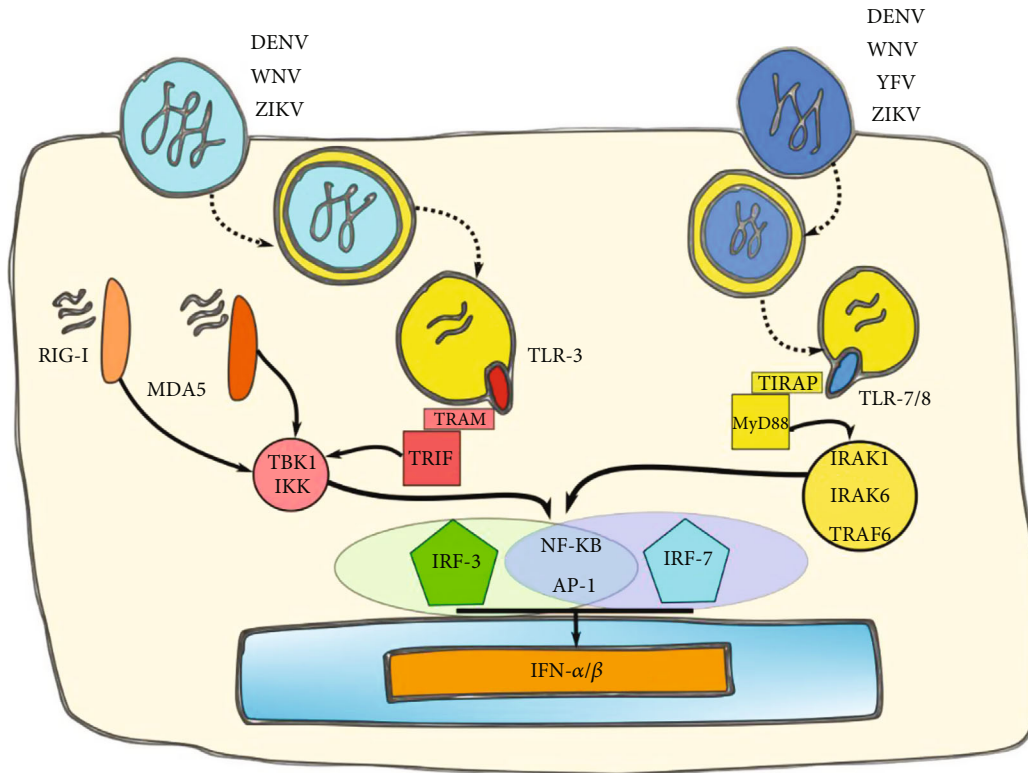
Figure 5: Induction of IFN-I by flaviviruses. The TLR and RLR signaling cascades converge with the activation of transcription factors, which are critical for the induction of IFN-α/β.
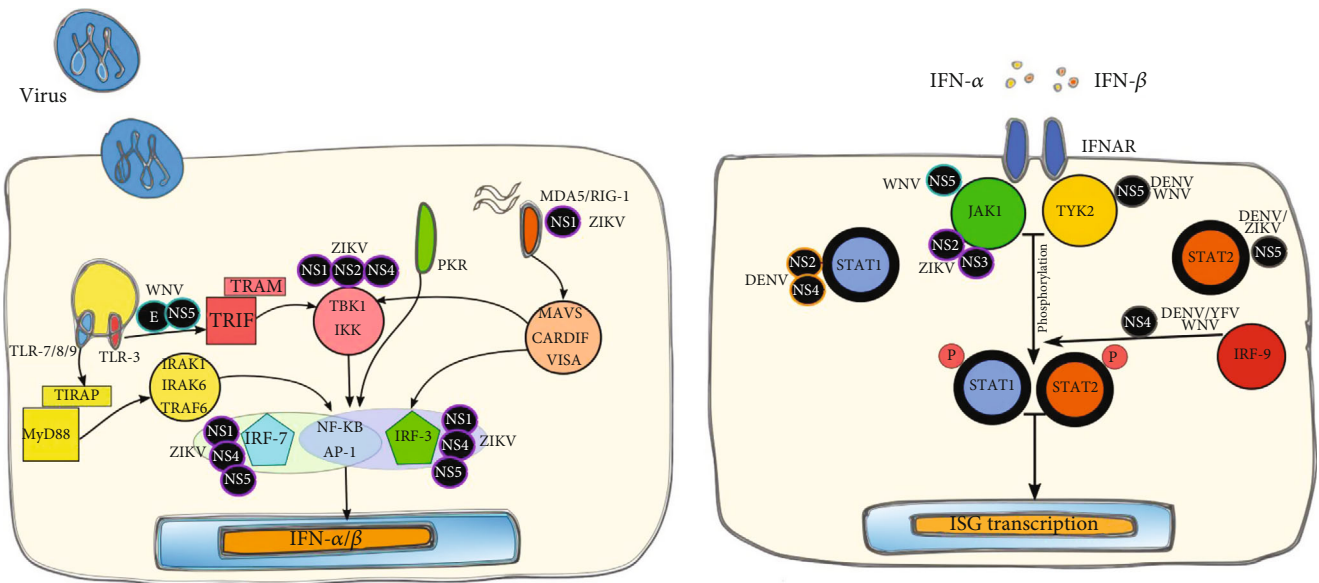
Figure 6: Inhibition of the IFN-I pathway by flaviviruses. Different viral proteins are involved in the modulation of the IFN-I activation pathway. Circular boxes in black background represent the viral protein involved in the pathway inhibition. Abbreviations: nonstructural protein (NS) 1, 2, 3, 4, and 5; E: envelope protein.

illness with myalgia and rash (formerly known as dengue fever (DF)) to severe forms of disease, characterized by plasma leakage and hemorrhage (dengue hemorrhagic fever (DHF)) leading to potentially life-threatening hypovolemic shock [169]. At present, several DENV vaccines are under development, including one that has been registered in

several countries with high endemicity due to the limited efficacy in naïve individuals and against all four DENV serotypes [170].

DENV has been reported to trigger a robust IFN-I response, which has been shown to be important in controlling DENV infection [171]. IFN-I has been reported to inhibit DENV infection in a variety of human cells, including hepatoma, fibroblasts, and myeloid cells [172, 173]. RIG-I and MDA5 sensing of DENV has been critical in the immune response [39], as knockdown of RIG-I and MDA5 in Huh7 cells resulted in increased DENV replication [174]. In addition to TLR-3, 7, and 8 [151], DENV has been reported to activate and upregulate the expression of TLR-2 and 6 in human PBMCs [175]. NLRs have also been shown to be activated in DENV and WNV infections leading to the formation of the inflammasome complex with subsequent production of inflammatory cytokines of the IL-1β family [176]. DENV sensing by PRRs results in the secretion of IFN-I which triggers the JAK/STAT pathway leading to the production of ISGs with diverse antiviral properties [151].

DENV nonstructural proteins have been involved in the downregulation of the IFN pathway in humans, targeting important signaling molecules downstream of PRRs, resulting in the inhibition of IFN-regulated gene expression [177]. NS4B in combination with NS2A and NS4A have been reported to block IFN-I signaling by decreasing STAT1 phosphorylation in human A549 cells [178]. Furthermore, the DENV NS2B-NS3 protease complex has been involved in the cleavage of the human adaptor molecule STING or MITA, inhibiting IFN-I production [179, 180]. Interestingly, DENV cleaves STING in humans but not in nonhuman primates that may serve as its sustaining reservoir in nature [181]. In addition, the DENV NS2B-NS3 protease interacts with the cellular IκB kinase, an important kinase involved in the IFN-I induction, disrupting RIG-I signaling and inhibiting the IFN production [182]. The DENV polymerase NS5 has also been reported as a potent and specific IFN-I antagonist, due to its binding to human STAT2 for ubiquitin-mediated proteasomal degradation [183, 184]. The ability of DENV NS5 to bind and degrade human but not mouse STAT2 may be the major reason behind DENV's efficient replication in human but not in wild-type mouse cells [185]. Recently, increased virulence of different type 1 DENV isolates has been associated with a higher capacity of NS proteins to suppress IFN signaling [186]. Likewise, sfRNA of DENV strains that are associated with greater epidemic potential prevents the ubiquitination-dependent activation of RIG-I by binding to the ubiquitin ligase tripartite motif protein 25 (TRIM25), subverting the RIG-I pathway, and consequently impairing IFN-I induction [162].

The viral control and immune regulation exerted by IFN-I in dengue patients and human cells infected with DENV have been studied in several reports [172, 173, 187–189]. Even though a strong IFN-I response has been described in dengue patients, the association of this response with disease severity is controversial [188, 190–192]. While some studies reported similar serum levels of IFN-α or IFN-β in DF and DHF in Thai and Mexican patients [188, 191], several other reports from Brazil, Mexico,

Colombia, Taiwan, India, and Thailand showed higher levels of IFN-α in patients with milder dengue disease [77, 190, 193–196], suggesting that a robust production of IFN-α may be correlated with a better clinical condition with respect to dengue infection and disease progression. Conversely, a recent study from Paraguay reported higher levels of IFN-β in severe vs. nonsevere dengue children [197]. Similarly, a study from Northeast Brazil described higher levels of IFN-β in primary DHF patients compared to those with primary DF [198]. Considering the preexisting immunity of patients to DENV, several reports described higher levels of IFN-α in patients undergoing primary infection compared to those with secondary DENV infections [77, 190, 192, 195], while other studies observed a similar expression of IFN-α levels in both types of infections [197, 198]. Furthermore, different serum levels of IFN-α and IFN-β have been reported in DENV-infected patients [192], probably due to the differential kinetics of IFN-I induction during DENV infection of human cells [199, 200].

2.2.2. Zika Virus (ZIKV). ZIKV is a newly emerging flavivirus transmitted to humans by *Aedes* spp. mosquitoes, including *Aedes aegypti* [201]. Since the first human ZIKV infection was reported in Uganda in 1964 [202], human ZIKV infections had remained sporadic and limited to small-scale epidemics in Africa and Southeast Asia for decades [203, 204], until 2007, when a large outbreak of Zika fever was reported on Yap Island (Micronesia) [205]. Over the next seven years, outbreaks were reported in other Pacific Islands [206]. In 2015, the largest outbreak of ZIKV ever reported began in Brazil, with a rapid expansion of the virus in South and Central America [207–209]. Typically, ZIKV infections can be asymptomatic or manifested as a self-limiting febrile illness characterized by rash, headache, conjunctivitis, arthralgia, and myalgia [204–207, 209]. However, recent outbreaks in the South Pacific and Latin America reported severe neurological complications, including Guillain-Barré syndrome (GBS) in adults and microcephaly in newborns [210–212]. ZIKV has been shown to cross the placental barrier, disrupting brain development [213]. Recently, ZIKV spread by sexual contact has also been documented [214]. At present, there is an urgent need for vaccines and therapeutics to combat ZIKV.

The induction of antiviral immune responses after ZIKV infection of human cells has been reported in several studies [215–219]. The innate immune response after ZIKV infection of skin cells and endometrial stromal cells was characterized by a strongly enhanced IFN-β gene expression and the induction of ISGs, including OAS, ISG15, and MX1 [215, 216]. Moreover, the transcription of PRRs such as TLR-3, RIG-I, and MDA5 has been reported to be induced upon ZIKV infection of human skin cells [215]. Similar to DENV, NLR (inflammasome) activation has also been reported in ZIKV infection of monocytes [220]. Elevated secretion of IFN-β was also reported in human lung epithelial cells, which may delay the apoptosis exerted by ZIKV infection [217]. Schwann cells (SC), which play a central role in peripheral nerve disease and can be the target for damage in GBS, were susceptible to infection with ZIKV and YFV, but not DENV,

and ZIKV infection of SC induced expression of IL-6, IFN-β, IFN-λ, IFIT-1, TNF-α and IL-23A mRNAs, and negative regulators of IFN signaling [218]. Furthermore, ZIKV infection of human DCs produced a strong induction of the RLR signaling pathway at the RNA transcription level, but ZIKV was able to block IFN-I signaling by targeting STAT1 and STAT2 phosphorylation [219].

The mechanisms by which ZIKV antagonizes IFN-I induction and signaling in human cells, which might contribute to the broad cellular tropism and persistence of ZIKV, have been reported in recent studies [159, 221–223]. ZIKV NS1 has been reported to interact with RIG-I and downregulate the antiviral signaling pathway [224]. In addition, Donald et al. reported that sfRNA in ZIKV acts as the antagonist of RIG-I-dependent IFN production [161]. Furthermore, ZIKV NS4A impairs RLR-MAVS interaction and subsequent induction of antiviral immune responses by binding to the caspase activation and recruitment domain (CARD) of MAVS and thus blocking its accessibility by RLRs [225, 226]. ZIKV NS3 has been shown to antagonize antiviral gene induction by RIG-I and MDA-5 by binding to and sequestering the scaffold proteins 14-3-3ε/η [227]. ZIKV NS1, NS2A, NS2B, and NS4B have been shown to interact directly with TANK-binding kinase 1 (TBK1), required for the phosphorylation of IRF-3 [159, 228]. In addition, the NS2B-NS3 protease complex of ZIKV has been reported to impair the JAK/STAT signaling pathway by promoting the degradation of JAK1 and to block RLR-triggered apoptotic cell death. Furthermore, the cooperation between NS1, NS4B, and NS2B-NS3 further attenuates antiviral immunity, by impairing IFN-induced degradation of NS2B-NS3 [159]. Similar to DENV, ZIKV NS5 has been reported as a potent antagonist of IFN-I responses by targeting human but not mouse STAT2 for ubiquitin-mediated proteasomal degradation [183, 185, 221, 222]. However, unlike DENV, ZIKV NS5 did not require the E3 ubiquitin ligase UBR4 interaction, exhibiting a virus-specific mechanism [222]. In addition, ZIKV NS1 and NS5 interact with NLRP3 and promote assembly of the NLRP3 inflammasome complex resulting in IL-1β production and stimulated ZIKV replication [229–231].

Recently, ZIKV microcephaly in newborns has been associated with rs3775291 single nucleotide polymorphisms (SNP) at TLR-3 reducing the activation of NF-κB and thus decreasing IFN-I responses in mothers infected by ZIKV during pregnancy [232]. Furthermore, comparative analysis of African and Asian lineage-derived ZIKV strains revealed pronounced differences in the activation of innate immune signaling and inhibition of viral replication which may be related to differential pathogenesis [233]. Compared to the replication of other ZIKV strains, the replication of Asian ZIKV strain Brazil Fortaleza 2015, which was associated with neurodevelopmental disorders, was less sensitive to the antiviral actions of IFN-I, while infection with this strain induced weaker and delayed innate immune responses *in vitro*.

2.2.3. West Nile Virus (WNV).

WNV is a mosquito-borne flavivirus of international health concern. WNV is maintained in a natural cycle involving primarily *Culex* spp. mosquitoes and avian hosts, but it also infects humans, horses, and other animals [234]. WNV is endemic in parts of Asia, Africa, and Europe, and it was first introduced to North America in 1999 [235]. Since then, the virus has spread rapidly throughout the United States and has been associated with over 21,000 encephalitis/meningitis cases and 1,800 deaths [236]. However, no effective prophylactic or therapeutic measures are currently available [234]. Infection with WNV in humans remains asymptomatic and/or subclinical in most cases and causes symptoms in 20-30% of WNV-infected individuals. The clinical manifestations range from a mild flu-like illness to more severe neuroinvasive disease, associated with significant morbidity and mortality [234, 237].

The protective role of IFN-I after WNV infection has been extensively demonstrated in mice by the marked increase in mortality in infected IFN-α/β receptor-deficient mice [166]. The studies of IFN-I protection after WNV infection are more limited in human cells. Recently, the PI3K, which play an important role in the induction of IFN-I antiviral responses, have been shown to control West Nile virus infection in human and mouse cells [238]. The presence of PI3K inhibitors blocked the translocation of IRF-7 from the cytosol to the nuclei, reduced IFN-I mRNA and protein expression, and decreased the secretion of IFN-I. Recently, in human monocyte-derived DCs early activation of RLR or IFN-I signaling could block WNV infection [239]. Furthermore, in human glioblastoma cells, WNV replication was regulated by early IFN-β induction, while in human neuroblastoma cells, a delayed IFN-β response, due to the concealing of viral dsRNA in intracellular membranes, resulted in efficient WNV replication [240]. In addition, differences in replication and induction of IFN-I responses between attenuated and virulent WNV strains in human monocyte-derived DCs accounted for the differing virulence in humans [241].

WNV NS4B and NS5 have been reported as important IFN-I antagonists [178, 242]. Similar to DENV, NS4B inhibited the JAK/STAT signaling pathway by decreasing STAT1 phosphorylation [178]. The NS5 of WNV strain NY99 blocked IFN-I responses by inhibition of STAT1 phosphorylation [242]. The helicase domain of WNV NS3 has been reported to inhibit IFN-I signaling and differences in virulence between the NY99 and NSW2011 strains of WNV have been attributed to differential inhibition of the helicase domain of NS3 [243]. Furthermore, WNV NS1 has been demonstrated to antagonize IFN-β production by suppressing RLR activation [244].

The human studies evaluating the IFN-I response to WNV infection and its relationship with disease severity are scarce [245]. Recently, a study with WNV-infected blood donors revealed that symptom development was positively correlated with early, potent IFN-α production [246]. However, this robust IFN-I response was associated with an inability to maintain continuing immunity during WNV infection, suggesting that an increased initial inflammation may negatively impact the adaptive-T cell responses.

2.2.4. Yellow Fever Virus (YFV).

YFV, the causative agent of yellow fever, is endemic in the tropical regions of Africa and the Americas and is transmitted to humans and

nonhuman primates by *Aedes* spp. mosquitoes, including *Aedes aegypti* [150]. Despite the presence of an effective vaccine YF17D [247], YFV infection has remained a public health concern in restricted parts of the world, with an incidence of 200,000 cases per year, leading to about 30,000 deaths [150]. In humans, YFV infection produces variable clinical manifestations, ranging from asymptomatic infection to mild febrile illness and to a possibly fatal disease characterized by severe hepatitis, renal failure, hemorrhage, and shock [248].

IFN-I induction has been shown in YF17D-vaccinated human subjects [249, 250] and combined IFN-I and -III are crucial for controlling YFV infection in mice [251]. Recently, it has been reported that cells infected with YFV stimulated pDCs to produce IFN-I in a TLR7- and cell contact-dependent manner. Cells producing immature particles as well as capsid-free viral RNA participated in pDC stimulation [252].

NS4B and NS5 of YFV have been reported as IFN-I antagonists. YFV NS4B interacts with STING, blocking IFN-I stimulation [253]. Moreover, YFV NS5 inhibits IFN-I signaling via binding and inhibition of STAT2 following IFN-I-induced phosphorylation of STAT1 and requires K6 ubiquitination [254]. This IFN-induced ubiquitination of YFV NS5 is absent in murine cells resulting in a lack of binding of YFV NS5 and human STAT2 in murine cells, highlighting the importance of YFV ubiquitination in determining the host cell range for YFV [255, 256].

Gene expression microarray analysis in PBMCs from YF17D-vaccinated humans revealed an immune profile related to antiviral IFN-I responses [249], suggesting that IFN-I may play a role in effective protection in vaccinated subjects. Moreover, human DCs infected with vaccine virus YF17D and a chimeric YF17D/DENV2 produced higher levels of IFN-α than those infected with DENV-2 [193], also indicating an induction of IFN-I production by YF17D vaccine virus. Recently, it has been reported that an inherited IFNAR1 deficiency resulted in life-threatening complications of vaccination with the YF vaccine in a previously healthy individual [257].

The dual role of IFNs in protection against and pathogenesis of viral infections was suggested using a gene overexpression screening approach in human cells [258]. In this study, several ISGs (ADAR, FAM46C, LY6E, and MCOLN2) were identified as inhibitors of YFV, WNV, HCV, HIV, chikungunya virus (CHIKV), and Venezuelan equine encephalitis virus (VEEV) replication. Conversely, several ISGs were found to enhance the replication of YFV, WNV, CHIKV, and VEEV, highlighting the complexity of the IFN-I system.

2.2.5. Japanese Encephalitis Virus (JEV). JEV is a mosquito-borne flavivirus causing severe neurologic disease, characterized by flaccid paralysis, meningitis, and encephalitis [150]. JEV is transmitted to humans by *Culex* spp. mosquitoes and is maintained in a zoonotic cycle involving pigs as the major reservoir and water birds as carriers [259]. JEV infections primarily occur in Asia, where 35,000-50,000 cases and 10,000-15,000 deaths are reported annually [150].

JEV infection of human microglial cells has been shown to induce an innate immune response characterized by the production of IFN-β via IRF-3 activation and phosphorylation. The overexpression of the ubiquitin ligase TRIM21, which interacts with IRF-3 negatively, regulated this innate immune response by targeting IRF-3-mediated IFN-β production [260]. Recently, neuronal transcriptomic responses to JEV infection showed the upregulation of RIG-I and MDA5, suggesting that neuronal cells play a significant role in immunity against JEV [261].

The antagonist activity of JEV NS5 in IFN-I responses has been reported. The JEV NS5 has been shown to block IFN-I signaling by reducing the phosphorylation of TYK2 and STAT1 and subsequently inhibiting STAT1 nuclear localization [262, 263]. Furthermore, JEV NS5 inhibited the nuclear translocation of IRF-3 and NF-κB by binding to nuclear transport proteins KPNA3 and KPNA4, impairing the production of IFN-β [264].

The IFN-I response during JEV infection in humans has not been fully characterized. A study of proinflammatory profile in humans with JE revealed that nonsurviving patients with JE showed higher levels of IFN-α in cerebrospinal fluid than those from survivors during the first days of illness, suggesting that it may be associated with higher viral load [265].

2.2.6. Saint Louis Encephalitis Virus (SLEV). SLEV is a mosquito-borne flavivirus transmitted to humans by *Culex* spp. mosquitoes, first discovered in 1933 when a large epidemic of encephalitis occurred in St. Louis, Missouri [150]. SLEV distribution ranges from Canada to Argentina and across North America [266]. SLEV produces a mild febrile disease in children and young adults and severe neurologic manifestations that are more frequently observed in elderly and immunocompromised patients.

Information about IFN-I induction and signaling as well as IFN-I evasion by viral proteins after SLEV infection in human cells is currently very limited. Former studies have shown a variable effect of human IFN in primary human fetal glial cell cultures [267]. Similar to DENV, ZIKV, and WNV, the protective role of IFN-I against SLEV has been demonstrated in mice since IFN signaling-deficient mice are more susceptible to infection by SLEV than in immunocompetent mice [268]. Specifically, the protective effects of IFN-α have been shown in mice by reducing mortality from SLEV delivered by the aerosol and subcutaneous routes [269].

3. IFN-I Therapy and Pathogenic Effects

IFNs are increasingly recognized as therapeutic agents. Three different types of human IFN (α, β, and γ) are widely used for the treatment of various diseases due to their immunomodulating, antiviral, and antiproliferative properties [270, 271]. However, the optimal dose and duration of IFNs as therapeutic agents have not been established [272]. This is important since IFNs administered in pharmacological doses produce considerable toxicity that is dose-related and that may require cessation of therapy. Common side effects due to IFN-α include flu-like symptoms (fatigue, fever, myalgias, and headaches), pulmonary toxicity, gastrointestinal symptoms,

neurotoxicity, and depression [273–275]. Lethal toxicity associated with IFN-α regimen is rare and severe toxicity due to IFN-α is manageable if recognized expeditiously [276, 277].

The role of endogenous and/or exogenous IFN in the viral infections discussed in this review has been partially explored in human cell lines, animal models, and clinical studies. Depending on the virus, the model used, and the time of infection, IFNs can be either beneficial or deleterious (Table 1).

Regarding influenza disease, a pathogenic role for endogenous IFN-I has been suggested in children with influenza infection, in whom clinical disease severity was associated with an increased level of IFN-α [278]. In agreement with this, another study associated severity with epithelial cell damage mediated by TNF-α related apoptosis-inducing ligand (TRAIL) [279] whose expression can be induced by IFN-α and IFN-β [280]. Moreover, recent evidence showed that the upregulation of TRAIL expression by monocytes and death receptor 5 (DR5) expression by epithelial cells contributed to the pathogenic mechanism induced by IFN-α/β, where enhanced production of this molecule can contribute to immunopathology in severe infections [64].

Concerning RSV infections, intranasal administration of recombinant IFN to infants with RSV infection has shown to be safe and to decrease the duration of symptoms without affecting the time of viral shedding [281–283].

During the 1980s, different studies showed that the use of high doses of intranasal IFN-α were useful in the prevention of HRV infection [284–286]. However, it was also found that the use of this molecule (in high doses) was associated with the occurrence of undesirable effects, as described above. Although low-dose treatment has proven to be better tolerated, it has been ineffective as postexposure prophylaxis [287]. Further investigation regarding the role of IFN-I during HRV infections needs to be completed.

Dual effects of IFN-I have been observed in HCoV infections, as mentioned previously. A series of case studies describing moderate and severe MERS-CoV infections in adults found that moderate cases had no IFN-α response, while severe cases had variable levels of IFN-α [288]. However, other studies have shown that MERS-CoV and SARS-CoV patients improved with IFN-α therapy [289, 290].

Regarding DENV infections, the studies evaluating the association of IFN-I response with disease severity in humans have yielded mixed results [188, 190–192], as described previously. Recent studies in dengue patients in South America have suggested that high levels of IFN-β might accompany a worsened progression of the disease [197, 198]. In addition, monocytes of individuals with past severe dengue (SD) exhibited a significant upregulation of IFNB-1, RIG-I, and NLRP3 genes compared to those with past non-SD that was accompanied with higher viral loads, suggesting that initial innate immune responses may influence disease outcome [291].

With regard to ZIKV, IFN-I treatment has not been described in ZIKV patients. A recent murine model of ZIKV infection using dexamethasone-immunosuppressed mice showed that IFN-I treatment improved clinical outcomes, reducing viral load and inflammation in different visceral organs including testicles, suggesting the consideration for evaluating the effects of recombinant IFN treatment in patients at high risk for ZIKV-associated complications [292].

Concerning WNV-infected patients, the use of IFN-α is limited to case reports of meningoencephalitis, where different outcomes have been described [293–296]. IFN-α treatment was well tolerated and might have potential beneficial effects. However, the dynamic course of WNV neuroinvasive disease prevented the determination of whether the beneficial effects were due to the experimental therapy or to chance. Further randomized, double-blinded, placebo-controlled clinical trials are needed to define the role of IFN-α treatment in WNV-infected patients.

The antiviral effects of IFN-α against JEV have been originally described in cell cultures [297]. In humans, IFN-α2a treatment against JE was also evaluated in a randomized double-blind placebo-controlled trial in Vietnamese children with JEV infection [298]. Intramuscular IFN-α administration did not show beneficial effects on hospital death or severe sequelae at discharge, suggesting that higher doses, alternative administration routes, or combination with other antiviral drugs might be needed.

Similar to WNV and JEV, the use of IFN-α was evaluated in human subjects with SLEV severe neurologic disease. A nonrandomized, unblinded, interventional pilot study evaluated IFN-α2b therapy for meningoencephalitis produced by SLEV and suggested a beneficial effect on the early neurologic course of the disease, indicating that two weeks of treatment were well tolerated [299]. A subsequent study in solid organ transplant recipients with SLEV meningoencephalitis showed that even with delayed administration, the combination treatment with IFN-α2b and intravenous immunoglobulin G was associated with a potential clinical improvement [300]. These studies support the conduction of a subsequent randomized, double-blinded, placebo-controlled trial of IFN-α2a therapy for SLEV meningoencephalitis.

4. Therapeutic Potential of ISG Effector Mechanisms in Human Viral Infectious Diseases

Upon recognition of viral infection, the cell, through PRRs, mediates the production of IFN-I leading to the transcription of hundreds of ISGs by the JAK/STAT pathway [301]. ISGs are the effectors of cell-autonomous antiviral defense and have been shown to be very effective at resisting and controlling pathogens. ISGs are induced to vastly different levels during viral infection or IFN treatment, and expression levels are often dependent on time, dose, and cell type. They act at different stages of the viral life cycle, from entry, replication, assembly, and release, providing adequate cellular immunity against both RNA and DNA viruses [302]. Microarray studies have identified between 50–1000 ISGs, with 200–500 genes typical of many cell types. Representative and well-studied ISG members with specific or broad antiviral activities include IRF1, IRF3, IRF7, IRF9, IFITM3, ISG15, and OASL. The products of these ISGs exert numerous antiviral

TABLE 2: Some interferon-stimulated genes that inhibit respiratory virus and flavivirus infections.

ISG	Viruses shown to be susceptible	Mechanism/component of innate immune system augmented by ISG	References
	Respiratory viruses		
Viperin	Influenza A virus HRV RSV	TLR-7/9 (IRAK1/TRAF6) NF-κB1/p50, AP-1	[305, 306]
ZAP	Influenza A virus	RIG-I	[307]
TRIM56	Influenza A and B viruses HCoV OC43	STING, TLR-3/TRIF	[308, 309]
TRIM21	HRV	cGAS, RIG-I,	[310]
ISG20	Influenza A and B viruses	Nucleoprotein blockade	[311, 312]
TDRD7	RSV PIV	Autophagy inhibition	[313]
	Flaviviruses		
IFITM2/3	DENV WNV	Entry and/or uncoating disruption	[314, 316]
Viperin	DENV	Restriction of early DENV RNA production/accumulation, via interaction with DENV NS3 and replication complexes	[315]
RyDEN	DENV	Interference with DENV translation via interaction with viral RNA and cellular mRNA-binding proteins	[317]
OAS	DENV	Blockade in DENV replication	[318]
ISG15	DENV ZIKV WNV JEV	Inhibition of viral replication	[321–324]
IFI6	YFV WNV DENV	Prevention of virus-induced ER membrane invaginations formation, that house replication machinery	[320]
TRIM69	DENV	DENV NS3 ubiquitination and degradation, thus interrupting DENV replication	[319]
Schlafen 11	WNV DENV ZIKV	Viral replication restriction by regulating tRNA abundance	[325]

effector functions, many of which are still not fully described. However, as the mechanisms of more IFN effectors are uncovered, it is likely that their modes of action will collectively span the majority of virus life cycle stages [303]. In addition to having potent antiviral activity, ISGs also augment the innate immune response to viral infection, thereby strengthening this response [304]. This has driven special attention in the attempt to advance novel therapeutics to control viral infection and their pathogenesis.

Currently, there are vaccines for influenza virus, YFV and JEV; however, neither effective vaccines nor specific therapies are available for RSV, hMPV, PIV, HRV, HCoV, DENV, ZIKV, WNV, or SLEV. Although several vaccines are under diverse phases of development for these viruses, there is a need for alternative antiviral therapeutic approaches.

Several ISGs have been described in the setting of respiratory viruses (Table 2). Recent work has shown that the egress of the influenza virus is also targeted by an ISG. Viperin interacts with the cellular enzyme farnesyl diphosphate-synthase to perturb lipid rafts, resulting in inhibition of influenza and rhinovirus release [305, 306]. In addition, ZAP, which can be upregulated independently from IFN production, limits influenza virus replication via the enhancement of RIG-I [307]. TRIM56 is another ISG with broad antiviral activity. Although its upregulation is dependent on IFN-I, it abrogates influenza virus A and B and HCoV infection through STING and TLR-3/TRIF [308, 309]. TRIM21 has shown activity against rhinovirus by intercepting incoming antibody-opsonized virions during cellular infection, mediating efficient postentry neutralization [310]. A new human IFN-induced gene that we have termed ISG20 codes for a $3'$ to $5'$ exonuclease with specificity for single-stranded RNA and, to a lesser extent, for DNA. ISG20-overexpressing HeLa cells showed resistance to infections by the influenza virus. The mechanism has been shown to be related to the impairment of the polymerase activity, inhibiting both the replication and transcription [311, 312]. Finally, TDRD7 (tudor domain containing 7) has shown activity against several

paramyxoviruses, such as HPIV3 and RSV by interfering with the activation of AMP-dependent kinase (AMPK). The activation of AMPK is required for efficient replication. TDRD7 interferes with the activation of AMPK, limiting viral replication [313].

Several ISGs have been reported to inhibit flavivirus infection through different mechanisms (Table 2). The expression of IFN-I inducible ISGs such as IFITM2/3, viperin, ISG15, ISG20, OAS, BST2, RyDEN, TRIM69, and IFI6 has been shown to block DENV infection [314–320] at multiple steps of the viral cycle. IFITM2 and IFITM3 disrupted the early steps (entry and/or uncoating) of DENV and WNV infection [314, 316]. In contrast, three IFN-induced cellular enzymes, viperin, ISG20, and dsRNA-activated protein kinase, inhibited the steps of the DENV and WNV cycle in viral proteins and/or RNA biosynthesis [314]. DENV infection-induced viperin which has antiviral properties residing in the C-terminal region of the protein that acts to restrict early DENV RNA production/accumulation, potentially via the interaction of viperin with DENV NS3 and replication complexes [315]. The expression of the Repressor of yield of DENV (RyDEN) conferred resistance to all serotypes of DENV in human cells. RyDEN is likely to interfere with the translation of DENV via interaction with viral RNA and cellular mRNA-binding proteins, resulting in the inhibition of virus replication in infected cells [317]. OAS and its downstream effector RNase L have been reported to block DENV replication and likely contributed to host defense against DENV infection playing a role in determining the outcomes of DENV disease severity [318]. ZIKV has been reported to induce the expression of ISG15 in primary human corneal epithelial cells, and the silencing of ISG15 increased ZIKV infectivity [321]. ISG15 has also inhibited the replication of several flaviviruses, including DENV, WNV, and JEV [322–324]. IFN-α-inducible protein 6 (IFI6), an endoplasmic reticulum- (ER-) localized integral membrane effector, prophylactically protected uninfected cells by preventing the formation of virus-induced ER membrane invaginations that house flavivirus (YFV, WNV, and DENV) replication machinery [320]. TRIM69 interacts with DENV NS3 directly and mediates its ubiquitination and degradation, thus, interrupting DENV replication [319]. Recently, Schlafen 11, an ISG that controls the synthesis of proteins by regulating tRNA abundance, has been reported to restrict WNV, DENV, and ZIKV replication by impairing viral infectivity [325].

5. Conclusions

After 60 years of research, the protective role of IFN-I has been demonstrated from cell culture to animal models and in human subjects. More recently, pathogenic effects of IFN-I have been described during viral infections highlighting the vast and intricate interactions of IFN-I in the immune response. Further insights into the effector mechanisms of individual ISGs in the complex signaling networks in human viral diseases are needed in order to design more specific and effective therapeutic strategies.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

PLA, ABB, DRH, and LBT contributed to the literature search and paper preparation.

Acknowledgments

References

[1] A. Isaacs and J. Lindenmann, "Virus interference. I. The interferon," *Journal of Interferon Research*, vol. 7, no. 5, pp. 429–438, 1957.

[2] J. Vilcek, "Fifty years of interferon research: aiming at a moving target," *Immunity*, vol. 25, no. 3, pp. 343–348, 2006.

[3] A. Isaacs, J. Lindenmann, and R. C. Valentine, "Virus interference. II. Some properties of interferon," *Proceedings of the Royal Society of London-Series B: Biological Sciences*, vol. 147, no. 927, pp. 268–273, 1957.

[4] S. Pestka, C. D. Krause, and M. R. Walter, "Interferons, interferon-like cytokines, and their receptors," *Immunological Reviews*, vol. 202, pp. 8–32, 2004.

[5] A. N. Theofilopoulos, R. Baccala, B. Beutler, and D. H. Kono, "Type I interferons (alpha/beta) in immunity and autoimmunity," *Annual Review of Immunology*, vol. 23, pp. 307–336, 2005.

[6] T. Kawai and S. Akira, "Toll-like receptors and their crosstalk with other innate receptors in infection and immunity," *Immunity*, vol. 34, no. 5, pp. 637–650, 2011.

[7] R. K. Durbin, S. V. Kotenko, and J. E. Durbin, "Interferon induction and function at the mucosal surface," *Immunological Reviews*, vol. 255, no. 1, pp. 25–39, 2013.

[8] I. Ioannidis, B. McNally, M. Willette et al., "Plasticity and virus specificity of the airway epithelial cell immune response during respiratory virus infection," *Journal of Virology*, vol. 86, no. 10, pp. 5422–5436, 2012.

[9] A. Iwasaki and R. Medzhitov, "Toll-like receptor control of the adaptive immune responses," *Nature Immunology*, vol. 5, no. 10, pp. 987–995, 2004.

[10] B. Beutler, "Inferences, questions and possibilities in toll-like receptor signalling," *Nature*, vol. 430, no. 6996, pp. 257–263, 2004.

[11] P. N. Moynagh, "The NF-B pathway," *Journal of Cell Science*, vol. 118, no. 20, pp. 4589–4592, 2005.

[12] K. Honda, A. Takaoka, and T. Taniguchi, "Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors," *Immunity*, vol. 25, no. 3, pp. 349–360, 2006.

[13] L. Malmgaard, "Induction and regulation of IFNs during viral infections," *Journal of Interferon & Cytokine Research*, vol. 24, no. 8, pp. 439–454, 2004.

[14] T. Taniguchi and A. Takaoka, "The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors," *Current Opinion in Immunology*, vol. 14, no. 1, pp. 111–116, 2002.

[15] L. B. Ivashkiv and L. T. Donlin, "Regulation of type I interferon responses," *Nature Reviews Immunology*, vol. 14, no. 1, pp. 36–49, 2014.

[16] C. E. Samuel, "Antiviral actions of interferons," *Clinical Microbiology Reviews*, vol. 14, no. 4, pp. 778–809, 2001.

[17] N. Yan and Z. J. Chen, "Intrinsic antiviral immunity," *Nature Immunology*, vol. 13, no. 3, pp. 214–222, 2012.

[18] A. Pindel and A. Sadler, "The role of protein kinase R in the interferon response," *Journal of Interferon & Cytokine Research*, vol. 31, no. 1, pp. 59–70, 2011.

[19] F. Gebauer and M. W. Hentze, "Molecular mechanisms of translational control," *Nature Reviews Molecular Cell Biology*, vol. 5, no. 10, pp. 827–835, 2004.

[20] R. H. Silverman, "Viral encounters with 2',5'-oligoadenylate synthetase and RNase L during the interferon antiviral response," *Journal of Virology*, vol. 81, no. 23, pp. 12720–12729, 2007.

[21] I. C. Huang, C. C. Bailey, J. L. Weyer et al., "Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus," *PLoS Pathogens*, vol. 7, no. 1, article e1001258, 2011.

[22] D. G. Murphy, K. Dimock, and C. Y. Kang, "Numerous transitions in human parainfluenza virus 3 RNA recovered from persistently infected cells," *Virology*, vol. 181, no. 2, pp. 760–763, 1991.

[23] O. Haller and G. Kochs, "Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity," *Journal of Interferon & Cytokine Research*, vol. 31, no. 1, pp. 79–87, 2011.

[24] R. Y. Chang, T. W. Hsu, Y. L. Chen et al., "Japanese encephalitis virus non-coding RNA inhibits activation of interferon by blocking nuclear translocation of interferon regulatory factor 3," *Veterinary Microbiology*, vol. 166, no. 1-2, pp. 11–21, 2013.

[25] P. Tailor, T. Tamura, and K. Ozato, "IRF family proteins and type I interferon induction in dendritic cells," *Cell Research*, vol. 16, no. 2, pp. 134–140, 2006.

[26] A. P. Liao, M. Salajegheh, C. Morehouse et al., "Human plasmacytoid dendritic cell accumulation amplifies their type 1 interferon production," *Clinical Immunology*, vol. 136, no. 1, pp. 130–138, 2010.

[27] F. P. Siegal, N. Kadowaki, M. Shodell et al., "The nature of the principal type 1 interferon-producing cells in human blood," *Science*, vol. 284, no. 5421, pp. 1835–1837, 1999.

[28] P. Fitzgerald-Bocarsly and D. Feng, "The role of type I interferon production by dendritic cells in host defense," *Biochimie*, vol. 89, no. 6-7, pp. 843–855, 2007.

[29] V. Brinkmann, T. Geiger, S. Alkan, and C. H. Heusser, "Interferon alpha increases the frequency of interferon gamma-producing human CD4+ T cells," *The Journal of Experimental Medicine*, vol. 178, no. 5, pp. 1655–1663, 1993.

[30] P. Marrack, J. Kappler, and T. Mitchell, "Type I interferons keep activated T cells alive," *The Journal of Experimental Medicine*, vol. 189, no. 3, pp. 521–530, 1999.

[31] J. M. Curtsinger, J. O. Valenzuela, P. Agarwal, D. Lins, and M. F. Mescher, "Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation," *Journal of Immunology*, vol. 174, no. 8, pp. 4465–4469, 2005.

[32] J. Martinez, X. Huang, and Y. Yang, "Direct action of type I IFN on NK cells is required for their activation in response to vaccinia viral infection in vivo," *Journal of Immunology*, vol. 180, no. 3, pp. 1592–1597, 2008.

[33] A. Le Bon and D. F. Tough, "Links between innate and adaptive immunity via type I interferon," *Current Opinion in Immunology*, vol. 14, no. 4, pp. 432–436, 2002.

[34] E. R. Gaunt, H. Harvala, C. McIntyre, K. E. Templeton, and P. Simmonds, "Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model," *Journal of Clinical Virology*, vol. 52, no. 3, pp. 215–221, 2011.

[35] J. S. Tregoning and J. Schwarze, "Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology," *Clinical Microbiology Reviews*, vol. 23, no. 1, pp. 74–98, 2010.

[36] K. T. Yeo, R. de la Puerta, N. W. S. Tee, K. C. Thoon, V. S. Rajadurai, and C. F. Yung, "Burden, etiology, and risk factors of respiratory virus infections among symptomatic preterm infants in the tropics: a retrospective single-center cohort study," *Clinical Infectious Diseases*, vol. 67, no. 10, pp. 1603–1609, 2018.

[37] Y. B. Seo, J. Y. Song, M. J. Choi et al., "Etiology and clinical outcomes of acute respiratory virus infection in hospitalized adults," *Infection & Chemotherapy*, vol. 46, no. 2, pp. 67–76, 2014.

[38] M. T. Caballero, M. E. Serra, P. L. Acosta et al., "TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization," *The Journal of Clinical Investigation*, vol. 125, no. 2, pp. 571–582, 2015.

[39] Y. M. Loo, J. Fornek, N. Crochet et al., "Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity," *Journal of Virology*, vol. 82, no. 1, pp. 335–345, 2008.

[40] A. L. Blasius and B. Beutler, "Intracellular toll-like receptors," *Immunity*, vol. 32, no. 3, pp. 305–315, 2010.

[41] N. A. Jewell, N. Vaghefi, S. E. Mertz et al., "Differential type I interferon induction by respiratory syncytial virus and influenza a virus in vivo," *Journal of Virology*, vol. 81, no. 18, pp. 9790–9800, 2007.

[42] J. A. Soto, N. M. S. Gálvez, F. M. Benavente et al., "Human metapneumovirus: mechanisms and molecular targets used by the virus to avoid the immune system," *Frontiers in Immunology*, vol. 9, p. 2466, 2018.

[43] A. Garcia-Sastre, "Induction and evasion of type I interferon responses by influenza viruses," *Virus Research*, vol. 162, no. 1-2, pp. 12–18, 2011.

[44] A. J. W. Te Velthuis and E. Fodor, "Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis," *Nature Reviews Microbiology*, vol. 14, no. 8, pp. 479–493, 2016.

[45] M. Yoneyama, M. Kikuchi, T. Natsukawa et al., "The RNA helicase RIG-I has an essential function in double-stranded RNA- induced innate antiviral responses," *Nature Immunology*, vol. 5, no. 7, pp. 730–737, 2004.

[46] H. Kato, O. Takeuchi, S. Sato et al., "Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses," *Nature*, vol. 441, no. 7089, pp. 101–105, 2006.

[47] H. Kato, O. Takeuchi, E. Mikamo-Satoh et al., "Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5," *The Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1601–1610, 2008.

[48] K. S. Childs, R. E. Randall, and S. Goodbourn, "LGP2 plays a critical role in sensitizing mda-5 to activation by double-stranded RNA," *PLoS One*, vol. 8, no. 5, article e64202, 2013.

[49] R. Le Goffic, J. Pothlichet, D. Vitour et al., "Cutting edge: influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells," *Journal of Immunology*, vol. 178, no. 6, pp. 3368–3372, 2007.

[50] J. M. Lund, L. Alexopoulou, A. Sato et al., "Recognition of single-stranded RNA viruses by toll-like receptor 7," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 15, pp. 5598–5603, 2004.

[51] B. G. Hale, R. E. Randall, J. Ortín, and D. Jackson, "The multifunctional NS1 protein of influenza A viruses," *The Journal of General Virology*, vol. 89, Part 10, pp. 2359–2376, 2008.

[52] B. G. Hale, J. Steel, R. A. Medina et al., "Inefficient control of host gene expression by the 2009 pandemic H1N1 influenza A virus NS1 protein," *Journal of Virology*, vol. 84, no. 14, pp. 6909–6922, 2010.

[53] A. Nogales, L. Martinez-Sobrido, D. Topham, and M. DeDiego, "Modulation of innate immune responses by the influenza A NS1 and PA-X proteins," *Viruses*, vol. 10, no. 12, p. 708, 2018.

[54] R. M. Krug, "Functions of the influenza A virus NS1 protein in antiviral defense," *Current Opinion in Virology*, vol. 12, pp. 1–6, 2015.

[55] J. D. Bloom, L. I. Gong, and D. Baltimore, "Permissive secondary mutations enable the evolution of influenza oseltamivir resistance," *Science*, vol. 328, no. 5983, pp. 1272–1275, 2010.

[56] A. Pichlmair, O. Schulz, C. P. Tan et al., "RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates," *Science*, vol. 314, no. 5801, pp. 997–1001, 2006.

[57] M. Bergmann, A. Garcia-Sastre, E. Carnero et al., "Influenza virus NS1 protein counteracts PKR-mediated inhibition of replication," *Journal of Virology*, vol. 74, no. 13, pp. 6203–6206, 2000.

[58] K. L. Schierhorn, F. Jolmes, J. Bespalowa et al., "Influenza A virus virulence depends on two amino acids in the N-terminal domain of its NS1 protein to facilitate inhibition of the RNA-dependent protein kinase PKR," *Journal of Virology*, vol. 91, no. 10, 2017.

[59] D. M. Knipe and P. M. Howley, *Fields Virology*, Lippincott Williams & Wilkins, Philadelphia, PA, 6th edition, 2013.

[60] C. Ehrhardt and S. Ludwig, "A new player in a deadly game: influenza viruses and the PI3K/Akt signalling pathway," *Cellular Microbiology*, vol. 11, no. 6, pp. 863–871, 2009.

[61] I. Koerner, G. Kochs, U. Kalinke, S. Weiss, and P. Staeheli, "Protective role of beta interferon in host defense against influenza A virus," *Journal of Virology*, vol. 81, no. 4, pp. 2025–2030, 2007.

[62] K. J. Szretter, S. Gangappa, J. A. Belser et al., "Early control of H5N1 influenza virus replication by the type I interferon response in mice," *Journal of Virology*, vol. 83, no. 11, pp. 5825–5834, 2009.

[63] G. E. Price, A. Gaszewska-Mastarlarz, and D. Moskophidis, "The role of alpha/beta and gamma interferons in development of immunity to influenza A virus in mice," *Journal of Virology*, vol. 74, no. 9, pp. 3996–4003, 2000.

[64] S. Davidson, S. Crotta, T. M. McCabe, and A. Wack, "Pathogenic potential of interferon $\alpha\beta$ in acute influenza infection," *Nature Communications*, vol. 5, no. 1, p. 3864, 2014.

[65] C. B. Hall, R. G. Douglas Jr., R. L. Simons, and J. M. Geiman, "Interferon production in children with respiratory syncytial, influenza, and parainfluenza virus infections," *The Journal of Pediatrics*, vol. 93, no. 1, pp. 28–32, 1978.

[66] G. A. Melendi, S. Coviello, N. Bhat, J. Zea-Hernandez, F. M. Ferolla, and F. P. Polack, "Breastfeeding is associated with the production of type I interferon in infants infected with influenza virus," *Acta Paediatrica*, vol. 99, no. 10, pp. 1517–1521, 2010.

[67] M. I. Klein, E. Bergel, L. Gibbons et al., "Differential gender response to respiratory infections and to the protective effect of breast milk in preterm infants," *Pediatrics*, vol. 121, no. 6, pp. e1510–e1516, 2008.

[68] D. H. Canaday, N. A. Amponsah, L. Jones, D. J. Tisch, T. R. Hornick, and L. Ramachandra, "Influenza-induced production of interferon-alpha is defective in geriatric individuals," *Journal of Clinical Immunology*, vol. 30, no. 3, pp. 373–383, 2010.

[69] R. L. Forbes, P. A. Wark, V. E. Murphy, and P. G. Gibson, "Pregnant women have attenuated innate interferon responses to 2009 pandemic influenza A virus subtype H1N1," *The Journal of Infectious Diseases*, vol. 206, no. 5, pp. 646–653, 2012.

[70] A. R. Everitt, S. Clare, T. Pertel et al., "IFITM3 restricts the morbidity and mortality associated with influenza," *Nature*, vol. 484, no. 7395, pp. 519–523, 2012.

[71] J. Chen, M. Wu, F. Wang et al., "Hepatitis B virus spliced variants are associated with an impaired response to interferon therapy," *Scientific Reports*, vol. 5, no. 1, p. 16459, 2015.

[72] C. B. Hall, G. A. Weinberg, M. K. Iwane et al., "The burden of respiratory syncytial virus infection in young children," *The New England Journal of Medicine*, vol. 360, no. 6, pp. 588–598, 2009.

[73] A. R. Falsey, P. A. Hennessey, M. A. Formica, C. Cox, and E. E. Walsh, "Respiratory syncytial virus infection in elderly and high-risk adults," *The New England Journal of Medicine*, vol. 352, no. 17, pp. 1749–1759, 2005.

[74] J. F. Bermejo-Martin, M. C. Garcia-Arevalo, R. O. de Lejarazu et al., "Predominance of Th2 cytokines, CXC chemokines and innate immunity mediators at the mucosal level during severe respiratory syncytial virus infection in children," *European Cytokine Network*, vol. 18, no. 3, pp. 162–167, 2007.

[75] N. Goutagny, Z. Jiang, J. Tian et al., "Cell type-specific recognition of human metapneumoviruses (HMPVs) by retinoic acid-inducible gene I (RIG-I) and TLR7 and viral interference of RIG-I ligand recognition by HMPV-B1 phosphoprotein," *Journal of Immunology*, vol. 184, no. 3, pp. 1168–1179, 2010.

[76] S. M. Belgnaoui, S. Paz, and J. Hiscott, "Orchestrating the interferon antiviral response through the mitochondrial antiviral signaling (MAVS) adapter," *Current Opinion in Immunology*, vol. 23, no. 5, pp. 564–572, 2011.

[77] R. F. Chen, K. D. Yang, L. Wang, J. W. Liu, C. C. Chiu, and J. T. Cheng, "Different clinical and laboratory manifestations

between dengue haemorrhagic fever and dengue fever with bleeding tendency," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 101, no. 11, pp. 1106–1113, 2007.

[78] A. Sabbah, T. H. Chang, R. Harnack et al., "Activation of innate immune antiviral responses by Nod2," *Nature Immunology*, vol. 10, no. 10, pp. 1073–1080, 2009.

[79] N. J. Roberts Jr., J. Hiscott, and D. J. Signs, "The limited role of the human interferon system response to respiratory syncytial virus challenge: analysis and comparison to influenza virus challenge," *Microbial Pathogenesis*, vol. 12, no. 6, pp. 409–414, 1992.

[80] K. McIntosh, "Interferon in nasal secretions from infants with viral respiratory tract infections," *The Journal of Pediatrics*, vol. 93, no. 1, pp. 33–36, 1978.

[81] J. Elliott, O. T. Lynch, Y. Suessmuth et al., "Respiratory syncytial virus NS1 protein degrades STAT2 by using the Elongin-Cullin E3 ligase," *Journal of Virology*, vol. 81, no. 7, pp. 3428–3436, 2007.

[82] M. Ramaswamy, L. Shi, S. M. Varga, S. Barik, M. A. Behlke, and D. C. Look, "Respiratory syncytial virus nonstructural protein 2 specifically inhibits type I interferon signal transduction," *Virology*, vol. 344, no. 2, pp. 328–339, 2006.

[83] J. Ren, T. Liu, L. Pang et al., "A novel mechanism for the inhibition of interferon regulatory factor-3-dependent gene expression by human respiratory syncytial virus NS1 protein," *The Journal of General Virology*, vol. 92, Part 9, pp. 2153–2159, 2011.

[84] Z. Ling, K. C. Tran, and M. N. Teng, "Human respiratory syncytial virus nonstructural protein NS2 antagonizes the activation of beta interferon transcription by interacting with RIG-I," *Journal of Virology*, vol. 83, no. 8, pp. 3734–3742, 2009.

[85] H. Jin, H. Zhou, X. Cheng, R. Tang, M. Munoz, and N. Nguyen, "Recombinant respiratory syncytial viruses with deletions in the NS1, NS2, SH, and M2-2 genes are attenuated in vitro and in vivo," *Virology*, vol. 273, no. 1, pp. 210–218, 2000.

[86] W. Zhang, H. Yang, X. Kong et al., "Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene," *Nature Medicine*, vol. 11, no. 1, pp. 56–62, 2005.

[87] M. Ramaswamy, L. Shi, M. M. Monick, G. W. Hunninghake, and D. C. Look, "Specific inhibition of type I interferon signal transduction by respiratory syncytial virus," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 6, pp. 893–900, 2004.

[88] M. S. Lo, R. M. Brazas, and M. J. Holtzman, "Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness," *Journal of Virology*, vol. 79, no. 14, pp. 9315–9319, 2005.

[89] N. Marr, T. I. Wang, S. H. Y. Kam et al., "Attenuation of respiratory syncytial virus-induced and RIG-I-dependent type I IFN responses in human neonates and very young children," *Journal of Immunology*, vol. 192, no. 3, pp. 948–957, 2014.

[90] R. S. Thwaites, M. Coates, K. Ito et al., "Reduced nasal viral load and IFN responses in infants with respiratory syncytial virus bronchiolitis and respiratory failure," *American Journal of Respiratory and Critical Care Medicine*, vol. 198, no. 8, pp. 1074–1084, 2018.

[91] B. G. van den Hoogen, J. C. de Jong, J. Groen et al., "A newly discovered human pneumovirus isolated from young children with respiratory tract disease," *Nature Medicine*, vol. 7, no. 6, pp. 719–724, 2001.

[92] N. Shafagati and J. Williams, "Human metapneumovirus - what we know now," *F1000Research*, vol. 7, p. 135, 2018.

[93] F. Feuillet, B. Lina, M. Rosa-Calatrava, and G. Boivin, "Ten years of human metapneumovirus research," *Journal of Clinical Virology*, vol. 53, no. 2, pp. 97–105, 2012.

[94] J. S. Kahn, "Epidemiology of human metapneumovirus," *Clinical Microbiology Reviews*, vol. 19, no. 3, pp. 546–557, 2006.

[95] S. Liao, X. Bao, T. Liu et al., "Role of retinoic acid inducible gene-I in human metapneumovirus-induced cellular signalling," *The Journal of General Virology*, vol. 89, Part 8, pp. 1978–1986, 2008.

[96] J. Ren, D. Kolli, T. Liu et al., "Human metapneumovirus inhibits IFN-β signaling by downregulating Jak1 and Tyk2 cellular levels," *PLoS One*, vol. 6, no. 9, article e24496, 2011.

[97] D. L. Dinwiddie and K. S. Harrod, "Human metapneumovirus inhibits IFN-α signaling through inhibition of STAT1 phosphorylation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 38, no. 6, pp. 661–670, 2008.

[98] J. Ren, Q. Wang, D. Kolli et al., "Human metapneumovirus M2-2 protein inhibits innate cellular signaling by targeting MAVS," *Journal of Virology*, vol. 86, no. 23, pp. 13049–13061, 2012.

[99] Y. Chen, X. Deng, J. Deng et al., "Functional motifs responsible for human metapneumovirus M2-2-mediated innate immune evasion," *Virology*, vol. 499, pp. 361–368, 2016.

[100] X. Bao, D. Kolli, T. Liu, Y. Shan, R. P. Garofalo, and A. Casola, "Human metapneumovirus small hydrophobic protein inhibits NF-kappaB transcriptional activity," *Journal of Virology*, vol. 82, no. 16, pp. 8224–8229, 2008.

[101] J. Malmo, N. Moe, S. Krokstad et al., "Cytokine profiles in human metapneumovirus infected children: identification of genes involved in the antiviral response and pathogenesis," *PLoS One*, vol. 11, no. 5, article e0155484, 2016.

[102] A. K. Hastings, J. J. Erickson, J. E. Schuster et al., "Role of type I interferon signaling in human metapneumovirus pathogenesis and control of viral replication," *Journal of Virology*, vol. 89, no. 8, pp. 4405–4420, 2015.

[103] H. Komada, M. Tsurudome, M. Ueda, M. Nishio, H. Bando, and Y. Ito, "Isolation and characterization of monoclonal antibodies to human parainfluenza virus type 4 and their use in revealing antigenic relation between subtypes 4A and 413," *Virology*, vol. 171, no. 1, pp. 28–37, 1989.

[104] G. A. Weinberg, C. B. Hall, M. K. Iwane et al., "Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization," *The Journal of Pediatrics*, vol. 154, no. 5, pp. 694–699, 2009.

[105] A. Sabbah and S. Bose, "Retinoic acid inducible gene I activates innate antiviral response against human parainfluenza virus type 3," *Virology Journal*, vol. 6, no. 1, p. 200, 2009.

[106] P. Luthra, D. Sun, R. H. Silverman, and B. He, "Activation of IFN-β expression by a viral mRNA through RNase L and MDA5," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 5, pp. 2118–2123, 2011.

[107] A. Schaap-Nutt, C. D'Angelo, M. A. Scull et al., "Human parainfluenza virus type 2 V protein inhibits interferon

production and signaling and is required for replication in non-human primates," *Virology*, vol. 397, no. 2, pp. 285–298, 2010.

[108] J. B. Boonyaratanakornkit, E. J. Bartlett, E. Amaro-Carambot, P. L. Collins, B. R. Murphy, and A. C. Schmidt, "The C proteins of human parainfluenza virus type 1 (HPIV1) control the transcription of a broad array of cellular genes that would otherwise respond to HPIV1 infection," *Journal of Virology*, vol. 83, no. 4, pp. 1892–1910, 2009.

[109] J. Boonyaratanakornkit, E. Bartlett, H. Schomacker et al., "The C proteins of human parainfluenza virus type 1 limit double-stranded RNA accumulation that would otherwise trigger activation of MDA5 and protein kinase R," *Journal of Virology*, vol. 85, no. 4, pp. 1495–1506, 2011.

[110] W. Van Cleve, E. Amaro-Carambot, S. R. Surman et al., "Attenuating mutations in the P/C gene of human parainfluenza virus type 1 (HPIV1) vaccine candidates abrogate the inhibition of both induction and signaling of type I interferon (IFN) by wild-type HPIV1," *Virology*, vol. 352, no. 1, pp. 61–73, 2006.

[111] A. G. Malur, M. A. Hoffman, and A. K. Banerjee, "The human parainfluenza virus type 3 (HPIV 3) C protein inhibits viral transcription," *Virus Research*, vol. 99, no. 2, pp. 199–204, 2004.

[112] H. Schomacker, R. M. Hebner, J. Boonyaratanakornkit et al., "The C proteins of human parainfluenza virus type 1 block IFN signaling by binding and retaining Stat1 in perinuclear aggregates at the late endosome," *PLoS One*, vol. 7, no. 2, article e28382, 2012.

[113] K. Childs, N. Stock, C. Ross et al., "mda-5, but not RIG-I, is a common target for paramyxovirus V proteins," *Virology*, vol. 359, no. 1, pp. 190–200, 2007.

[114] J. Andrejeva, K. S. Childs, D. F. Young et al., "The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter," *Proceedings of the National Academy of Sciences*, vol. 101, no. 49, pp. 17264–17269, 2004.

[115] J. Andrejeva, D. F. Young, S. Goodbourn, and R. E. Randall, "Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons," *Journal of Virology*, vol. 76, no. 5, pp. 2159–2167, 2002.

[116] D. R. Yang and H. Z. Zhu, "Hepatitis C virus and antiviral innate immunity: who wins at tug-of-war?," *World Journal of Gastroenterology*, vol. 21, no. 13, pp. 3786–3800, 2015.

[117] A. Schaap-Nutt, R. Liesman, E. J. Bartlett et al., "Human parainfluenza virus serotypes differ in their kinetics of replication and cytokine secretion in human tracheobronchial airway epithelium," *Virology*, vol. 433, no. 2, pp. 320–328, 2012.

[118] J. E. Gern, "The ABCs of rhinoviruses, wheezing, and asthma," *Journal of Virology*, vol. 84, no. 15, pp. 7418–7426, 2010.

[119] A. M. Fendrick, "Viral respiratory infections due to rhinoviruses: current knowledge, new developments," *American Journal of Therapeutics*, vol. 10, no. 3, pp. 193–202, 2003.

[120] K. Triantafilou, E. Vakakis, E. A. J. Richer, G. L. Evans, J. P. Villiers, and M. Triantafilou, "Human rhinovirus recognition in non-immune cells is mediated by toll-like receptors and MDA-5, which trigger a synergetic pro-inflammatory immune response," *Virulence*, vol. 2, no. 1, pp. 22–29, 2011.

[121] C. A. Hewson, A. Jardine, M. R. Edwards, V. Laza-Stanca, and S. L. Johnston, "Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells," *Journal of Virology*, vol. 79, no. 19, pp. 12273–12279, 2005.

[122] Q. Wang, D. J. Miller, E. R. Bowman et al., "MDA5 and TLR3 initiate pro-inflammatory signaling pathways leading to rhinovirus-induced airways inflammation and hyperresponsiveness," *PLoS Pathogens*, vol. 7, no. 5, article e1002070, 2011.

[123] L. Slater, N. W. Bartlett, J. J. Haas et al., "Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium," *PLoS Pathogens*, vol. 6, no. 11, article e1001178, 2010.

[124] N. W. Bartlett, L. Slater, N. Glanville et al., "Defining critical roles for NF-κB p65 and type I interferon in innate immunity to rhinovirus," *EMBO Molecular Medicine*, vol. 4, no. 12, pp. 1244–1260, 2012.

[125] J. Drahos and V. R. Racaniello, "Cleavage of IPS-1 in cells infected with human rhinovirus," *Journal of Virology*, vol. 83, no. 22, pp. 11581–11587, 2009.

[126] P. M. Barral, D. Sarkar, P. B. Fisher, and V. R. Racaniello, "RIG-I is cleaved during picornavirus infection," *Virology*, vol. 391, no. 2, pp. 171–176, 2009.

[127] M. R. Edwards, N. Regamey, M. Vareille et al., "Impaired innate interferon induction in severe therapy resistant atopic asthmatic children," *Mucosal Immunology*, vol. 6, no. 4, pp. 797–806, 2013.

[128] Y. Xi, A. Finlayson, O. J. White, M. L. Carroll, and J. W. Upham, "Rhinovirus stimulated IFN-α production: how important are plasmacytoid DCs, monocytes and endosomal pH?," *Clin Transl Immunology*, vol. 4, no. 10, article e46, 2015.

[129] A. Sykes, M. R. Edwards, J. Macintyre et al., "Rhinovirus 16-induced IFN-α and IFN-β are deficient in bronchoalveolar lavage cells in asthmatic patients," *The Journal of Allergy and Clinical Immunology*, vol. 129, no. 6, pp. 1506–1514.e6, 2012, e6.

[130] T. M. Becker, S. R. Durrani, Y. A. Bochkov, M. K. Devries, V. Rajamanickam, and D. J. Jackson, "Effect of exogenous interferons on rhinovirus replication and airway inflammatory responses," *Annals of Allergy, Asthma & Immunology*, vol. 111, no. 5, pp. 397–401, 2013.

[131] J. A. Cakebread, Y. Xu, C. Grainge et al., "Exogenous IFN-β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus," *The Journal of Allergy and Clinical Immunology*, vol. 127, no. 5, pp. 1148–1154.e9, 2011.

[132] J. L. Kennedy, M. Shaker, V. McMeen et al., "Comparison of viral load in individuals with and without asthma during infections with rhinovirus," *American Journal of Respiratory and Critical Care Medicine*, vol. 189, no. 5, pp. 532–539, 2014.

[133] L. van der Hoek, "Human coronaviruses: what do they cause?," *Antiviral Therapy*, vol. 12, no. 4 Part B, pp. 651–658, 2007.

[134] P. S. Masters, "The molecular biology of coronaviruses," *Advances in Virus Research*, vol. 66, pp. 193–292, 2006.

[135] A. E. Gorbalenya, E. J. Snijder, and W. J. Spaan, "Severe acute respiratory syndrome coronavirus phylogeny: toward consensus," *Journal of Virology*, vol. 78, no. 15, pp. 7863–7866, 2004.

[136] Y. Chen, Q. Liu, and D. Guo, "Emerging coronaviruses: genome structure, replication, and pathogenesis," *Journal of Medical Virology*, vol. 92, no. 4, pp. 418–423, 2020.

[137] S. Jensen and A. R. Thomsen, "Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion," *Journal of Virology*, vol. 86, no. 6, pp. 2900–2910, 2012.

[138] D. E. Levy, I. J. Marie, and J. E. Durbin, "Induction and function of type I and III interferon in response to viral infection," *Current Opinion in Virology*, vol. 1, no. 6, pp. 476–486, 2011.

[139] F. McNab, K. Mayer-Barber, A. Sher, A. Wack, and A. O'Garra, "Type I interferons in infectious disease," *Nature Reviews Immunology*, vol. 15, no. 2, pp. 87–103, 2015.

[140] V. D. Menachery, A. J. Eisfeld, A. Schäfer et al., "Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses," *MBio*, vol. 5, no. 3, p. e01174, 2014.

[141] R. Züst, L. Cervantes-Barragan, M. Habjan et al., "Ribose 2'- _O_ -methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5," *Nature Immunology*, vol. 12, no. 2, pp. 137–143, 2011.

[142] M. Frieman, K. Ratia, R. E. Johnston, A. D. Mesecar, and R. S. Baric, "Severe acute respiratory syndrome coronavirus papain-like protease ubiquitin-like domain and catalytic domain regulate antagonism of IRF3 and NF-kappaB signaling," *Journal of Virology*, vol. 83, no. 13, pp. 6689–6705, 2009.

[143] S. G. Devaraj, N. Wang, Z. Chen et al., "Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus," *The Journal of Biological Chemistry*, vol. 282, no. 44, pp. 32208–32221, 2007.

[144] A. M. Mielech, A. Kilianski, Y. M. Baez-Santos, A. D. Mesecar, and S. C. Baker, "MERS-CoV papain-like protease has deISGylating and deubiquitinating activities," *Virology*, vol. 450-451, pp. 64–70, 2014.

[145] K. L. Siu, K. H. Kok, M. H. Ng et al., "Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3.TANK.TBK1/IKKepsilon complex," *The Journal of Biological Chemistry*, vol. 284, no. 24, pp. 16202–16209, 2009.

[146] C. S. Shi, H. Y. Qi, C. Boularan et al., "SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome," *Journal of Immunology*, vol. 193, no. 6, pp. 3080–3089, 2014.

[147] V. A. Scheuplein, J. Seifried, A. H. Malczyk et al., "High secretion of interferons by human plasmacytoid dendritic cells upon recognition of Middle East respiratory syndrome coronavirus," *Journal of Virology*, vol. 89, no. 7, pp. 3859–3869, 2015.

[148] M. J. Cameron, L. Ran, L. Xu et al., "Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome," *Journal of Virology*, vol. 81, no. 16, pp. 8692–8706, 2007.

[149] E. Gould, J. Pettersson, S. Higgs, R. Charrel, and X. de Lamballerie, "Emerging arboviruses: why today?," *One Health*, vol. 4, pp. 1–13, 2017.

[150] C. P. Pierson and M. S. Diamond, "Flaviviruses," in *Fields Virology*, D. M. Knipe and P. M. Howley, Eds., pp. 747–794, Lippincott-Raven, Philadelphia, 2013.

[151] M. U. Gack and M. S. Diamond, "Innate immune escape by dengue and West Nile viruses," *Current Opinion in Virology*, vol. 20, pp. 119–128, 2016.

[152] S. L. Cumberworth, J. J. Clark, A. Kohl, and C. L. Donald, "Inhibition of type I interferon induction and signalling by mosquito-borne flaviviruses," *Cellular Microbiology*, vol. 19, no. 5, p. e12737, 2017.

[153] M. S. Diamond, "Mechanisms of evasion of the type I interferon antiviral response by flaviviruses," *Journal of Interferon & Cytokine Research*, vol. 29, no. 9, pp. 521–530, 2009.

[154] J. H. Lai, M. Y. Wang, C. Y. Huang et al., "Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells," *EMBO Reports*, vol. 19, no. 8, 2018.

[155] J. W. Schoggins, D. MacDuff, N. Imanaka et al., "Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity," *Nature*, vol. 505, no. 7485, pp. 691–695, 2014.

[156] S. Assil, S. Coléon, C. Dong et al., "Plasmacytoid dendritic cells and infected cells form an interferogenic synapse required for antiviral responses," *Cell Host & Microbe*, vol. 25, no. 5, pp. 730–745.e6, 2019, e6.

[157] J. A. Castillo Ramirez and S. Urcuqui-Inchima, "Dengue virus control of Type I IFN responses: a history of manipulation and control," *Journal of Interferon & Cytokine Research*, vol. 35, no. 6, pp. 421–430, 2015.

[158] S. M. Best, "The many faces of the flavivirus NS5 protein in antagonism of type I interferon signaling," *Journal of Virology*, vol. 91, no. 3, 2017.

[159] Y. Wu, Q. Liu, J. Zhou et al., "Zika virus evades interferon-mediated antiviral response through the co- operation of multiple nonstructural proteins *in vitro*," *Cell Discovery*, vol. 3, no. 1, p. 17006, 2017.

[160] E. Schnettler, M. G. Sterken, J. Y. Leung et al., "Noncoding flavivirus RNA displays RNA interference suppressor activity in insect and Mammalian cells," *Journal of Virology*, vol. 86, no. 24, pp. 13486–13500, 2012.

[161] C. L. Donald, B. Brennan, S. L. Cumberworth et al., "Full genome sequence and sfRNA interferon antagonist activity of Zika virus from Recife, Brazil," *PLoS Neglected Tropical Diseases*, vol. 10, no. 10, article e0005048, 2016.

[162] G. Manokaran, E. Finol, C. Wang et al., "Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness," *Science*, vol. 350, no. 6257, pp. 217–221, 2015.

[163] S. Orozco, M. A. Schmid, P. Parameswaran et al., "Characterization of a model of lethal dengue virus 2 infection in C57BL/6 mice deficient in the alpha/beta interferon receptor," *The Journal of General Virology*, vol. 93, Part 10, pp. 2152–2157, 2012.

[164] R. M. Zellweger, T. R. Prestwood, and S. Shresta, "Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease," *Cell Host & Microbe*, vol. 7, no. 2, pp. 128–139, 2010.

[165] H. M. Lazear, J. Govero, A. M. Smith et al., "A mouse model of Zika virus pathogenesis," *Cell Host & Microbe*, vol. 19, no. 5, pp. 720–730, 2016.

[166] M. A. Samuel and M. S. Diamond, "Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival," *Journal of Virology*, vol. 79, no. 21, pp. 13350–13361, 2005.

[167] M. G. Guzman and E. Harris, "Dengue," *Lancet*, vol. 385, no. 9966, pp. 453–465, 2015.

[168] S. Bhatt, P. W. Gething, O. J. Brady et al., "The global distribution and burden of dengue," *Nature*, vol. 496, no. 7446, pp. 504–507, 2013.

[169] B. E. Martina, P. Koraka, and A. D. Osterhaus, "Dengue virus pathogenesis: an integrated view," *Clinical Microbiology Reviews*, vol. 22, no. 4, pp. 564–581, 2009.

[170] M. G. Guzman, D. J. Gubler, A. Izquierdo, E. Martinez, and S. B. Halstead, "Dengue infection," *Nature Reviews Disease Primers*, vol. 2, no. 1, p. 16055, 2016.

[171] A. E. Ngono and S. Shresta, "Immune response to dengue and Zika," *Annual Review of Immunology*, vol. 36, pp. 279–308, 2018.

[172] M. S. Diamond and E. Harris, "Interferon inhibits dengue virus infection by preventing translation of viral RNA through a PKR-independent mechanism," *Virology*, vol. 289, no. 2, pp. 297–311, 2001.

[173] M. S. Diamond, T. G. Roberts, D. Edgil, B. Lu, J. Ernst, and E. Harris, "Modulation of dengue virus infection in human cells by alpha, beta, and gamma interferons," *Journal of Virology*, vol. 74, no. 11, pp. 4957–4966, 2000.

[174] A. M. Nasirudeen, H. H. Wong, P. Thien, S. Xu, K. P. Lam, and D. X. Liu, "RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection," *PLoS Neglected Tropical Diseases*, vol. 5, no. 1, article e926, 2011.

[175] J. Chen, M. M. Ng, and J. J. Chu, "Activation of TLR2 and TLR6 by dengue NS1 protein and its implications in the immunopathogenesis of dengue virus infection," *PLoS Pathogens*, vol. 11, no. 7, article e1005053, 2015.

[176] M. S. Suthar, M. S. Diamond, and M. Gale Jr., "West Nile virus infection and immunity," *Nature Reviews Microbiology*, vol. 11, no. 2, pp. 115–128, 2013.

[177] S. Pagni and A. Fernandez-Sesma, "Evasion of the human innate immune system by dengue virus," *Immunologic Research*, vol. 54, no. 1-3, pp. 152–159, 2012.

[178] J. L. Munoz-Jordan, G. G. Sanchez-Burgos, M. Laurent-Rolle, and A. Garcia-Sastre, "Inhibition of interferon signaling by dengue virus," *Proceedings of the National Academy of Sciences*, vol. 100, no. 24, pp. 14333–14338, 2003.

[179] C. Y. Yu, T. H. Chang, J. J. Liang et al., "Dengue virus targets the adaptor protein MITA to subvert host innate immunity," *PLoS Pathogens*, vol. 8, no. 6, article e1002780, 2012.

[180] S. Aguirre, A. M. Maestre, S. Pagni et al., "DENV inhibits type I IFN production in infected cells by cleaving human STING," *PLoS Pathogens*, vol. 8, no. 10, article e1002934, 2012.

[181] A. C. Stabell, N. R. Meyerson, R. C. Gullberg et al., "Dengue viruses cleave STING in humans but not in nonhuman primates, their presumed natural reservoir," *eLife*, vol. 7, 2018.

[182] Y. I. Anglero-Rodriguez, P. Pantoja, and C. A. Sariol, "Dengue virus subverts the interferon induction pathway via NS2B/3 protease-IκB kinase epsilon interaction," *Clinical and Vaccine Immunology*, vol. 21, no. 1, pp. 29–38, 2014.

[183] J. Ashour, M. Laurent-Rolle, P. Y. Shi, and A. García-Sastre, "NS5 of dengue virus mediates STAT2 binding and degrada-

tion," *Journal of Virology*, vol. 83, no. 11, pp. 5408–5418, 2009.

[184] M. Mazzon, M. Jones, A. Davidson, B. Chain, and M. Jacobs, "Dengue virus NS5 inhibits interferon-α signaling by blocking signal transducer and activator of transcription 2 phosphorylation," *The Journal of Infectious Diseases*, vol. 200, no. 8, pp. 1261–1270, 2009.

[185] J. Ashour, J. Morrison, M. Laurent-Rolle et al., "Mouse STAT2 restricts early dengue virus replication," *Cell Host & Microbe*, vol. 8, no. 5, pp. 410–421, 2010.

[186] C. Zou, C. Huang, J. Zhang et al., "Virulence difference of five type I dengue viruses and the intrinsic molecular mechanism," *PLoS Neglected Tropical Diseases*, vol. 13, no. 3, article e0007202, 2019.

[187] J. R. Rodriguez-Madoz, D. Bernal-Rubio, D. Kaminski, K. Boyd, and A. Fernandez-Sesma, "Dengue virus inhibits the production of type I interferon in primary human dendritic cells," *Journal of Virology*, vol. 84, no. 9, pp. 4845–4850, 2010.

[188] I. Kurane, B. L. Innis, S. Nimmannitya, A. Nisalak, F. A. Ennis, and A. Meager, "High levels of interferon alpha in the sera of children with dengue virus infection," *The American Journal of Tropical Medicine and Hygiene*, vol. 48, no. 2, pp. 222–229, 1993.

[189] P. Becquart, N. Wauquier, D. Nkoghe et al., "Acute dengue virus 2 infection in Gabonese patients is associated with an early innate immune response, including strong interferon alpha production," *BMC Infectious Diseases*, vol. 10, no. 1, p. 356, 2010.

[190] S. I. D. L. C. Hernández, H. Puerta-Guardo, H. Flores-Aguilar et al., "A strong interferon response correlates with a milder dengue clinical condition," *Journal of Clinical Virology*, vol. 60, no. 3, pp. 196–199, 2014.

[191] R. E. Pech Torres, R. M. Cedillo Rivera, M. A. Loroño Pino, and G. G. Sánchez Burgos, "Serum levels of IFN-β are associated with days of evolution but not with severity of dengue," *Journal of Medical Virology*, vol. 88, no. 3, pp. 395–399, 2016.

[192] Y. Tang, Z. Kou, F. Zhang et al., "Both viremia and cytokine levels associate with the lack of severe disease in secondary dengue 1 infection among adult Chinese patients," *PLoS One*, vol. 5, no. 12, article e15631, 2010.

[193] M. Gandini, S. R. Reis, A. Torrentes-Carvalho et al., "Dengue-2 and yellow fever 17DD viruses infect human dendritic cells, resulting in an induction of activation markers, cytokines and chemokines and secretion of different TNF-α and IFN-α profiles," *Memórias do Instituto Oswaldo Cruz*, vol. 106, no. 5, pp. 594–605, 2011.

[194] D. H. Libraty, T. P. Endy, H. S. Houng et al., "Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections," *The Journal of Infectious Diseases*, vol. 185, no. 9, pp. 1213–1221, 2002.

[195] M. Singla, M. Kar, T. Sethi et al., "Immune response to dengue virus infection in pediatric patients in New Delhi, India–association of viremia, inflammatory mediators and monocytes with disease severity," *PLoS Neglected Tropical Diseases*, vol. 10, no. 3, article e0004497, 2016.

[196] F. Perdomo-Celis, D. M. Salgado, and C. F. Narvaez, "Selective dysfunction of subsets of peripheral blood mononuclear cells during pediatric dengue and its relationship with clinical outcome," *Virology*, vol. 507, pp. 11–19, 2017.

[197] L. B. Talarico, A. B. Byrne, S. Amarilla et al., "Characterization of type I interferon responses in dengue and severe dengue in children in Paraguay," *Journal of Clinical Virology*, vol. 97, pp. 10–17, 2017.

[198] R. A. Oliveira, M. M. Silva, C. E. Calzavara-Silva et al., "Primary dengue haemorrhagic fever in patients from northeast of Brazil is associated with high levels of interferon-β during acute phase," *Memórias do Instituto Oswaldo Cruz*, vol. 111, no. 6, pp. 378–384, 2016.

[199] T. H. Chang, C. L. Liao, and Y. L. Lin, "Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kappaB activation," *Microbes and Infection*, vol. 8, no. 1, pp. 157–171, 2006.

[200] I. Kurane and F. A. Ennis, "Production of interferon alpha by dengue virus-infected human monocytes," *The Journal of General Virology*, vol. 69, no. 2, pp. 445–449, 1988.

[201] M. I. Li, P. S. Wong, L. C. Ng, and C. H. Tan, "Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (Linnaeus) to Zika virus," *PLoS Neglected Tropical Diseases*, vol. 6, no. 8, article e1792, 2012.

[202] D. I. Simpson, "Zika virus infection in man," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 58, pp. 335–338, 1964.

[203] A. D. Haddow, A. J. Schuh, C. Y. Yasuda et al., "Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage," *PLoS Neglected Tropical Diseases*, vol. 6, no. 2, article e1477, 2012.

[204] E. B. Hayes, "Zika virus outside Africa," *Emerging Infectious Diseases*, vol. 15, no. 9, pp. 1347–1350, 2009.

[205] M. R. Duffy, T. H. Chen, W. T. Hancock et al., "Zika virus outbreak on Yap Island, Federated States of Micronesia," *The New England Journal of Medicine*, vol. 360, no. 24, pp. 2536–2543, 2009.

[206] W. T. Hancock, M. Marfel, and M. Bel, "Zika virus, French Polynesia, South Pacific, 2013," *Emerging Infectious Diseases*, vol. 20, no. 11, p. 1960, 2014.

[207] C. Zanluca, V. C. A. Melo, A. L. P. Mosimann, G. I. V. Santos, C. N. D. Santos, and K. Luz, "First report of autochthonous transmission of Zika virus in Brazil," *Memórias do Instituto Oswaldo Cruz*, vol. 110, no. 4, pp. 569–572, 2015.

[208] A. Enfissi, J. Codrington, J. Roosblad, M. Kazanji, and D. Rousset, "Zika virus genome from the Americas," *Lancet*, vol. 387, no. 10015, pp. 227-228, 2016.

[209] G. S. Campos, A. C. Bandeira, and S. I. Sardi, "Zika virus outbreak, Bahia, Brazil," *Emerging Infectious Diseases*, vol. 21, no. 10, pp. 1885-1886, 2015.

[210] O. Dyer, "Zika virus spreads across Americas as concerns mount over birth defects," *BMJ*, vol. 351, p. h6983, 2015.

[211] L. C. Rodrigues, "Microcephaly and Zika virus infection," *Lancet*, vol. 387, no. 10033, pp. 2070–2072, 2016.

[212] D. W. Smith and J. Mackenzie, "Zika virus and Guillain-Barré syndrome: another viral cause to add to the list," *Lancet*, vol. 387, no. 10027, pp. 1486–1488, 2016.

[213] R. B. Martines, J. Bhatnagar, M. K. Keating et al., "Notes from the field: evidence of Zika virus infection in brain and placental tissues from two congenitally infected newborns and two fetal losses–Brazil, 2015," *MMWR Morbidity and Mortality Weekly Report*, vol. 65, no. 6, pp. 159-160, 2016.

[214] E. D'Ortenzio, S. Matheron, Y. Yazdanpanah et al., "Evidence of sexual transmission of Zika virus," *The New England Journal of Medicine*, vol. 374, no. 22, pp. 2195–2198, 2016.

[215] R. Hamel, O. Dejarnac, S. Wichit et al., "Biology of Zika virus infection in human skin cells," *Journal of Virology*, vol. 89, no. 17, pp. 8880–8896, 2015.

[216] I. Pagani, S. Ghezzi, A. Ulisse et al., "Human endometrial stromal cells are highly permissive to productive infection by Zika virus," *Scientific Reports*, vol. 7, no. 1, p. 44286, 2017.

[217] E. Frumence, M. Roche, P. Krejbich-Trotot et al., "The South Pacific epidemic strain of Zika virus replicates efficiently in human epithelial A549 cells leading to IFN-β production and apoptosis induction," *Virology*, vol. 493, pp. 217–226, 2016.

[218] G. Dhiman, R. Abraham, and D. E. Griffin, "Human Schwann cells are susceptible to infection with Zika and yellow fever viruses, but not dengue virus," *Scientific Reports*, vol. 9, no. 1, p. 9951, 2019.

[219] J. R. Bowen, K. M. Quicke, M. S. Maddur et al., "Zika virus antagonizes type I interferon responses during infection of human dendritic cells," *PLoS Pathogens*, vol. 13, no. 2, article e1006164, 2017.

[220] S. F. Khaiboullina, T. Uppal, R. Sarkar, A. Gorzalski, S. St Jeor, and S. C. Verma, "ZIKV infection regulates inflammasomes pathway for replication in monocytes," *Scientific Reports*, vol. 7, no. 1, p. 16050, 2017.

[221] A. Kumar, S. Hou, A. M. Airo et al., "Zika virus inhibits type-I interferon production and downstream signaling," *EMBO Reports*, vol. 17, no. 12, pp. 1766–1775, 2016.

[222] A. Grant, S. S. Ponia, S. Tripathi et al., "Zika virus targets human STAT2 to inhibit type I interferon signaling," *Cell Host & Microbe*, vol. 19, no. 6, pp. 882–890, 2016.

[223] J. F. V. López, P. A. Velilla, and S. Urcuqui-Inchima, "Chikungunya virus and Zika virus, two different viruses examined with a common aim: role of pattern recognition receptors on the inflammatory response," *Journal of Interferon & Cytokine Research*, vol. 39, no. 9, pp. 507–521, 2019.

[224] J. A. Kim, R. K. Seong, S. W. Son, and O. S. Shin, "Insights into ZIKV-mediated innate immune responses in human dermal fibroblasts and epidermal keratinocytes," *The Journal of Investigative Dermatology*, vol. 139, no. 2, pp. 391–399, 2019.

[225] J. Ma, H. Ketkar, T. Geng et al., "Zika virus non-structural protein 4A blocks the RLR-MAVS signaling," *Frontiers in Microbiology*, vol. 9, p. 1350, 2018.

[226] Y. Hu, X. Dong, Z. He et al., "Zika virus antagonizes interferon response in patients and disrupts RIG-I-MAVS interaction through its CARD-TM domains," *Cell & Bioscience*, vol. 9, no. 1, p. 46, 2019.

[227] W. Riedl, D. Acharya, J. H. Lee et al., "Zika virus NS3 mimics a cellular 14-3-3-binding motif to antagonize RIG-I- and MDA5-mediated innate immunity," *Cell Host & Microbe*, vol. 26, no. 4, pp. 493–503.e6, 2019.

[228] H. Xia, H. Luo, C. Shan et al., "An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction," *Nature Communications*, vol. 9, no. 1, p. 414, 2018.

[229] P. M. Tricarico, I. Caracciolo, S. Crovella, and P. D'Agaro, "Zika virus induces inflammasome activation in the glial cell line U87-MG," *Biochemical and Biophysical Research Communications*, vol. 492, no. 4, pp. 597–602, 2017.

[230] W. Wang, G. Li, de Wu et al., "Zika virus infection induces host inflammatory responses by facilitating NLRP3 inflammasome assembly and interleukin-1β secretion," *Nature Communications*, vol. 9, no. 1, p. 106, 2018.

[231] Y. Zheng, Q. Liu, Y. Wu et al., "Zika virus elicits inflammation to evade antiviral response by cleaving cGAS via NS1-caspase-1 axis," *The EMBO Journal*, vol. 37, no. 18, 2018.

[232] C. N. O. Santos, D. R. Ribeiro, J. Cardoso Alves et al., "Association between Zika virus microcephaly in newborns with the rs3775291 variant in toll-like receptor 3 and rs1799964 variant at tumor necrosis factor-α gene," *The Journal of Infectious Diseases*, vol. 220, no. 11, pp. 1797–1801, 2019.

[233] K. Esser-Nobis, L. D. Aarreberg, J. A. Roby, M. R. Fairgrieve, R. Green, and M. Gale Jr., "Comparative analysis of African and Asian lineage-derived Zika virus strains reveals differences in activation of and sensitivity to antiviral innate immunity," *Journal of Virology*, vol. 93, no. 13, 2019.

[234] T. J. Gray and C. E. Webb, "A review of the epidemiological and clinical aspects of West Nile virus," *Int J Gen Med*, vol. 7, pp. 193–203, 2014.

[235] F. Mostashari, M. L. Bunning, P. T. Kitsutani et al., "Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey," *Lancet*, vol. 358, no. 9278, pp. 261–264, 2001.

[236] "Centers for Disease Control and Prevention, West Nile Virus, Statistics and Maps," https://www.cdc.gov/westnile/statsmaps/index.html.

[237] E. B. Hayes and D. J. Gubler, "West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States," *Annual Review of Medicine*, vol. 57, no. 1, pp. 181–194, 2006.

[238] L. Wang, L. Yang, E. Fikrig, and P. Wang, "An essential role of PI3K in the control of West Nile virus infection," *Scientific Reports*, vol. 7, no. 1, p. 3724, 2017.

[239] M. G. Zimmerman, J. R. Bowen, C. E. McDonald, B. Pulendran, and M. S. Suthar, "West Nile virus infection blocks inflammatory response and T cell costimulatory capacity of human monocyte-derived dendritic cells," *Journal of Virology*, vol. 93, no. 23, 2019.

[240] Y. Takamatsu, L. Uchida, and K. Morita, "Delayed IFN response differentiates replication of West Nile virus and Japanese encephalitis virus in human neuroblastoma and glioblastoma cells," *The Journal of General Virology*, vol. 96, no. 8, pp. 2194–2199, 2015.

[241] D. J. Rawle, Y. X. Setoh, J. H. Edmonds, and A. A. Khromykh, "Comparison of attenuated and virulent West Nile virus strains in human monocyte-derived dendritic cells as a model of initial human infection," *Virology Journal*, vol. 12, no. 1, p. 46, 2015.

[242] M. Laurent-Rolle, E. F. Boer, K. J. Lubick et al., "The NS5 protein of the virulent West Nile virus NY99 strain is a potent antagonist of type I interferon-mediated JAK-STAT signaling," *Journal of Virology*, vol. 84, no. 7, pp. 3503–3515, 2010.

[243] Y. Setoh, P. Periasamy, N. Peng, A. Amarilla, A. Slonchak, and A. Khromykh, "Helicase domain of West Nile virus NS3 protein plays a role in inhibition of type I interferon signalling," *Viruses*, vol. 9, no. 11, p. 326, 2017.

[244] H. L. Zhang, H. Q. Ye, S. Q. Liu et al., "West Nile virus NS1 antagonizes interferon beta production by targeting RIG-I and MDA5," *Journal of Virology*, vol. 91, no. 18, 2017.

[245] L. H. Tobler, M. J. Cameron, M. C. Lanteri et al., "Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors," *The Journal of Infectious Diseases*, vol. 198, no. 7, pp. 979–983, 2008.

[246] K. W. Hoffman, D. Sachs, S. V. Bardina et al., "Differences in early cytokine production are associated with development of a greater number of symptoms following West Nile virus infection," *The Journal of Infectious Diseases*, vol. 214, no. 4, pp. 634–643, 2016.

[247] T. P. Monath, "Yellow fever: an update," *The Lancet Infectious Diseases*, vol. 1, no. 1, pp. 11–20, 2001.

[248] T. P. Monath, "Treatment of yellow fever," *Antiviral Research*, vol. 78, no. 1, pp. 116–124, 2008.

[249] T. D. Querec, R. S. Akondy, E. K. Lee et al., "Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans," *Nature Immunology*, vol. 10, no. 1, pp. 116–125, 2009.

[250] D. Gaucher, R. Therrien, N. Kettaf et al., "Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses," *The Journal of Experimental Medicine*, vol. 205, no. 13, pp. 3119–3131, 2008.

[251] F. Douam, Y. E. Soto Albrecht, G. Hrebikova et al., "Type III interferon-mediated signaling is critical for controlling live attenuated yellow fever virus infection in vivo," *MBio*, vol. 8, no. 4, 2017.

[252] L. Sinigaglia, S. Gracias, E. Décembre et al., "Immature particles and capsid-free viral RNA produced by yellow fever virus-infected cells stimulate plasmacytoid dendritic cells to secrete interferons," *Scientific Reports*, vol. 8, no. 1, p. 10889, 2018.

[253] H. Ishikawa, Z. Ma, and G. N. Barber, "STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity," *Nature*, vol. 461, no. 7265, pp. 788–792, 2009.

[254] M. Laurent-Rolle, J. Morrison, R. Rajsbaum et al., "The interferon signaling antagonist function of yellow fever virus NS5 protein is activated by type I interferon," *Cell Host & Microbe*, vol. 16, no. 3, pp. 314–327, 2014.

[255] M. Laurent-Rolle and J. Morrison, "The role of NS5 protein in determination of host cell range for yellow fever virus," *DNA and Cell Biology*, vol. 38, no. 12, pp. 1414–1417, 2019.

[256] L. Miorin, M. Laurent-Rolle, G. Pisanelli et al., "Host-specific NS5 ubiquitination determines yellow fever virus tropism," *Journal of Virology*, vol. 93, no. 14, 2019.

[257] N. Hernandez, G. Bucciol, L. Moens et al., "Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines," *The Journal of Experimental Medicine*, vol. 216, no. 9, pp. 2057–2070, 2019.

[258] J. W. Schoggins, S. J. Wilson, M. Panis et al., "A diverse range of gene products are effectors of the type I interferon antiviral response," *Nature*, vol. 472, no. 7344, pp. 481–485, 2011.

[259] S. K. Unni, D. Růžek, C. Chhatbar, R. Mishra, M. K. Johri, and S. K. Singh, "Japanese encephalitis virus: from genome to infectome," *Microbes and Infection*, vol. 13, no. 4, pp. 312–321, 2011.

[260] G. Manocha, R. Mishra, N. Sharma, K. Kumawat, A. Basu, and S. K. Singh, "Regulatory role of TRIM21 in the type-I interferon pathway in Japanese encephalitis virus-infected human microglial cells," *Journal of Neuroinflammation*, vol. 11, no. 1, p. 24, 2014.

[261] S. P. Yu, K. C. Ong, D. Perera, and K. T. Wong, "Neuronal transcriptomic responses to Japanese encephalitis virus infection with a special focus on chemokine CXCL11 and pattern recognition receptors RIG-1 and MDA5," *Virology*, vol. 527, pp. 107–115, 2019.

[262] R. J. Lin, B. L. Chang, H. P. Yu, C. L. Liao, and Y. L. Lin, "Blocking of interferon-induced Jak-Stat signaling by Japanese encephalitis virus NS5 through a protein tyrosine phosphatase-mediated mechanism," *Journal of Virology*, vol. 80, no. 12, pp. 5908–5918, 2006.

[263] R. J. Lin, C. L. Liao, E. Lin, and Y. L. Lin, "Blocking of the alpha interferon-induced Jak-Stat signaling pathway by Japanese encephalitis virus infection," *Journal of Virology*, vol. 78, no. 17, pp. 9285–9294, 2004.

[264] J. Ye, Z. Chen, Y. Li et al., "Japanese encephalitis virus NS5 inhibits type I interferon (IFN) production by blocking the nuclear translocation of IFN regulatory factor 3 and NF-κB," *Journal of Virology*, vol. 91, no. 8, 2017.

[265] P. M. Winter, N. M. Dung, H. T. Loan et al., "Proinflammatory cytokines and chemokines in humans with Japanese encephalitis," *The Journal of Infectious Diseases*, vol. 190, no. 9, pp. 1618–1626, 2004.

[266] W. K. Reisen, "Epidemiology of St. Louis encephalitis virus," *Advances in Virus Research*, vol. 61, pp. 139–183, 2003.

[267] J. P. Luby, "Sensitivities of neurotropic arboviruses to human interferon," *The Journal of Infectious Diseases*, vol. 132, no. 4, pp. 361–367, 1975.

[268] R. E. Marques, J. del Sarto, R. P. Rocha et al., "Development of a model of Saint Louis encephalitis infection and disease in mice," *Journal of Neuroinflammation*, vol. 14, no. 1, p. 61, 2017.

[269] T. J. Brooks and R. J. Phillpotts, "Interferon-alpha protects mice against lethal infection with St Louis encephalitis virus delivered by the aerosol and subcutaneous routes," *Antiviral Research*, vol. 41, no. 1, pp. 57–64, 1999.

[270] V. Bocci, "Evaluation of routes of administration of interferon in cancer: a review and a proposal," *Cancer Drug Delivery*, vol. 1, no. 4, pp. 337–351, 1984.

[271] L. Westfechtel, R. N. Werner, C. Dressler, M. Gaskins, and A. Nast, "Adjuvant treatment of anogenital warts with systemic interferon: a systematic review and meta-analysis," *Sexually Transmitted Infections*, vol. 94, no. 1, pp. 21–29, 2018.

[272] R. Di Trolio, E. Simeone, G. Di Lorenzo, C. Buonerba, and P. A. Ascierto, "The use of interferon in melanoma patients: a systematic review," *Cytokine & Growth Factor Reviews*, vol. 26, no. 2, pp. 203–212, 2015.

[273] L. Savale, C. Sattler, S. Günther et al., "Pulmonary arterial hypertension in patients treated with interferon," *The European Respiratory Journal*, vol. 44, no. 6, pp. 1627–1634, 2014.

[274] M. Schmidinger, "Understanding and managing toxicities of vascular endothelial growth factor (VEGF) inhibitors," *EJC Supplements*, vol. 11, no. 2, pp. 172–191, 2013.

[275] A. Caraceni, L. Gangeri, C. Martini et al., "Neurotoxicity of interferon-? in melanoma therapy," *Cancer*, vol. 83, no. 3, pp. 482–489, 1998.

[276] H. Gogas, D. Bafaloukos, J. Ioannovich et al., "Tolerability of adjuvant high-dose interferon alfa-2b: 1 month versus 1 year–a Hellenic Cooperative Oncology Group study," *Anticancer Research*, vol. 24, no. 3b, pp. 1947–1952, 2004.

[277] T. Minami, T. Kishikawa, M. Sato, R. Tateishi, H. Yoshida, and K. Koike, "Meta-analysis: mortality and serious adverse events of peginterferon plus ribavirin therapy for chronic hepatitis C," *Journal of Gastroenterology*, vol. 48, no. 2, pp. 254–268, 2013.

[278] C. M. Oshansky, A. J. Gartland, S. S. Wong et al., "Mucosal immune responses predict clinical outcomes during influenza infection independently of age and viral load," *American Journal of Respiratory and Critical Care Medicine*, vol. 189, no. 4, pp. 449–462, 2014.

[279] S. Herold, M. Steinmueller, W. von Wulffen et al., "Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand," *The Journal of Experimental Medicine*, vol. 205, no. 13, pp. 3065–3077, 2008.

[280] K. Högner, T. Wolff, S. Pleschka et al., "Macrophage-expressed IFN-β contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia," *PLoS Pathogens*, vol. 9, no. 2, article e1003188, 2013.

[281] R. Y. Sung, J. Yin, S. J. Oppenheimer, J. S. Tam, and J. Lau, "Treatment of respiratory syncytial virus infection with recombinant interferon alfa-2a," *Archives of Disease in Childhood*, vol. 69, no. 4, pp. 440–442, 1993.

[282] J. Portnoy, R. Hicks, F. Pacheco, and L. Olson, "Pilot study of recombinant interferon alpha-2a for treatment of infants with bronchiolitis induced by respiratory syncytial virus," *Antimicrobial Agents and Chemotherapy*, vol. 32, no. 4, pp. 589–591, 1988.

[283] J. Dubois, L. Hershon, L. Carmant, S. Bélanger, J. M. Leclerc, and M. David, "Toxicity profile of interferon alfa-2b in children: a prospective evaluation," *The Journal of Pediatrics*, vol. 135, no. 6, pp. 782–785, 1999.

[284] B. M. Farr, Gwaltney JM Jr, K. F. Adams, and F. G. Hayden, "Intranasal interferon-alpha 2 for prevention of natural rhinovirus colds," *Antimicrobial Agents and Chemotherapy*, vol. 26, no. 1, pp. 31–34, 1984.

[285] F. G. Hayden and J. M. Gwaltney, "Intranasal interferon 2 for prevention of rhinovirus infection and illness," *The Journal of Infectious Diseases*, vol. 148, no. 3, pp. 543–550, 1983.

[286] F. G. Hayden, J. K. Albrecht, D. L. Kaiser, and Gwaltney JM Jr, "Prevention of natural colds by contact prophylaxis with intranasal alpha 2-interferon," *The New England Journal of Medicine*, vol. 314, no. 2, pp. 71–75, 1986.

[287] A. S. Monto, S. A. Schwartz, and J. K. Albrecht, "Ineffectiveness of postexposure prophylaxis of rhinovirus infection with low-dose intranasal alpha 2b interferon in families," *Antimicrobial Agents and Chemotherapy*, vol. 33, no. 3, pp. 387–390, 1989.

[288] E. S. Kim, P. G. Choe, W. B. Park et al., "Clinical progression and cytokine profiles of Middle East respiratory syndrome coronavirus infection," *Journal of Korean Medical Science*, vol. 31, no. 11, pp. 1717–1725, 2016.

[289] A. S. Omrani, M. M. Saad, K. Baig et al., "Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study," *The Lancet Infectious Diseases*, vol. 14, no. 11, pp. 1090–1095, 2014.

[290] M. R. Loutfy, L. M. Blatt, K. A. Siminovitch et al., "Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study," *JAMA*, vol. 290, no. 24, pp. 3222–3228, 2003.

[291] A. Kamaladasa, L. Gomes, A. Wijesinghe et al., "Altered monocyte response to the dengue virus in those with varying

severity of past dengue infection," *Antiviral Research*, vol. 169, p. 104554, 2019.

[292] J. F. W. Chan, A. J. Zhang, C. C. S. Chan et al., "Zika virus infection in dexamethasone-immunosuppressed mice demonstrating disseminated infection with multi-organ involvement including orchitis effectively treated by recombinant type I interferons," *eBioMedicine*, vol. 14, pp. 112–122, 2016.

[293] A. C. Kalil, M. P. Devetten, S. Singh et al., "Use of interferon-alpha in patients with West Nile encephalitis: report of 2 cases," *Clinical Infectious Diseases*, vol. 40, no. 5, pp. 764–766, 2005.

[294] J. Hwang, H. S. Ryu, H. Kim, and S. A. Lee, "The first reported case of West Nile encephalitis in Korea," *Journal of Korean Medical Science*, vol. 30, no. 3, pp. 343–345, 2015.

[295] K. M. Chan-Tack and G. Forrest, "Failure of interferon alpha-2b in a patient with West Nile virus meningoencephalitis and acute flaccid paralysis," *Scandinavian Journal of Infectious Diseases*, vol. 37, no. 11-12, pp. 944–946, 2005.

[296] M. Lewis and J. R. Amsden, "Successful treatment of West Nile virus infection after approximately 3 weeks into the disease course," *Pharmacotherapy*, vol. 27, no. 3, pp. 455–458, 2007.

[297] C. Harinasuta, C. Wasi, and S. Vithanomsat, "The effect of interferon on Japanese encephalitis virus in vitro," *The Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 15, no. 4, pp. 564–568, 1984.

[298] T. Solomon, N. M. Dung, B. Wills et al., "Interferon alfa-2a in Japanese encephalitis: a randomised double-blind placebo-controlled trial," *Lancet*, vol. 361, no. 9360, pp. 821–826, 2003.

[299] J. J. Rahal, J. Anderson, C. Rosenberg, T. Reagan, and L. L. Thompson, "Effect of interferon-alpha2b therapy on St. Louis viral meningoencephalitis: clinical and laboratory results of a pilot study," *The Journal of Infectious Diseases*, vol. 190, no. 6, pp. 1084–1087, 2004.

[300] C. A. Hartmann, H. R. Vikram, M. T. Seville et al., "Neuroinvasive St. Louis encephalitis virus infection in solid organ transplant recipients," *American Journal of Transplantation*, vol. 17, no. 8, pp. 2200–2206, 2017.

[301] J. W. Schoggins and C. M. Rice, "Interferon-stimulated genes and their antiviral effector functions," *Current Opinion in Virology*, vol. 1, no. 6, pp. 519–525, 2011.

[302] W. M. Schneider, M. D. Chevillotte, and C. M. Rice, "Interferon-stimulated genes: a complex web of host defenses," *Annual Review of Immunology*, vol. 32, pp. 513–545, 2014.

[303] W. Wang, L. Xu, J. Su, M. P. Peppelenbosch, and Q. Pan, "Transcriptional regulation of antiviral interferon-stimulated genes," *Trends in Microbiology*, vol. 25, no. 7, pp. 573–584, 2017.

[304] K. M. Crosse, E. A. Monson, M. R. Beard, and K. J. Helbig, "Interferon-stimulated genes as enhancers of antiviral innate immune signaling," *Journal of Innate Immunity*, vol. 10, no. 2, pp. 85–93, 2018.

[305] X. Wang, E. R. Hinson, and P. Cresswell, "The interferon-inducible protein viperin inhibits influenza virus release by perturbing lipid rafts," *Cell Host & Microbe*, vol. 2, no. 2, pp. 96–105, 2007.

[306] K. J. Helbig and M. R. Beard, "The role of viperin in the innate antiviral response," *Journal of Molecular Biology*, vol. 426, no. 6, pp. 1210–1219, 2014.

[307] Y. Zhu and G. Gao, "ZAP-mediated mRNA degradation," *RNA Biology*, vol. 5, no. 2, pp. 65–67, 2008.

[308] B. Liu, N. L. Li, Y. Shen et al., "The C-terminal tail of TRIM56 dictates antiviral restriction of influenza A and B viruses by impeding viral RNA synthesis," *Journal of Virology*, vol. 90, no. 9, pp. 4369–4382, 2016.

[309] B. Liu, N. L. Li, J. Wang et al., "Overlapping and distinct molecular determinants dictating the antiviral activities of TRIM56 against flaviviruses and coronavirus," *Journal of Virology*, vol. 88, no. 23, pp. 13821–13835, 2014.

[310] R. E. Watkinson, W. A. McEwan, J. C. H. Tam, M. Vaysburd, and L. C. James, "TRIM21 promotes cGAS and RIG-I sensing of viral genomes during infection by antibody-opsonized virus," *PLoS Pathogens*, vol. 11, no. 10, article e1005253, 2015.

[311] L. Espert, G. Degols, C. Gongora et al., "ISG20, a new interferon-induced RNase specific for single-stranded RNA, defines an alternative antiviral pathway against RNA genomic viruses," *The Journal of Biological Chemistry*, vol. 278, no. 18, pp. 16151–16158, 2003.

[312] H. Qu, J. Li, L. Yang, L. Sun, W. Liu, and H. He, "Influenza A virus-induced expression of ISG20 inhibits viral replication by interacting with nucleoprotein," *Virus Genes*, vol. 52, no. 6, pp. 759–767, 2016.

[313] G. Subramanian, T. Kuzmanovic, Y. Zhang et al., "A new mechanism of interferon's antiviral action: induction of autophagy, essential for paramyxovirus replication, is inhibited by the interferon stimulated gene, TDRD7," *PLoS Pathogens*, vol. 14, no. 1, article e1006877, 2018.

[314] D. Jiang, J. M. Weidner, M. Qing et al., "Identification of five interferon-induced cellular proteins that inhibit west nile virus and dengue virus infections," *Journal of Virology*, vol. 84, no. 16, pp. 8332–8341, 2010.

[315] K. J. Helbig, J. M. Carr, J. K. Calvert et al., "Viperin is induced following dengue virus type-2 (DENV-2) infection and has anti-viral actions requiring the C-terminal end of viperin," *PLoS Neglected Tropical Diseases*, vol. 7, no. 4, article e2178, 2013.

[316] A. L. Brass, I. C. Huang, Y. Benita et al., "The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus," *Cell*, vol. 139, no. 7, pp. 1243–1254, 2009.

[317] Y. Suzuki, W. X. Chin, Q. Han et al., "Characterization of RyDEN (C19orf66) as an interferon-stimulated cellular inhibitor against dengue virus replication," *PLoS Pathogens*, vol. 12, no. 1, article e1005357, 2016.

[318] R. J. Lin, H. P. Yu, B. L. Chang, W. C. Tang, C. L. Liao, and Y. L. Lin, "Distinct antiviral roles for human 2′,5′-oligoadenylate synthetase family members against dengue virus infection," *Journal of Immunology*, vol. 183, no. 12, pp. 8035–8043, 2009.

[319] K. Wang, C. Zou, X. Wang et al., "Interferon-stimulated TRIM69 interrupts dengue virus replication by ubiquitinating viral nonstructural protein 3," *PLoS Pathogens*, vol. 14, no. 8, article e1007287, 2018.

[320] R. B. Richardson, M. B. Ohlson, J. L. Eitson et al., "A CRISPR screen identifies IFI6 as an ER-resident interferon effector that blocks flavivirus replication," *Nature Microbiology*, vol. 3, no. 11, pp. 1214–1223, 2018.

[321] P. K. Singh, S. Singh, D. Farr, and A. Kumar, "Interferon-stimulated gene 15 (ISG15) restricts Zika virus replication

in primary human corneal epithelial cells," *The Ocular Surface*, vol. 17, no. 3, pp. 551–559, 2019.

[322] J. Dai, W. Pan, and P. Wang, "ISG15 facilitates cellular antiviral response to dengue and West Nile virus infection in vitro," *Virology Journal*, vol. 8, no. 1, p. 468, 2011.

[323] D. J. Morales and D. J. Lenschow, "The antiviral activities of ISG15," *Journal of Molecular Biology*, vol. 425, no. 24, pp. 4995–5008, 2013.

[324] T. Hishiki, Q. Han, K. Arimoto et al., "Interferon-mediated ISG15 conjugation restricts dengue virus 2 replication," *Biochemical and Biophysical Research Communications*, vol. 448, no. 1, pp. 95–100, 2014.

[325] F. Valdez, J. Salvador, P. M. Palermo et al., "Schlafen 11 restricts flavivirus replication," *Journal of Virology*, vol. 93, no. 15, 2019.

Review Article

The Role of Innate Leukocytes during Influenza Virus Infection

Prem P. Lamichhane[1,2] and Amali E. Samarasinghe [1,2]

[1]*Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN 38103, USA*
[2]*Children's Foundation Research Institute, Memphis, TN 38103, USA*

Correspondence should be addressed to Amali E. Samarasinghe; asamaras@uthsc.edu

Influenza virus infection is a serious threat to humans and animals, with the potential to cause severe pneumonia and death. Annual vaccination strategies are a mainstay to prevent complications related to influenza. However, protection from the emerging subtypes of influenza A viruses (IAV) even in vaccinated individuals is challenging. Innate immune cells are the first cells to respond to IAV infection in the respiratory tract. Virus replication-induced production of cytokines from airway epithelium recruits innate immune cells to the site of infection. These leukocytes, namely, neutrophils, monocytes, macrophages, dendritic cells, eosinophils, natural killer cells, innate lymphoid cells, and $\gamma\delta$ T cells, become activated in response to IAV, to contain the virus and protect the airway epithelium while triggering the adaptive arm of the immune system. This review addresses different anti-influenza virus schemes of innate immune cells and how these cells fine-tune the balance between immunoprotection and immunopathology during IAV infection. Detailed understanding on how these innate responders execute anti-influenza activity will help to identify novel therapeutic targets to halt IAV replication and associated immunopathology.

1. Introduction

Respiratory viruses infect millions around the world each year causing a range of symptoms and claiming thousands of lives [1–3]. Some viruses such as respiratory syncytial virus are lethal to the very young but harmless to most adults [4]. Other viruses, such as rhinovirus, cause essentially the same symptoms (predominantly a runny nose) in any age group [5]. Still others like influenza A virus (IAV) can cause severe infections in patients across age groups during one season, mild symptoms in another year or be lethal in yet another season [6]. In fact, IAV infections cause seasonal epidemics and global pandemics and are a major cause for public health concern. Although generally milder than pandemics, seasonal influenza epidemics can cause around 650,000 deaths globally each year [7]. Despite mainstay vaccination strategies to minimize IAV infections, influenza pandemics have occurred once every 10-30 years, primarily due to cross-species transmission and antigenic shifts in the virus [8, 9].

As a member of the Orthomyxoviridae family, IAV is an enveloped virus with an octasegmented negative sense,

single-stranded RNA genome [6]. At present, 18 hemagglutinin (HA) and 11 neuraminidase (NA) subtypes of IAV are documented to circulate in nature [10]. Only two HA subtypes of IAV (H1N1 and H3N2) and two lineages of influenza B viruses (Victoria and Yamagata) cause annual epidemics in humans [6]. However, IAVs dominate, inducing more severe morbidity and mortality compared to influenza B viruses, therefore, will be the focus of this review [11–13].

Rapid changes in IAV surface antigens through antigenic shift have resulted in three pandemics during the 20th century [14]. Avian IAV subtypes (H5N1, H7N1, H7N2, H7N3, H7N9, H9N2, and H10N8) can cross the species barrier to infect humans [8, 15, 16] and cause severe, lethal disease. In fact, H5N1 highly pathogenic avian influenza (HPAI) and the H7N9 low pathogenic avian influenza viruses pose a serious public health concern due to their respective high fatality rates of 52.79% and 39.42% [17]. The most severe H1N1 influenza pandemic occurred in 1918 claiming over 50 million lives [18] while the last H1N1 pandemic in 2009 (pH1N1) is estimated to have

claimed ~200,000 lives across the globe [19]. While annual vaccinations are highly encouraged by public health agencies, poor adherence and low efficacy increase the need for better strategies to understand host responses during IAV pathogenesis in order to delineate other mechanisms which enhance antiviral immunity.

Primary and secondary immune barriers play a crucial role in safeguarding the host against influenza. Physical barriers of the immune system including soluble components like mucus, collectins, and antimicrobial peptides provide the first line of defense by mitigating virus exposure to underlying airway epithelial cells which are the principal site for IAV replication [20, 21]. Upon breaching these physical barriers, IAVs bind to sialic acid receptors on airway epithelial cells and enter these cells to complete replicative cycles within, destroying infected cells in the process [22, 23]. Predilections for specific sialic acid linkages may restrict IAV to the upper respiratory tract. However, alterations in the sialic acid linkage preferences, particularly in reassortant viruses, can render IAV more adept at lower respiratory tract infection and dissemination [24, 25]. Infection of the epithelium activates the innate branch of the immune system which consists of humoral/soluble as well as cellular components.

Infected airway epithelial cells trigger innate immune responses in two ways. First, viral RNAs within infected cells are sensed by pattern recognition receptors such as toll-like receptors, nucleotide-binding oligomerization domain-like receptors, or retinoic acid-inducible gene (RIG)-I like receptors. Signaling through these receptors induces the production of antiviral soluble factors, including interferons (IFNs), which act on adjacent healthy cells in a paracrine manner to trigger antiviral gene transcription. Activation of mediators like protein kinase R, $2'5'$-oligoadenylate synthetase, RNaseL, cleavage and polyadenylation factor [26, 27] in otherwise healthy cells induces an antiviral state thereby limiting viral replication [28]. IFNs also induce expression of interferon-stimulated genes (like myxovirus-1) that have strong anti-influenza activity [29]. Furthermore, infected alveolar epithelial cells in the airway secrete proinflammatory cytokines like TNFα, IL-1β, IL-6, CCL2, CCL5, CXCL8, and CXCL10 [30] in superfluity, which support innate immune cell recruitment [20, 31] to contain viral spread. However, the production of inflammatory cytokines is not always protective as cytokine storms arise when they are secreted in excess as observed during HPAI H5N1 virus infection [32–34] often leading to severe pneumonia and death. Secondly, phagocytosis of infected cells by antigen-presenting cells (APCs) can activate adaptive immune responses [20, 28] that help to eliminate the infection.

Innate immune cells provide the first line of cellular defense to combat IAV infection. Leukocytes like neutrophils, monocytes, eosinophils, natural killer (NK) cells, innate lymphoid cells (ILCs), and $\gamma\delta$ T cells provide anti-influenza host protection by releasing preformed cytokines and granule contents that either directly or indirectly help the host to eliminate the threat posed by replicating virus. On the other hand, macrophages and dendritic cells (DCs) can phagocytose IAV-infected cells and present viral antigens to T cells, initiating adaptive immune responses including B cell-mediated humoral immunity. The aim of this review is to provide insight into the function of these innate leukocytes in immune protection and repair of damaged airways upon IAV infection.

2. Neutrophils: The First Recruits

Neutrophils are among the first innate cells to be recruited during IAV infection. Neutrophils are granulocytes derived from hematopoietic stem cells influenced by IL-3, M-CSF, and G-CSF. While neutrophils are first to respond to any noxious stimuli, their role during influenza is complex and accumulation in the lungs is impacted both by virus strain and dose [35, 36]. Secretion of several chemotactic factors such as CCL3 [37], CXCL-1 (IL-8 in humans), and CXCL-2 [38] acts as chemotactic signals for neutrophil generation and recruitment. Neutrophils release MMP-9 to digest type IV collagen in the pulmonary endothelial basement membrane [37] and enter the lung tissue through CXCR2 engagement [39] (Figure 1).

Neutropenia has been demonstrated to increase the pulmonary virus titer and mortality rate upon IAV infection [40] suggesting a protective role for neutrophils during influenza. Phagocytosis, release of granular contents, and production of cytokines are major effector functions of neutrophils. These granulocytes contain cationic antimicrobial peptides like defensins and cathelicidins that neutralize IAV [41, 42]. Cathelicidins have anti-influenza activity *in vivo* through the reduction of viral replication and hindering the production of inflammatory mediators [41], showcasing a promising role for neutrophil antimicrobial peptides during virus infections. Furthermore, proinflammatory cytokines produced by neutrophils limit virus replication and halt progression to severe disease [43, 44] (Figure 1). Neutrophils are susceptible to IAV infection [45–47], although infection has been demonstrated to be both productive (for pH1N1) [48] and abortive (for seasonal H1N1 and WSN33) [45, 49]. These discrepancies might be related to virus subtypes/strains used. Interestingly, mice treated with β-defensin had reduced IAV burden and an associated reduction in neutrophils in the lungs [21], underscoring the importance of a prudently adjudicated immune response to host protection. Nonetheless, IAV-infected neutrophils upregulate IFNs and other antiviral factors [45, 46] that limit viral replication (Figure 1).

In addition to these conventional roles, neutrophils also utilize more sophisticated strategies to safeguard the host during influenza. One such scheme is the production of neutrophil extracellular traps (NETs) through NETosis, a release of web like structures of nucleic acids coated with histones [50] which capture viral particles [51, 52] thereby preventing viral dissemination. Inflammatory mediators like TNFα and cathelicidins in the pulmonary environment trigger NETosis [53, 54] (Figure 1). Arginine in histones within NETs has differential aggregation properties for IAV wherein seasonal strains are more likely to be inhibited compared to pH1N1 [55]. However, mechanisms that dictate strain preferences for NETosis in response to IAV are unknown. Increased
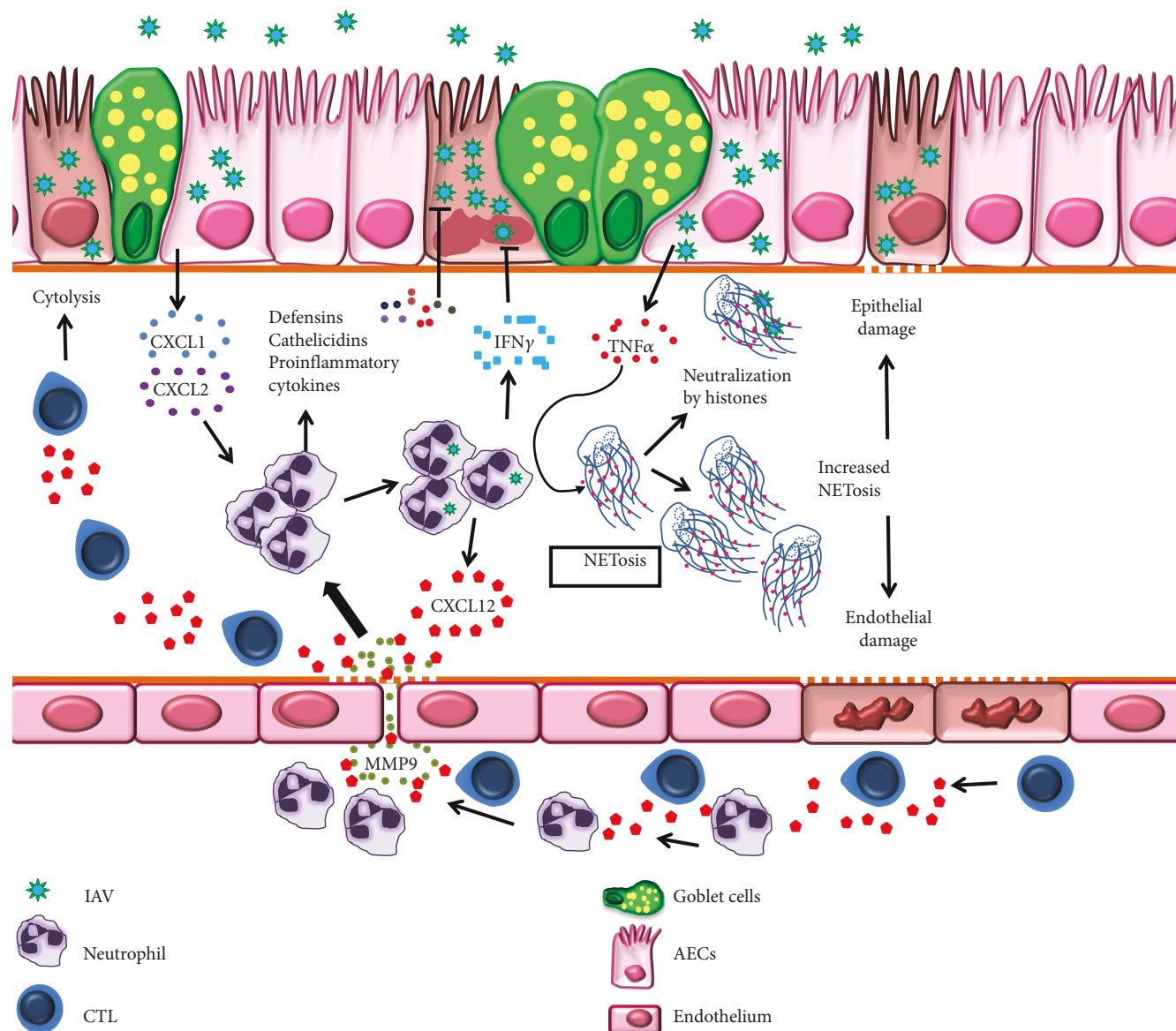
FIGURE 1: Schematic representation of neutrophil-mediated host defense mechanisms during influenza virus infection. Influenza A virus-(IAV)-infected airway epithelium releases neutrophil chemoattractants: CXCL1 and CXCL2. Neutrophils traffic into infected lungs by digesting endothelial basement membrane collagen. During trafficking, they release CXCL12-loaded vesicles/membranes which provide signals for CD8^{+} T cell migration and effector function. Once in the lungs, neutrophils secrete antimicrobial peptides and cytokines, including IFNγ, to inhibit IAV replication. TNFα produced by infected airway epithelium triggers the formation of neutrophil extracellular traps (NETs) which neutralize IAV particles. Enhanced NETosis damages airway epithelium and endothelium leading to severe pneumonia.

NET production, as observed in severe cases of H7N9 and pH1N1 infection [56], augmented damage to the pulmonary endothelia and epithelia [56, 57] leading to severe pneumonia suggesting that uncontrolled NET production may contribute to disease severity.

Neutrophils were also shown to guide CD8^{+} T cell activation and recruitment into the lungs during influenza emphasizing the reliance of adaptive immune cells on those from the innate branch. By presenting IAV antigens on the cell surface, neutrophils function as APCs to activate CD8^{+} T cells within the lungs [58]. In addition, neutrophil membrane-bound CXCL12 is used as a beacon by CD8^{+} T cells during migration into the IAV-infected lungs [59] (Figure 1). The importance of neutrophils in innate immune defenses against IAV is well defined. Yet, uncontrolled trafficking of neutrophils as observed during infection with highly pathogenic H5N1 and 1918 pandemic IAV [44, 60] results in the production of excessive reactive oxygen species [61, 62] that causes oxidative stress-mediated pulmonary damage. Additionally, neutrophil CXCL12 trails can recruit CD8^{+} T cells [59] in excess which may boost/perpetuate inflammation, lung damage and severe pathology through

cytolysis of infected epithelia during IAV infection. Thus, the abundance of neutrophils in the IAV-infected lungs may dictate their precise role in immunoprotection or immunopathology.

3. Monocytes: Recruited in Time of Need

Monocytes, peripheral blood phagocytes, are recruited into the lungs during IAV infection. Based on cell surface receptors, CD14 and CD16, circulating human monocytes can be subdivided into three distinct subsets: classical (CD14++/-CD16-), intermediate (CD14++/CD16+), and nonclassical (CD14+/CD16++) [63–65]. Compared to peripheral circulation (where classical monocytes are predominant), intermediate monocytes predominate in airways [66]. Similarly, murine monocytes (CD11b+CD115+) are divided into three subsets based on the expression of Ly6C as classical (Ly6C++), intermediate (Ly6C+), and nonclassical (Ly6C-) [67, 68].

The recruitment of these circulatory monocytes across the endothelial/epithelial barrier into the lungs has been extensively studied [69]. IAV-infected alveolar macrophages and epithelial cells secrete monocyte attracting chemokines, CCL2, CCL3, and CCL5 [69–71]. However, transepithelial migration of monocytes occurs through the CCL2/CCR2 axis, wherein CCR2-expressing monocytes move chemotactically towards CCL2 [69]. Interaction between monocyte integrins and endothelial adhesion molecules mediates transendothelial migration of monocytes [69, 72–75]. During IAV infection, monocyte transmigration across the endothelial barrier is predominantly dependent on interactions between VCAM-1 and β1 integrin (CD49d) and ICAM-1 interactions with β2 integrin (CD11a/CD18 or CD11b/CD18) [69] and promoted by TNFα secreted by infected alveolar macrophages (AMs) [69, 76], integrin-associated protein (CD47) [69, 76, 77], and junctional adhesion molecule 3 [69, 76, 78] (Figure 2).

Similar to neutrophils, monocytes are susceptible to IAV infection [79–81] irrespective of the subset [31], and infection rates may vary with the IAV subtype with the highest infectivity for H5N1 and H7N9 than pH1N1 [81]. Virus-infected monocytes secrete inflammatory cytokines and chemokines [31, 80] (Figure 2) in a virus dose-dependent manner [31]. Infected monocytes produce large amounts of IFNs [80, 82, 83] well known for their anti-influenza activity in addition to serving as a source for an array of cytokines and chemokines; for example, U937 promonocytic cells produce IL-1β, IL-8, IL-18, CCL3 [82], and IFN-α/β [80, 83]. Importantly, the microenvironment of infected lungs drives monocyte differentiation into macrophages and DCs (Figure 2) which play a pivotal role in phagocytosis of virus-infected cells thereby priming the adaptive immune system. During airway inflammation, migration and differentiation of blood monocytes contribute to the pool of pulmonary macrophages [20]. The absence of CD14 on monocytes/macrophages alters the immunopathogenesis during complex disease-disease interactions that occur when IAV infects hosts with underlying allergic states [84] suggesting that the function of each subset is regulated by the inflammatory milieu during influenza. The role of

monocyte-derived macrophages in protection from IAV infection is described elsewhere [85, 86].

Although monocytes provide immunoprotection against IAV infection, excessive infiltration of monocytes can induce immunopathology and mice exhibiting uncontrolled IAV replication have excessive monocyte recruitment into the lungs [87]. Conversely, monocyte-deficient CCR2-/- mice demonstrate increased survival following IAV infection [87–89], suggesting that monocytes can drive IAV-induced host pathology. Moreover, disease severity correlates with increased monocyte-derived cytokines [87] which in turn leads to immunopathology. Overall, the role in immune protection or pathology is determined by the number of infiltrating monocytes and the cytokine milieu.

4. Pulmonary Macrophages: Sentinels in Command?

The pulmonary macrophage population consists of alveolar macrophages (AMs) and interstitial macrophages (IMs) [86, 90]. Macrophages are phenotypically classified as proinflammatory (M1) and anti-inflammatory (M2). This classification is best suited for monocyte-derived macrophages wherein different cytokines and growth factors are used for differentiation but not AMs [86]. In relation to IAV, AMs are best studied while the role of IMs is ambiguous due to phenotypic similarity with monocytes and technical difficulty in liberating them from the lung tissue [91]. Irrespective of lower phagocytic ability compared to AMs [92], owing to their location in the interstitium, IMs phagocytose pathogens that have evaded AMs thereby providing a second line of defense within the tissue. The potential antiviral activity of IMs needs to be explored further.

As tissue resident professional phagocytes that safeguard the airway against intruding pulmonary pathogens, AMs maintain lung homeostasis [93, 94]. Cytokines, TGF-β and GM-CSF, promote differentiation of fetal monocytes into AMs [95–97]. With a prolonged half-life of about 1-5 weeks, the AM population is primarily maintained via self-renewal [98–100]. Pulmonary homeostasis is conserved in part by interaction of these AMs with alveolar epithelial cells (AECs). At steady state, AECs that express CD200 engage surface CD200R on AMs controlling the production of inflammatory cytokines [101]. However, IAV infection lowers the expression of CD200 and CD200R [101] compromising the CD200-CD200R axis which leads to macrophage activation and production of inflammatory mediators (Figure 2).

Pigs [102] and mice [103, 104] intranasally administered with clodronate liposome to deplete AMs have higher lung virus titers and severe disease characterized by impaired clearance of surfactant proteins, cellular debris, dead cells, pulmonary edema, and inflammation [102, 103]. Moreover, transgenic mice selectively depleted of AMs exhibit severe influenza [104, 105] suggesting a critical role for AMs in limiting virus-induced airway injury. However, the possible protective role of IMs may not be underestimated as they have more potent activators of antigen-specific T cells compared to AMs [90]. As most other leukocytes, AMs too secrete type I IFNs during IAV infection [106, 107] (Figure 2). IAV
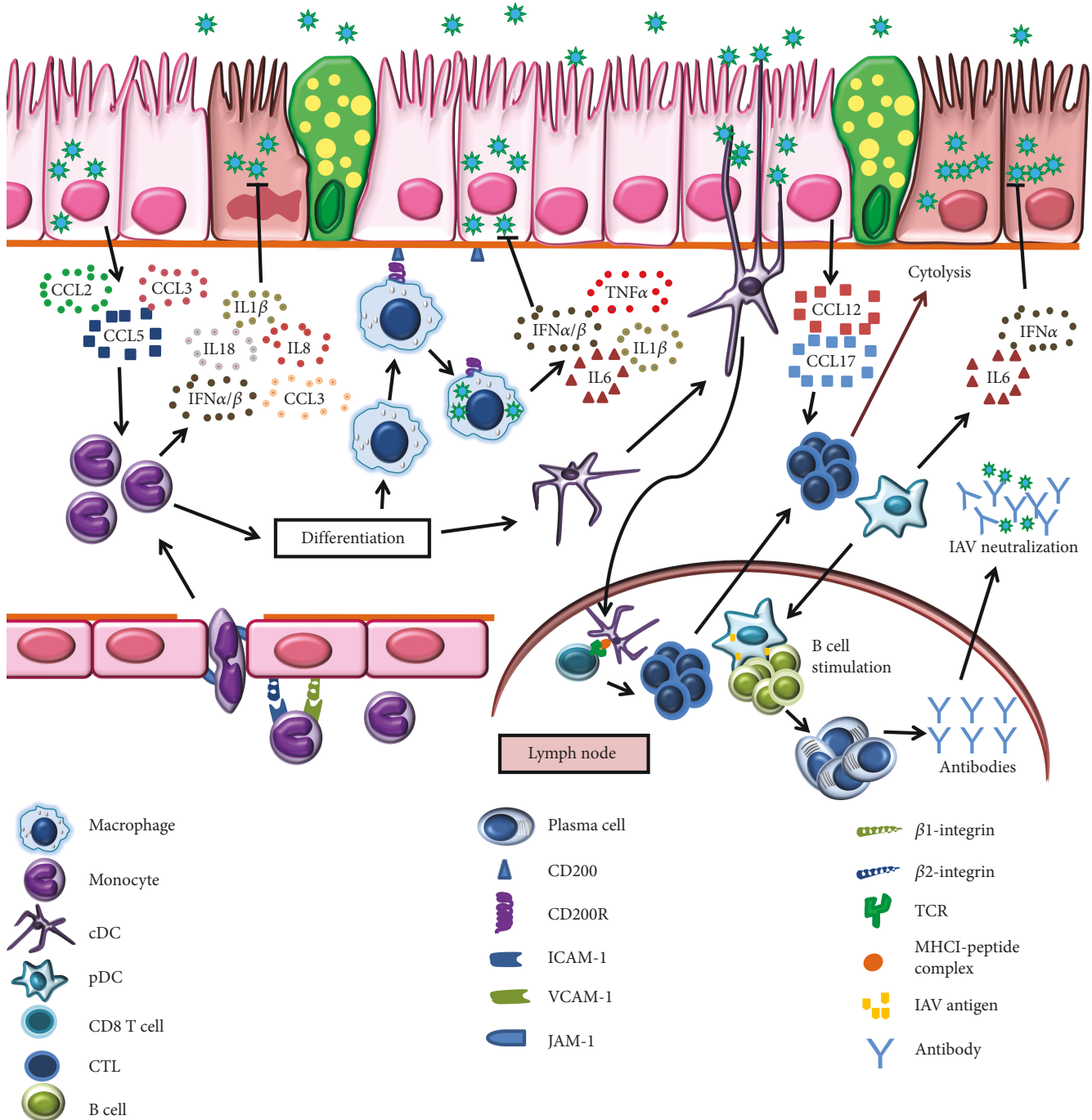
FIGURE 2: Schematic representation of monocyte-, macrophage-, and dendritic cell- (DC-) controlled immunoprotection and immunopathology during influenza virus infection. Influenza A virus- (IAV-) infected airway epithelium produces monocyte chemoattractants: CCL2, CCL3, and CCL5. Monocytes interact with endothelium through β1 integrin, VCAM-1, and β2 integrin, ICAM-1, binding, and subsequent entry into the lung tissue is assisted by JAM. These activated monocytes secrete inflammatory cytokines that inhibit IAV replication. Monocytes exert anti-IAV activity by differentiating into phagocytic cells like macrophages and DCs. IAV infection downregulates CD200-CD200R activating macrophages. These activated macrophages release an array of inflammatory cytokines that limit IAV replication. Both conventional and plasmacytoid DCs (cDCs and pDCs) exert anti-IAV activity in lungs. IAV-activated cDCs migrate to lymph node and present antigen to CD8^{+} T cells. Chemokines, CCL12 and CCL17, secreted by infected airway epithelium provide signals for the activated cytotoxic T cells trafficking into the lungs where they lyse IAV-infected cells. The other DC subset, pDC migrate to lymph node and stimulate B cell differentiation into antibody secreting plasma cells. These antibodies neutralize IAV particles conferring humoral protection.

infection of AMs showed species specificity where human AMs are not infected while the murine counterparts exhibit abortive infection [108, 109]. Since IAVs fail to infect human AMs [108], an alternative mechanism like phagocytosis of apoptotic cells may also trigger AM activation and cytokine secretion during infection [110].

Owing to the contribution of AMs in maintaining lung tissue integrity and pulmonary homeostasis, their role in protecting AECs from IAV-induced damage is important. Type I AECs are responsible for gas exchange, and their impairment during infection leads to pulmonary dysfunction. On the other hand, type II AECs can self-renew or divide to replenish damaged type I AECs [111]. Engagement of CysLT, a cell surface cysteinyl leukotriene receptor on type I AEC, enhances their susceptibility to IAV infection [104]. However, AMs suppress the CysLT pathway in type I AEC [104] conferring the protection to AECs during infection. Peroxisome proliferator-activated receptor gamma (PPAR-γ), highly expressed in AMs and critical during their development [112], restricts AMs from excessive proinflammatory cytokine production [113] thereby maintaining lung homeostasis. Parallelly, PPAR-γ stimulation lessens IAV-associated inflammation within airways [114] and increases the secretion of tissue remodeling (MMP7 and MMP9) and epiendothelial growth factors (EGF and VEGF) [115] suggesting a critical role for AMs as regulators of wound healing and tissue repair upon IAV infection.

The contribution of macrophages to immunopathology is evident from the fact that CCR2$^{-/-}$ mice are protected from IAV-induced pulmonary tissue destruction and mortality [89]. Furthermore, protective mechanisms in these macrophage deficient mice are traced to delayed migration of T cells [89] suggesting that macrophages alter cellular immune responses. The role of macrophages in IAV-mediated pathology is based on findings from monocyte-derived macrophages (MDMs). The induction of macrophage-derived cytokines, IFN-α/β, TNFα, CCL2, CCL3, CCL5, and CXCL10, is greater for highly pathogenic H5N1 virus than seasonal influenza viruses [116, 117] suggesting that these inflammatory mediators may contribute to IAV-induced immunopathology. In contrast, MDMs infected with H5N1 virus showed lower expression of cytokines, namely, IFN-α/β, TNFα, CCL5, and CCL8 at early times postinfection [118]. This may be representative of an immune evasion strategy of IAVs to prolong uncontrolled replication and replication-induced pathology to gain a foothold in the host. Cumulatively, these observations suggest that pulmonary dysfunction observed in fatal cases of influenza depends on how macrophages respond to the invading virus.

5. Dendritic Cells: Calling for Help

Dendritic cells (DCs) are professional APCs that patrol the body surfaces (skin, gut, or airway) for intruding microbes or insults. They play a key role in host immunity by bridging innate and adaptive arms of the immune system [119, 120]. DCs are broadly classified as CD11c$^+$ conventional DCs (cDCs) and CD11c$^-$ plasmacytoid DCs (pDCs) [121, 122]. Furthermore, two subsets of cDCs have been

identified in the airways: CD103$^+$CD11b$^-$ (CD103$^+$ cDCs) and CD103$^-$CD11b$^+$ (CD11b$^+$ cDCs) cells [123]; the latter of which resides in the lamina propria which lies immediately beneath the airway epithelium [124, 125]. In a steady state, the major DC population in the airway mucosa is CD103$^+$ cDCs followed by CD11b$^+$ cDCs and pDCs [124, 126, 127]. Humans also have more myeloid DCs (similar to cDCs in mice) in the airways than pDCs [66]. The CD103$^+$ cDCs can extend their dendrites or traverse the alveolar epithelia into the airway for surveillance and antigen capture [123] (Figure 2).

Acute IAV infection leads to a decrease in cDCs and pDCs in peripheral circulation [128–130] and a sustained increase in the respiratory tract [129, 130] (Figure 2) suggesting active trafficking of DC populations during influenza. Following intranasal inoculation of IAV (X31 H3N2) in mice, pulmonary cDC subsets demonstrated sustained increase while it was transient for pDC. Moreover, draining lymph nodes (DLNs) contained only cDCs [127] suggesting maturation and migration of cDCs to DLNs following IAV infection (Figure 2). Antimicrobial peptide, β-defensin, induces a reduction in lung cDCs during pH1N1 infection [21], emphasizing the complex interaction and dependence between the soluble and cellular factors of the innate immune system. Depletion of CD103$^+$ cDCs aggravated disease severity [127] suggesting a crucial role during influenza. Therefore, the totality of cDCs' contribution to lung health during IAV infection remains unknown, and since these subsets of cDCs secrete inflammatory mediators [131], a possible role in recruiting other leukocytes during influenza needs to be more fully explored.

Compared to other DC subsets, CD103$^+$ cDCs are highly efficient at viral antigen uptake and migration to DLNs [131] and are the most efficient cross-presenters of the immune system [132] as they differ in their antigen processing and presentation capabilities [133]. While CD103$^+$ cDCs process and present IAV antigens to CD8$^+$ T cells efficiently [127, 131], IAV-activated CD11b$^+$ cDCs fail to prime CD8$^+$ T cells [127] (Figure 2). This might be related to the ability of these cells to support IAV replication ex vivo; CD103$^+$ cDCs support productive IAV infection while CD11b$^+$ cDCs do not [134], providing an explanation for CD103$^+$ cDC's superior antigen-presenting capacity. However, whether CD103$^+$ cDCs support IAV replication in vivo is unclear with data suggesting that viral antigen is acquired through phagocytosis instead to allow cross-presentation to CD8$^+$ T cells [132].

The effective function of cDCs in priming the T cell-mediated immune response depends on (1) trafficking of IAV-activated DCs into DLNs, (2) presentation of antigen to specific CD8$^+$ T cells, and (3) lung homing capacity of activated CD8$^+$ T cells. Migration of pulmonary cDCs into DLNs depends on expression of CCR7, as ccr7-deficient mice lack DC trafficking into DLNs [133, 135]. Both CD103$^+$ cDCs and CD11b$^+$ cDCs express CCR7 [131] confirming the migratory nature of these cDCs. Once in the DLNs, these DCs prime IAV antigen-specific CD8$^+$ T cells, which migrate into infected lungs in a CCR4-dependent manner [136] to exert anti-influenza activity (Figure 2).

Following IAV infection, pDCs produce large amounts of chemoattractants, importantly CXCL1, CCL2, CCL5, and CXCL10 [137] (Figure 2). Mice selectively depleted of pDCs exhibited delayed recruitment of T cells into airways [138] suggesting that they regulate accumulation of T cells during early IAV infection. The ability of pDCs to support IAV replication is dose dependent [139, 140]; susceptibility observed with higher multiplicity of infections (MOIs) [139, 140] might not correlate with infection in humans. Since, at lower MOIs, pDCs do not support IAV replication [139], they fail to prime CD4^{+} or CD8^{+} T cells directly [127]. However, pDCs can uptake IAV antigens from virus-infected cells and upregulate CCR7 expression in response [141] suggesting that they too can migrate to DLNs and cross-present acquired IAV antigens. During IAV infection, pDCs induce differentiation of B cells into antibody-secreting plasma cells through IFNα and IL-6 [142] and the depletion of pDCs abrogates antibody secretion [127]. The activation of lung-resident B cells during pH1N1 infection [143] may be led by these DC subsets that encounter viral antigen thereby providing dual protection at the site of infection as well as by couriering a call for help (Figure 2).

As with most factors of the immune response, moderation is required for health and excessive cytokine levels can be detrimental to the host. Highly pathogenic H5N1 virus-infected pDCs produce higher amounts of IFNα than those infected with less virulent seasonal H1N1 and H3N2 strains [139, 144]. While IFNα secreted from pDCs does have antiviral functions [139, 145] (Figure 2), prolific production during IAV infection results in uncontrolled inflammation and host pathology [146], alluding that pDCs may also contribute to the cytokine storm during infection. As an antithesis to sublethal IAV infection, lethal infection causes the accumulation of pDCs in DLNs with enhanced expression of Fas ligand (FasL) [147, 148] which engages with Fas, a membrane protein of the death receptor family [149] expressed on IAV-specific CD8^{+} T cells eliminating them via Fas-mediated apoptosis [147, 148]. Conversely, since IAV infection in pDC-depleted mice leads to enhanced accumulation of inflammatory cells, particularly CD11b^{+} cDCs and macrophages which produce massive amounts of proinflammatory cytokines (TNFα and IL-6) [150], pDCs may be important immunoregulators during lethal IAV infection. The protective versus deleterious effect of pDCs depends on the infectious dose.

6. Eosinophils: Additional Sources of Host Defense

Eosinophils are not usually considered mediators of anti-influenza immunity. However, epidemiologic data associated with the 2009 H1N1 pandemic suggested that asthmatics, presumably with pulmonary eosinophilia, were less likely to suffer from IAV-induced morbidity and mortality [151–153]. Pediatric influenza patients who developed acute pneumonia demonstrated a rise in the serum IL-5 levels and peripheral eosinophilia [154], suggesting that eosinophil recruitment may be necessary for late-stage anti-influenza host defense. Pulmonary eosinophilia

has also been documented in IAV-infected mice [155–158] suggesting that cytokines like CCL5 [30] or IL-5 [155] produced during IAV infection may drive eosinophil migration (Figure 3). Based on known functions of eosinophils during parasite infections and allergy [159], it may be likely that their influx into the lung during mid-late infection is in support of reparative processes required after IAV-induced cytopathology.

Eosinophils are conducive to IAV infection and undergo piecemeal degranulation in response to IAV [160]. This selective release of granule proteins such as major basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin, in addition to immunoregulatory cytokines and chemokines [161, 162] at the infection site, may either immunomodulate other leukocytes or help contain the virus *in situ* (Figure 3). Additionally, mice suffering from acute allergy are protected from IAV-induced airway damage [157] suggesting that eosinophils are important mediators during anti-influenza immunity in special populations of patients such as those with a T_H2 bias. Our findings that eosinophils were capable of trafficking to DLNs following IAV infection and their putative function in IAV antigen presentation in the context of MHCI to activate CD8^{+} T cells [160] forecast multifaceted functions for eosinophils during influenza pathogenesis [163].

7. Natural Killer Cells: On-Site Killing

As large granular lymphocytes representing about 10% of lung resident lymphocytes, NK cells accumulate in the respiratory tract in response to IAV infection [164–167]. This increase correlates with an initial decrease in circulatory NK cells [166, 168] suggesting that during early IAV infection NK cells are recruited directly from blood [164] (Figure 3). NK cell recruitment to the site of infection depends on expression of chemokine receptors, namely, CCR2, CCR5, and CXCR3 [164, 167, 169] through interaction with ligands like CCL2, CCL5, CXCL9, CXCL10, and CXCL11 [164, 167, 169] (Figure 3).

Primary effector functions (cytolysis of infected cells and cytokine secretion [170]) of IAV-stimulated NK cells are facilitated through cytotoxic receptors, NKp46, NKp44, and NKp30, of which viral HA binds directly to sialic acids expressed on NKp46 [171–173] (Figure 3). Viral subtypes may dictate the strength of NK cell activation wherein H5N1 and 1918 H1N1 viruses induce stronger responses compared to the pH1N1 virus [174], suggesting that the difference in receptor binding preference of the infecting virus differentially regulates NK cell activation. Cytolysis of IAV-infected cells is achieved by degranulation and release of perforin and granzyme, as well as by lytic activity of IFNγ secreted by activated NK cells [174] (Figure 3).

Epithelial cytopathology is a principal outcome of IAV infection of the respiratory tract which NK cells can mitigate through IL-22 production [165]. Airway epithelial cells expressing IL-22R [175] respond to IL-22 through epithelial regeneration which reduces inflammation [165]. This
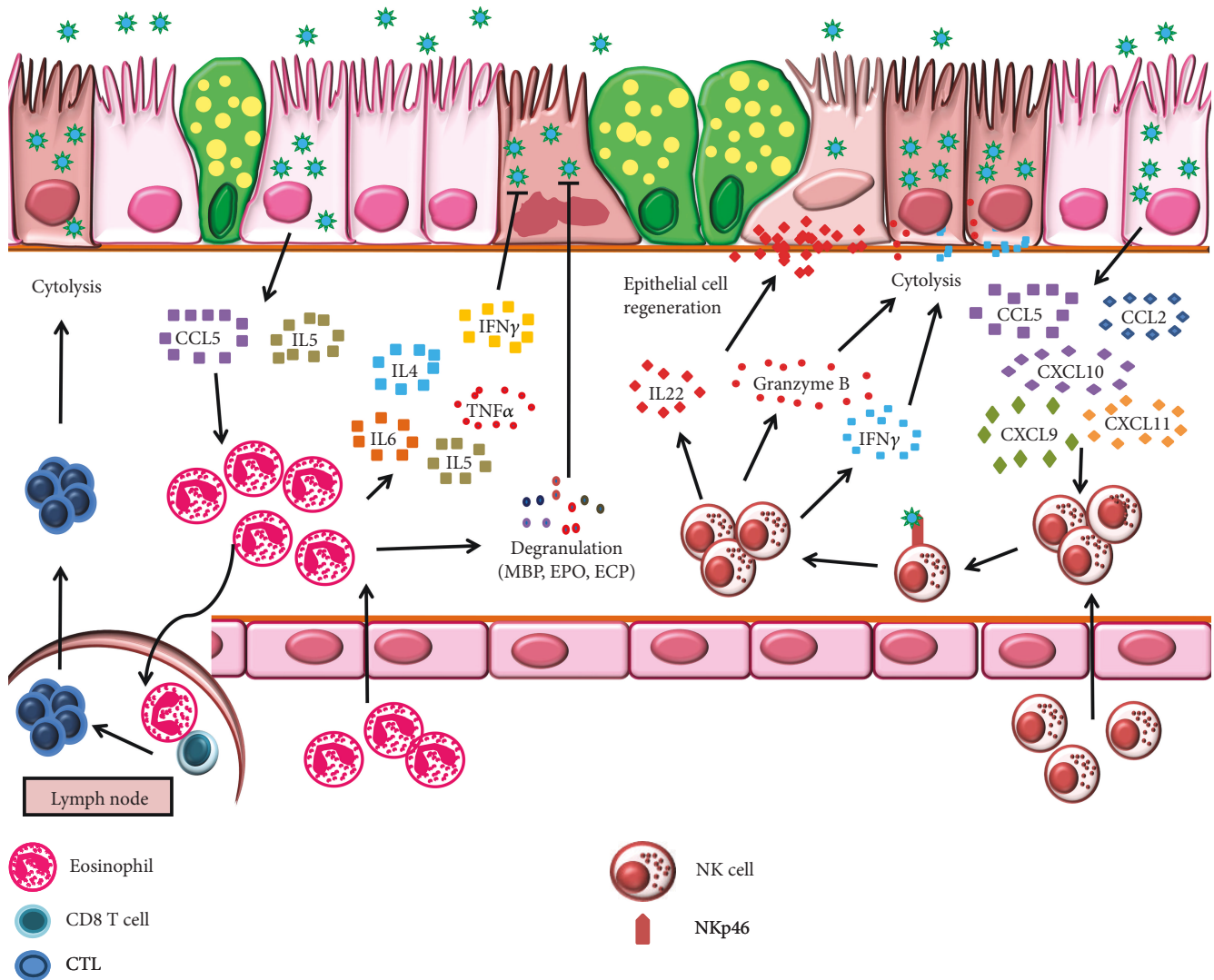
FIGURE 3: Immunoprotection and immunopathology mediated by eosinophils and natural killer (NK) cells during influenza. CCL5 and IL-5 released by IAV-infected airway epithelium mediate eosinophil trafficking into the lungs. Eosinophils undergo piecemeal degranulation and secrete inflammatory cytokines that exert anti-IAV activity. Additionally, IAV-infected eosinophils migrate to draining lymph nodes to present viral antigen to CD8$^+$ T cells. Once activated, cytotoxic T cells traffic to the lungs to lyse IAV-infected cells. NK cell recruitment is driven by CCL2, CCL5, CXCL9, CXCL10, and CXCL11 secreted by IAV-infected airway epithelium. Engagement of IAV with NK cell receptor NKp46 activates NK cell effector functions. Perforins, granzyme B, and IFNγ released by NK cell destroy IAV-infected cells while IL-22 aids in the regeneration of damaged epithelium.

demonstrated the pivotal role of the IL-22-IL-22R axis in maintaining epithelial integrity and tissue homeostasis upon IAV infection (Figure 3). In vitro studies suggest that NK cell apoptosis triggered during IAV replication within these cells [171] may be an immune evasion strategy empoyed by IAV. Since the full spectrum of interaction between NK cells and IAV is yet to be elucidated, additional studies that focus on NK cells are warranted.

Cells that coexpress the NK cell marker and the T cell receptor, termed NKT cells, have gained more attention from influenza virologists in recent years. These cells provide immunoprotection during IAV infection by reducing inflammation, primarily the accumulation of inflammatory

monocytes [176], reducing immunosuppressive activity of myeloid-derived suppressor cells [177], or through the production of IL-22 that controls lung epithelial damage [178].

8. Innate Lymphoid Cells: Immediate Depots of Cytokines

The ILC family consists of cytotoxic NK cells and three non-cytotoxic members, ILC1, ILC2, and ILC3, that are innate counterparts of T cells that do not express antigen receptors [179]. Various insults activate ILC subsets; ILC1 responds to viruses and intracellular bacteria, ILC2 to extracellular
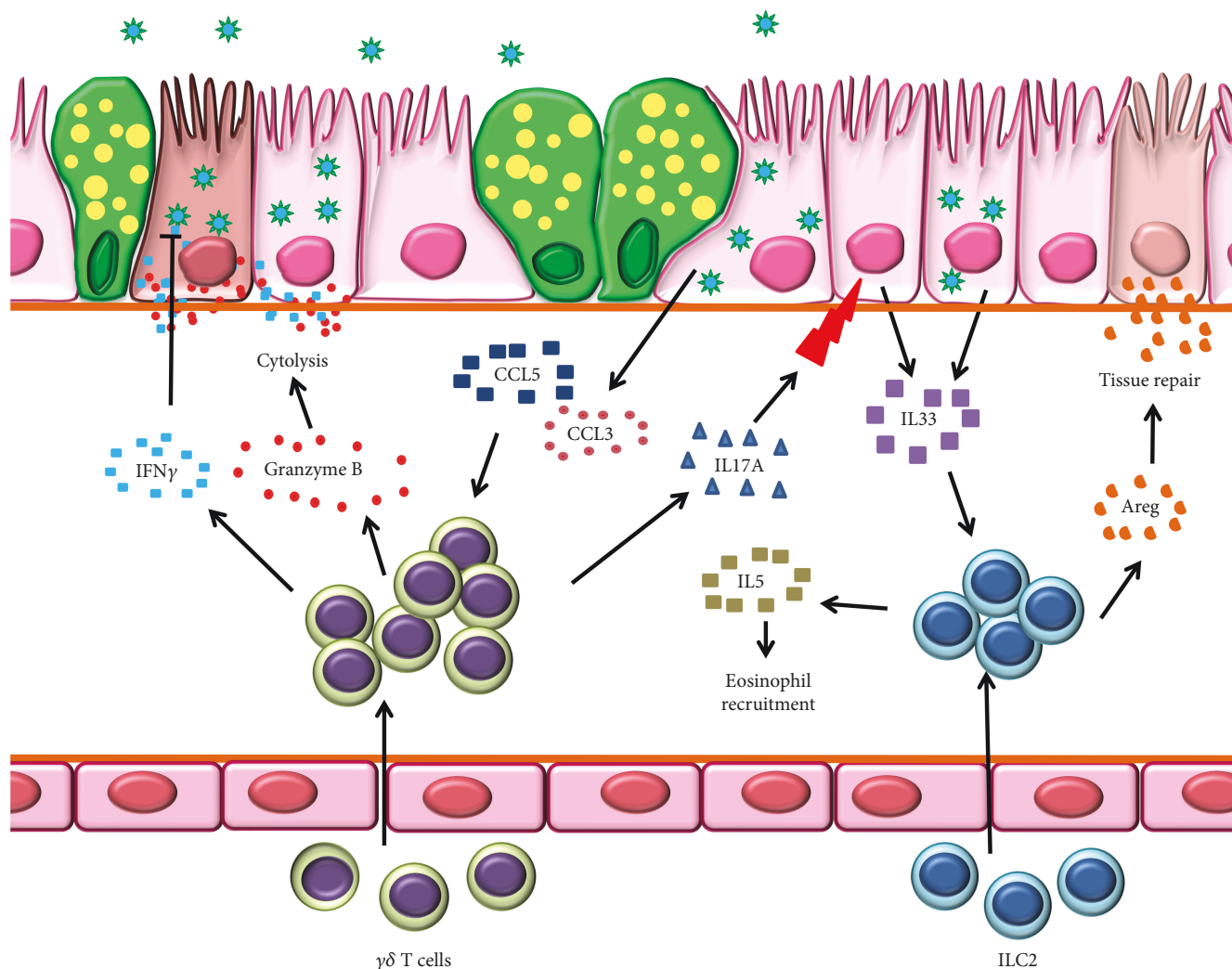
FIGURE 4: Innate lymphoid cells (ILCs) and $\gamma\delta$ T cells are important during early stages of influenza virus infection. Schematic representation of immunoprotection and immunopathology mediated by ILCs in the IAV-infected lungs. $\gamma\delta$ T cell migration into the infected lungs is driven along the CCR5-CCL5 axis. $\gamma\delta$ T cells destroy infected cells through the secretion of IFNγ and perforin-granzyme B pathway and secrete IL-17A to trigger airway epithelium release of IL-33, a chemoattractant for ILC2. In addition, IL-33 secreted by IAV-infected epithelium enhances the trafficking of ILC2 into the lungs. Activated ILC2s release amphiregulin (Areg) which helps to repair tissue damage and IL-5 which induces eosinophil trafficking.

parasites and allergens, and ILC3 to extracellular bacteria and fungi [179–182].

Although ILC2s are known to provide antihelminthic immunity, recent findings demonstrate their influx into lungs following IAV infection [183] most likely in response to alveolar epithelial- and macrophage-derived IL-33 [155, 184–187] sensed through surface-expressed IL-33R [188] (Figure 4). As potent producers of IL-5, ILC2s may regulate eosinophil infiltration during influenza [155, 188]. Within the lungs, ILC2s increase expression of genes encoding amphiregulin and extracellular matrix proteins asporin, decorin, and dermatopontin [188]. Strikingly, ILC2-derived amphiregulin is involved in tissue repair and remodeling [188] (Figure 4) suggesting that ILC2s are capable of lung tissue homeostasis during influenza. As major contributors of IFNγ, a crucial cytokine in the anti-influenza armamentar-

ium, ILC1s induce cytolysis of IAV-infected cells [179–182]. However, IFNγ can also enhance influenza disease severity possibly through suppression of ILC2 function [189].

9. $\gamma\delta$ T Cells: Holding Down the Fort

Innate-like T cells expressing γ and δ chains as receptors, $\gamma\delta$ T cells constitute around 1-5% of blood lymphocytes. Given that $\gamma\delta$ T cells respond antecedent to $\alpha\beta$ T cells during infection [190], they may serve a pivotal role in early-stage antiviral host defense during influenza. Intravenous adoptive transfer of $\gamma\delta$ T cells to IAV-infected mice inhibits viral replication and controls disease progression [191] suggesting that these cells can traffic to lungs (Figure 4). Moreover, IAV-activated $\gamma\delta$ T cells express high levels of chemokine receptors CXCR5, CCR1, and CCR5 [192] allowing their

migration towards CCL3 and CCL5 [193] abundant IAV-infected lungs [116] (Figure 4). As a means of self-promotion, IAV-activated $\gamma\delta$ T cells produce preferred chemokines including CCL5 [192] to chaperon circulatory $\gamma\delta$ T cell recruitment into the lungs. The production of CCL5 is dependent on virus subtypes (higher for avian viruses than human viruses [192]), indicating that infection with avian viruses promotes more robust $\gamma\delta$ T cell infiltration.

Similar to other innate lymphocytes, $\gamma\delta$ T cells exert anti-influenza activity either by direct killing [194, 195] or noncytolytic inhibition of virus replication through the secretion of IFNγ [192, 196] (Figure 4). The inhibition of human H1N1 virus replication is achieved by both noncytolytic and cytolytic mechanisms [192, 196]. In contrast, avian IAVs are resistant to the antiviral activity of IFNγ. The cytotoxicity of $\gamma\delta$ T cells involves cytolytic granules (perforin and granzymes), NKG2D, TRAIL, and Fas-FasL pathways [194, 195]. Furthermore, $\gamma\delta$ T cells effectively kill cells infected with various IAV subtypes [192, 195, 196] suggesting their role in the heterosubtypic immune response. It has been argued that IAV-infected macrophages and sentinel DCs alter the mevalonate pathway which in turn liberates isopentyl pyrophosphate (IPP), an antigen for $\gamma\delta$ T cells [196]. The production of IPP activates $\gamma\delta$ T cells, thereby conferring immune protection independent of incoming virus subtypes.

Subtypes of IAV differ in the degree of disease severity they elicit in the host. Apart from engaging the T cell receptor, IAV HA protein activates $\gamma\delta$ T cells [197, 198] through α-2,3 and α-2,6 sialic acid receptor engagement on the cell surface [198] suggesting that HA-mediated differential activation of $\gamma\delta$ T cells might be related to the degree of protection provided by these immune cells to IAVs with different sialic acid preferences.

The rapid secretion of IL-17A by $\gamma\delta$ T cells from neonatal mice promotes AECs to produce IL-33 (Figure 4), protecting neonates from severe influenza [199]. Since $\gamma\delta$ T cell-mediated IL-33 production serves as a cue for IL-33R expressing ILC recruitment [188], which in turn promotes tissue repair through amphiregulin secretion [199], the complex cell-cell interactions that occur even in the innate cell compartment of the immune system are necessary and important to anti-influenza host protection (Figure 4). These observations suggested that IL-17A and the timing of its release can modulate the balance between protection and pathogenicity in a host infected with IAV.

10. Conclusion

Despite annual vaccination strategies, IAV infections pose a continuous threat to human health. Development of better therapeutic options is required to tackle the growing burden of IAV infections. This requires a thorough understanding of virus pathogenesis and contribution of immediate responders during infection. Innate immune cells are critical to primary immunity against IAV infection at the respiratory barrier. However, the balance between innate immune cell-induced protection and pathology is governed by their abundance, the potency of secreted immune mediators, infecting viral strain/subtype, and immune status of the host (Figure 5).
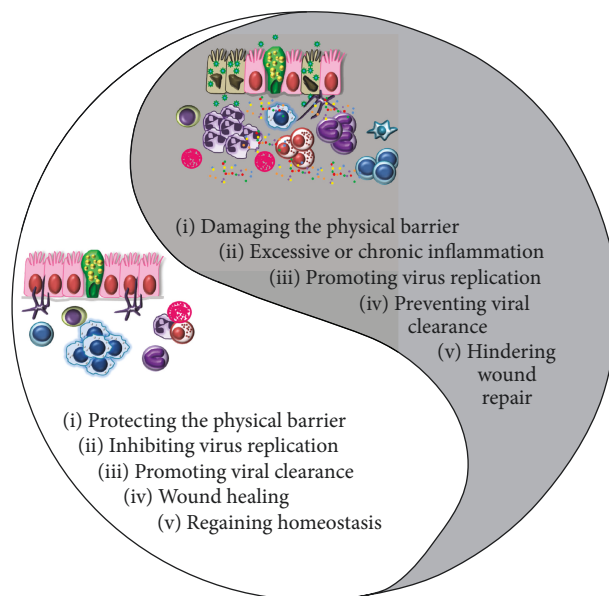

FIGURE 5: Yin and Yang of innate immune cell functions during influenza virus infection. At steady state, innate immune cells corporate to maintain pulmonary homeostasis while clearing IAV effectively with minimal tissue damage. When infected with a highly pathogenic virus or when immediate immune responses are unable to clear the infection, the immune system goes on overdrive causing excessive proinflammatory cytokine production and uncontrolled inflammation which can be pathogenic and detrimental to the host.

Hence, the identification of key regulators in innate leukocytes that mediate protection against IAV may provide broader options for therapeutic interventions.

Disclosure

Conflicts of Interest

The authors have no conflict of interest to declare.

Acknowledgments

References

[1] S. Ye and T. Wang, "Laboratory epidemiology of respiratory viruses in a large children's hospital," *Medicine*, vol. 97, no. 30, article e11385, 2018.

[2] E. R. Gaunt, H. Harvala, C. McIntyre, K. E. Templeton, and P. Simmonds, "Disease burden of the most commonly

detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model," *Journal of Clinical Virology*, vol. 52, no. 3, pp. 215–221, 2011.

[3] B. Visseaux, C. Burdet, G. Voiriot et al., "Prevalence of respiratory viruses among adults, by season, age, respiratory tract region and type of medical unit in Paris, France, from 2011 to 2016," *PLoS One*, vol. 12, no. 7, article e0180888, 2017.

[4] R. A. Karron and R. E. Black, "Determining the burden of respiratory syncytial virus disease: the known and the unknown," *The Lancet*, vol. 390, no. 10098, pp. 917-918, 2017.

[5] S. E. Jacobs, D. M. Lamson, K. St. George, and T. J. Walsh, "Human rhinoviruses," *Clinical Microbiology Reviews*, vol. 26, no. 1, pp. 135–162, 2013.

[6] D. M. Knipe, P. M. Howley, D. E. Griffin, Knipe, and Howley, "Fields virology," in *Orthomyxoviruses*, P. Wright, G. Neumann, and Y. Kawaoka, Eds., pp. 1186–1243, Lippincott Williams & Wilkins, Philadelphia, PA, USA, 6th edition, 2013.

[7] World Health Organization, *Influenza (Seasonal)*, WHO, Geneva, 2018.

[8] J. F.-W. Chan, K. K. W. To, H. Chen, and K. Y. Yuen, "Cross-species transmission and emergence of novel viruses from birds," *Current Opinion in Virology*, vol. 10, pp. 63–69, 2015.

[9] R. G. Webster and E. A. Govorkova, "Continuing challenges in influenza," *Annals of the New York Academy of Sciences*, vol. 1323, no. 1, pp. 115–139, 2014.

[10] S. Tong, X. Zhu, Y. Li et al., "New world bats harbor diverse influenza A viruses," *PLoS Pathogens*, vol. 9, no. 10, article e1003657, 2013.

[11] S. Caini, M. Kroneman, T. Wiegers, C. el Guerche-Séblain, and J. Paget, "Clinical characteristics and severity of influenza infections by virus type, subtype, and lineage: a systematic literature review," *Influenza and Other Respiratory Viruses*, vol. 12, no. 6, pp. 780–792, 2018.

[12] P. Wu, A. M. Presanis, H. S. Bond, E. H. Y. Lau, V. J. Fang, and B. J. Cowling, "A joint analysis of influenza-associated hospitalizations and mortality in Hong Kong, 1998-2013," *Scientific Reports*, vol. 7, no. 1, p. 929, 2017.

[13] A. Daley, R. Nallusamy, and D. Isaacs, "Comparison of influenza A and influenza B virus infection in hospitalized children," *Journal of Paediatrics and Child Health*, vol. 36, no. 4, pp. 332–335, 2000.

[14] N. J. Cox and K. Subbarao, "Global epidemiology of influenza: past and present," *Annual Review of Medicine*, vol. 51, no. 1, pp. 407–421, 2000.

[15] D. Kalthoff, A. Globig, and M. Beer, "(Highly pathogenic) avian influenza as a zoonotic agent," *Veterinary Microbiology*, vol. 140, no. 3-4, pp. 237–245, 2010.

[16] S. S. Y. Wong and K. Yuen, "Avian influenza virus infections in humans," *Chest*, vol. 129, no. 1, pp. 156–168, 2006.

[17] World Health Organization, *Influenza at the Human-Animal Interface: Monthly Risk Assessment and Summary*, WHO, Geneva, 2019.

[18] J. K. Taubenberger and D. M. Morens, "1918 influenza: the mother of all pandemics," *Emerging Infectious Diseases*, vol. 12, no. 1, pp. 15–22, 2006.

[19] L. Simonsen, P. Spreeuwenberg, R. Lustig et al., "Global mortality estimates for the 2009 influenza pandemic from the GLaMOR project: a modeling study," *PLoS Medicine*, vol. 10, no. 11, article e1001558, 2013.

[20] J. H. C. M. Kreijtz, R. A. M. Fouchier, and G. F. Rimmelzwaan, "Immune responses to influenza virus infection," *Virus Research*, vol. 162, no. 1-2, pp. 19–30, 2011.

[21] K. S. LeMessurier, Y. Lin, J. A. McCullers, and A. E. Samarasinghe, "Antimicrobial peptides alter early immune response to influenza A virus infection in C57BL/6 mice," *Antiviral Research*, vol. 133, pp. 208–217, 2016.

[22] W. Pan, Z. Dong, F. Li et al., "Visualizing influenza virus infection in living mice," *Nature Communications*, vol. 4, no. 1, article 2369, 2013.

[23] B. Manicassamy, S. Manicassamy, A. Belicha-Villanueva, G. Pisanelli, B. Pulendran, and A. Garcia-Sastre, "Analysis of in vivo dynamics of influenza virus infection in mice using a GFP reporter virus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 25, pp. 11531–11536, 2010.

[24] H. Tse, K. K. W. To, X. Wen et al., "Clinical and virological factors associated with viremia in pandemic influenza A/H1N1/2009 virus infection," *PLoS One*, vol. 6, no. 9, article e22534, 2011.

[25] J. K. Taubenberger and D. M. Morens, "The pathology of influenza virus infections," *Annual Review of Pathology*, vol. 3, no. 1, pp. 499–522, 2008.

[26] B. G. Hale, R. E. Randall, J. Ortin, and D. Jackson, "The multifunctional NS1 protein of influenza A viruses," *The Journal of General Virology*, vol. 89, no. 10, pp. 2359–2376, 2008.

[27] G. Kochs, A. Garcia-Sastre, and L. Martinez-Sobrido, "Multiple anti-interferon actions of the influenza A virus NS1 protein," *Journal of Virology*, vol. 81, no. 13, pp. 7011–7021, 2007.

[28] X. Chen, S. Liu, M. U. Goraya, M. Maarouf, S. Huang, and J. L. Chen, "Host immune response to influenza A virus infection," *Frontiers in Immunology*, vol. 9, p. 320, 2018.

[29] O. Haller and G. Kochs, "Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity," *Journal of Interferon & Cytokine Research*, vol. 31, no. 1, pp. 79–87, 2011.

[30] S. Matsukura, F. Kokubu, H. Kubo et al., "Expression of RANTES by normal airway epithelial cells after influenza virus A infection," *American Journal of Respiratory Cell and Molecular Biology*, vol. 18, no. 2, pp. 255–264, 1998.

[31] M. A. Hoeve, A. A. Nash, D. Jackson, R. E. Randall, and I. Dransfield, "Influenza virus A infection of human monocyte and macrophage subpopulations reveals increased susceptibility associated with cell differentiation," *PLoS One*, vol. 7, no. 1, article e29443, 2012.

[32] M. D. de Jong, C. P. Simmons, T. T. Thanh et al., "Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia," *Nature Medicine*, vol. 12, no. 10, pp. 1203–1207, 2006.

[33] J. S. M. Peiris, W. C. Yu, C. W. Leung et al., "Re-emergence of fatal human influenza A subtype H5N1 disease," *The Lancet*, vol. 363, no. 9409, pp. 617–619, 2004.

[34] K. F. To, P. K. S. Chan, K. F. Chan et al., "Pathology of fatal human infection associated with avian influenza A H5N1 virus," *Journal of Medical Virology*, vol. 63, no. 3, pp. 242–246, 2001.

[35] M. D. Tate, L. J. Ioannidis, B. Croker, L. E. Brown, A. G. Brooks, and P. C. Reading, "The role of neutrophils during mild and severe influenza virus infections of mice," *PLoS One*, vol. 6, no. 3, article e17618, 2011.

[36] M. D. Tate, A. G. Brooks, and P. C. Reading, "The role of neutrophils in the upper and lower respiratory tract during influenza virus infection of mice," *Respiratory Research*, vol. 9, no. 1, p. 57, 2008.

[37] L. M. Bradley, M. F. Douglass, D. Chatterjee, S. Akira, and B. J. G. Baaten, "Matrix metalloprotease 9 mediates neutrophil migration into the airways in response to influenza virus-induced toll-like receptor signaling," *PLoS Pathogens*, vol. 8, no. 4, article e1002641, 2012.

[38] F. G. Hayden, R. Fritz, M. C. Lobo, W. Alvord, W. Strober, and S. E. Straus, "Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense," *The Journal of Clinical Investigation*, vol. 101, no. 3, pp. 643–649, 1998.

[39] M. D. Wareing, A. L. Shea, C. A. Inglis, P. B. Dias, and S. R. Sarawar, "CXCR2 is required for neutrophil recruitment to the lung during influenza virus infection, but is not essential for viral clearance," *Viral Immunology*, vol. 20, no. 3, pp. 369–378, 2007.

[40] H. Fujisawa, "Neutrophils play an essential role in cooperation with antibody in both protection against and recovery from pulmonary infection with influenza virus in mice," *Journal of Virology*, vol. 82, no. 6, pp. 2772–2783, 2008.

[41] P. G. Barlow, P. Svoboda, A. Mackellar et al., "Antiviral activity and increased host defense against influenza infection elicited by the human cathelicidin LL-37," *PLoS One*, vol. 6, no. 10, article e25333, 2011.

[42] K. A. Daher, M. E. Selsted, and R. I. Lehrer, "Direct inactivation of viruses by human granulocyte defensins," *Journal of Virology*, vol. 60, no. 3, pp. 1068–1074, 1986.

[43] M. D. Tate, Y. M. Deng, J. E. Jones, G. P. Anderson, A. G. Brooks, and P. C. Reading, "Neutrophils ameliorate lung injury and the development of severe disease during influenza infection," *Journal of Immunology*, vol. 183, no. 11, pp. 7441–7450, 2009.

[44] L. A. Perrone, J. K. Plowden, A. García-Sastre, J. M. Katz, and T. M. Tumpey, "H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice," *PLoS Pathogens*, vol. 4, no. 8, article e1000115, 2008.

[45] N. Malachowa, B. Freedman, D. E. Sturdevant et al., "Differential ability of pandemic and seasonal H1N1 influenza A viruses to alter the function of human neutrophils," *mSphere*, vol. 3, no. 1, 2018.

[46] F. X. Ivan, K. S. Tan, M. C. Phoon et al., "Neutrophils infected with highly virulent influenza H3N2 virus exhibit augmented early cell death and rapid induction of type I interferon signaling pathways," *Genomics*, vol. 101, no. 2, pp. 101–112, 2013.

[47] Y. Zhao, M. Lu, L. T. Lau et al., "Neutrophils may be a vehicle for viral replication and dissemination in human H5N1 avian influenza," *Clinical Infectious Diseases*, vol. 47, no. 12, pp. 1575–1578, 2008.

[48] Z. Zhang, T. Huang, F. Yu et al., "Infectious progeny of 2009 A (H1N1) influenza virus replicated in and released from human neutrophils," *Scientific Reports*, vol. 5, no. 1, article 17809, 2015.

[49] L. F. Cassidy, D. S. Lyles, and J. S. Abramson, "Synthesis of viral proteins in polymorphonuclear leukocytes infected with influenza A virus," *Journal of Clinical Microbiology*, vol. 26, no. 7, pp. 1267–1270, 1988.

[50] B. G. Yipp and P. Kubes, "NETosis: how vital is it?," *Blood*, vol. 122, no. 16, pp. 2784–2794, 2013.

[51] C. N. Jenne, C. H. Y. Wong, F. J. Zemp et al., "Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps," *Cell Host & Microbe*, vol. 13, no. 2, pp. 169–180, 2013.

[52] T. Saitoh, J. Komano, Y. Saitoh et al., "Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1," *Cell Host & Microbe*, vol. 12, no. 1, pp. 109–116, 2012.

[53] S. Hemmers, J. R. Teijaro, S. Arandjelovic, and K. A. Mowen, "PAD4-mediated neutrophil extracellular trap formation is not required for immunity against influenza infection," *PLoS One*, vol. 6, no. 7, article e22043, 2011.

[54] S. Tripathi, A. Verma, E. J. Kim, M. R. White, and K. L. Hartshorn, "LL-37 modulates human neutrophil responses to influenza A virus," *Journal of Leukocyte Biology*, vol. 96, no. 5, pp. 931–938, 2014.

[55] M. Hoeksema, S. Tripathi, M. White et al., "Arginine-rich histones have strong antiviral activity for influenza A viruses," *Innate Immunity*, vol. 21, no. 7, pp. 736–745, 2015.

[56] L. Zhu, L. Liu, Y. Zhang et al., "High level of neutrophil extracellular traps correlates with poor prognosis of severe influenza A infection," *The Journal of Infectious Diseases*, vol. 217, no. 3, pp. 428–437, 2018.

[57] T. Narasaraju, E. Yang, R. P. Samy et al., "Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis," *The American Journal of Pathology*, vol. 179, no. 1, pp. 199–210, 2011.

[58] M. M. Hufford, G. Richardson, H. Zhou et al., "Influenza-infected neutrophils within the infected lungs act as antigen presenting cells for anti-viral CD8+ T cells," *PLoS One*, vol. 7, no. 10, article e46581, 2012.

[59] K. Lim, Y. M. Hyun, K. Lambert-Emo et al., "Neutrophil trails guide influenza-specific CD8+ T cells in the airways," *Science*, vol. 349, no. 6252, article aaa4352, 2015.

[60] T. M. Tumpey, A. Garcia-Sastre, J. K. Taubenberger et al., "Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice," *Journal of Virology*, vol. 79, no. 23, pp. 14933–14944, 2005.

[61] S. Yatmaz, H. J. Seow, R. C. Gualano et al., "Glutathione peroxidase-1 reduces influenza A virus-induced lung inflammation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 48, no. 1, pp. 17–26, 2013.

[62] R. Vlahos, J. Stambas, S. Bozinovski, B. R. S. Broughton, G. R. Drummond, and S. Selemidis, "Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation," *PLoS Pathogens*, vol. 7, no. 2, article e1001271, 2011.

[63] G. D. Thomas, A. A. J. Hamers, C. Nakao et al., "Human blood monocyte subsets: a new gating strategy defined using cell surface markers identified by mass cytometry," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 37, no. 8, pp. 1548–1558, 2017.

[64] L. Ziegler-Heitbrock, "Blood monocytes and their subsets: established features and open questions," *Frontiers in Immunology*, vol. 6, p. 423, 2015.

[65] L. Ziegler-Heitbrock, P. Ancuta, S. Crowe et al., "Nomenclature of monocytes and dendritic cells in blood," *Blood*, vol. 116, no. 16, pp. e74–e80, 2010.

[66] F. Baharom, S. Thomas, G. Rankin et al., "Dendritic cells and monocytes with distinct inflammatory responses reside in lung mucosa of healthy humans," *Journal of Immunology*, vol. 196, no. 11, pp. 4498–4509, 2016.

[67] G. Reynolds and M. Haniffa, "Human and mouse mononuclear phagocyte networks: a tale of two species?," *Frontiers in Immunology*, vol. 6, p. 330, 2015.

[68] J. Yang, L. Zhang, C. Yu, X. F. Yang, and H. Wang, "Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases," *Biomarker Research*, vol. 2, no. 1, p. 1, 2014.

[69] S. Herold, W. von Wulffen, M. Steinmueller et al., "Alveolar epithelial cells direct monocyte transepithelial migration upon influenza virus infection: impact of chemokines and adhesion molecules," *Journal of Immunology*, vol. 177, no. 3, pp. 1817–1824, 2006.

[70] D. Bußfeld, A. Kaufmann, R. G. Meyer, D. Gemsa, and H. Sprenger, "Differential mononuclear leukocyte attracting chemokine production after stimulation with active and inactivated influenza A virus," *Cellular Immunology*, vol. 186, no. 1, pp. 1–7, 1998.

[71] H. Sprenger, R. G. Meyer, A. Kaufmann, D. Bussfeld, E. Rischkowsky, and D. Gemsa, "Selective induction of monocyte and not neutrophil-attracting chemokines after influenza A virus infection," *Journal of Experimental Medicine*, vol. 184, no. 3, pp. 1191–1196, 1996.

[72] K. Zen and C. A. Parkos, "Leukocyte-epithelial interactions," *Current Opinion in Cell Biology*, vol. 15, no. 5, pp. 557–564, 2003.

[73] R. Kwiatkowski, J. H. Artrip, J. Ankersmit et al., "Importance of CD49d-VCAM interactions in human monocyte adhesion to porcine endothelium," *Xenotransplantation*, vol. 5, no. 1, pp. 67–74, 1998.

[74] X. C. Li, M. Miyasaka, and T. B. Issekutz, "Blood monocyte migration to acute lung inflammation involves both CD11/CD18 and very late activation antigen-4-dependent and independent pathways," *The Journal of Immunology*, vol. 161, no. 11, pp. 6258–6264, 1998.

[75] J. Meerschaert and M. B. Furie, "The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium," *The Journal of Immunology*, vol. 154, no. 8, pp. 4099–4112, 1995.

[76] S. Rosseau, J. Selhorst, K. Wiechmann et al., "Monocyte migration through the alveolar epithelial barrier: adhesion molecule mechanisms and impact of chemokines," *Journal of Immunology*, vol. 164, no. 1, pp. 427–435, 2000.

[77] E. J. Brown and W. A. Frazier, "Integrin-associated protein (CD47) and its ligands," *Trends in Cell Biology*, vol. 11, no. 3, pp. 130–135, 2001.

[78] A. Woodfin, M. B. Voisin, M. Beyrau et al., "The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo," *Nature Immunology*, vol. 12, no. 8, pp. 761–769, 2011.

[79] P. P. Lamichhane and P. Puthavathana, "PR8 virus harbouring H5N1 NS gene contributed for THP-1 cell tropism," *Virus*, vol. 29, no. 4, pp. 548–552, 2018.

[80] P. P. Lamichhane, K. Boonnak, D. Changsom et al., "H5N1 NS genomic segment distinctly governs the influenza virus infectivity and cytokine induction in monocytic cells," *Asian Pacific Journal of Allergy and Immunology*, vol. 36, no. 1, pp. 58–68, 2018.

[81] A. C. Y. Lee, K. K. W. To, H. Zhu et al., "Avian influenza virus A H7N9 infects multiple mononuclear cell types in peripheral blood and induces dysregulated cytokine responses and apoptosis in infected monocytes," *The Journal of General Virology*, vol. 98, no. 5, pp. 922–934, 2017.

[82] M. Vongsakul, J. Kasisith, P. Noisumdaeng, and P. Puthavathana, "The difference in IL-1β, MIP-1α, IL-8 and IL-18 production between the infection of PMA activated U937 cells with recombinant vaccinia viruses inserted 2004 H5N1 influenza HA genes and NS genes," *Asian Pacific Journal of Allergy and Immunology*, vol. 29, no. 4, pp. 349–356, 2011.

[83] S. Ludwig, C. Ehrhardt, E. R. Neumeier, M. Kracht, U. R. Rapp, and S. Pleschka, "Influenza virus-induced AP-1-dependent gene expression requires activation of the JNK signaling pathway," *The Journal of Biological Chemistry*, vol. 276, no. 14, pp. 10990–10998, 2001.

[84] M. Palipane, J. D. Snyder, K. S. LeMessurier, A. K. Schofield, S. N. Woolard, and A. E. Samarasinghe, "Macrophage CD14 impacts immune defenses against influenza virus in allergic hosts," *Microbial Pathogenesis*, vol. 127, pp. 212–219, 2019.

[85] T. D. Cline, D. Beck, and E. Bianchini, "Influenza virus replication in macrophages: balancing protection and pathogenesis," *The Journal of General Virology*, vol. 98, no. 10, pp. 2401–2412, 2017.

[86] M. Q. Nicol and B. M. Dutia, "The role of macrophages in influenza A virus infection," *Future Virology*, vol. 9, no. 9, pp. 847–862, 2014.

[87] S. J. Lin, M. Lo, R. L. Kuo et al., "The pathological effects of CCR2+ inflammatory monocytes are amplified by an IFNAR1-triggered chemokine feedback loop in highly pathogenic influenza infection," *Journal of Biomedical Science*, vol. 21, no. 1, p. 99, 2014.

[88] K. L. Lin, Y. Suzuki, H. Nakano, E. Ramsburg, and M. D. Gunn, "CCR2+ monocyte-derived dendritic cells and exudate macrophages produce influenza-induced pulmonary immune pathology and mortality," *The Journal of Immunology*, vol. 180, no. 4, pp. 2562–2572, 2008.

[89] T. C. Dawson, M. A. Beck, W. A. Kuziel, F. Henderson, and N. Maeda, "Contrasting effects of CCR5 and CCR2 deficiency in the pulmonary inflammatory response to influenza A virus," *The American Journal of Pathology*, vol. 156, no. 6, pp. 1951–1959, 2000.

[90] R. Zaynagetdinov, T. P. Sherrill, P. L. Kendall et al., "Identification of myeloid cell subsets in murine lungs using flow cytometry," *American Journal of Respiratory Cell and Molecular Biology*, vol. 49, no. 2, pp. 180–189, 2013.

[91] M. Liegeois, C. Legrand, C. J. Desmet, T. Marichal, and F. Bureau, "The interstitial macrophage: a long-neglected piece in the puzzle of lung immunity," *Cellular Immunology*, vol. 330, pp. 91–96, 2018.

[92] M. Fathi, A. Johansson, M. Lundborg, L. Orre, C. M. Sköld, and P. Camner, "Functional and morphological differences between human alveolar and interstitial macrophages," *Experimental and Molecular Pathology*, vol. 70, no. 2, pp. 77–82, 2001.

[93] M. Kopf, C. Schneider, and S. P. Nobs, "The development and function of lung-resident macrophages and dendritic cells," *Nature Immunology*, vol. 16, no. 1, pp. 36–44, 2015.

[94]   T. Hussell and T. J. Bell, "Alveolar macrophages: plasticity in a tissue-specific context," *Nature Reviews Immunology*, vol. 14, no. 2, pp. 81–93, 2014.

[95]   X. Yu, A. Buttgereit, I. Lelios et al., "The cytokine TGF-β promotes the development and homeostasis of alveolar macrophages," *Immunity*, vol. 47, no. 5, pp. 903–912.e4, 2017.

[96]   L. van de Laar, W. Saelens, S. de Prijck et al., "Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages," *Immunity*, vol. 44, no. 4, pp. 755–768, 2016.

[97]   M. Guilliams, I. de Kleer, S. Henri et al., "Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF," *Journal of Experimental Medicine*, vol. 210, no. 10, pp. 1977–1992, 2013.

[98]   E. Gomez Perdiguero, K. Klapproth, C. Schulz et al., "Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors," *Nature*, vol. 518, no. 7540, pp. 547–551, 2015.

[99]   J. D. Tarling, H. S. Lin, and S. Hsu, "Self-renewal of pulmonary alveolar macrophages: evidence from radiation chimera studies," *Journal of Leukocyte Biology*, vol. 42, no. 5, pp. 443–446, 1987.

[100] R. T. Sawyer, "The cytokinetic behavior of pulmonary alveolar macrophages in monocytopenic mice," *Journal of Leukocyte Biology*, vol. 39, no. 1, pp. 89–99, 1986.

[101] R. J. Snelgrove, J. Goulding, A. M. Didierlaurent et al., "A critical function for CD200 in lung immune homeostasis and the severity of influenza infection," *Nature Immunology*, vol. 9, no. 9, pp. 1074–1083, 2008.

[102] H. M. Kim, Y. W. Lee, K. J. Lee et al., "Alveolar macrophages are indispensable for controlling influenza viruses in lungs of pigs," *Journal of Virology*, vol. 82, no. 9, pp. 4265–4274, 2008.

[103] M. D. Tate, D. L. Pickett, N. van Rooijen, A. G. Brooks, and P. C. Reading, "Critical role of airway macrophages in modulating disease severity during influenza virus infection of mice," *Journal of Virology*, vol. 84, no. 15, pp. 7569–7580, 2010.

[104] A. Cardani, A. Boulton, T. S. Kim, and T. J. Braciale, "Alveolar macrophages prevent lethal influenza pneumonia by inhibiting infection of type-1 alveolar epithelial cells," *PLoS Pathogens*, vol. 13, no. 1, article e1006140, 2017.

[105] C. Schneider, S. P. Nobs, A. K. Heer et al., "Alveolar macrophages are essential for protection from respiratory failure and associated morbidity following influenza virus infection," *PLoS Pathogens*, vol. 10, no. 4, article e1004053, 2014.

[106] J. Wang, M. P. Nikrad, E. A. Travanty et al., "Innate immune response of human alveolar macrophages during influenza A infection," *PLoS One*, vol. 7, no. 3, article e29879, 2012.

[107] W. C. L. Yu, R. W. Y. Chan, J. Wang et al., "Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses," *Journal of Virology*, vol. 85, no. 14, pp. 6844–6855, 2011.

[108] D. B. Ettensohn, M. W. Frampton, J. E. Nichols, and N. J. Roberts Jr., "Human alveolar macrophages may not be susceptible to direct infection by a human influenza virus," *The Journal of Infectious Diseases*, vol. 214, no. 11, pp. 1658–1665, 2016.

[109] S. L. Londrigan, K. R. Short, J. Ma et al., "Infection of mouse macrophages by seasonal influenza viruses can be restricted at the level of virus entry and at a late stage in the virus life cycle," *Journal of Virology*, vol. 89, no. 24, pp. 12319–12329, 2015.

[110] G. Trinchieri, "Type I interferon: friend or foe?," *Journal of Experimental Medicine*, vol. 207, no. 10, pp. 2053–2063, 2010.

[111] H. E. Ward and T. E. Nicholas, "Alveolar type I and type II cells," *Australian and New Zealand Journal of Medicine*, vol. 14, 5, Supplement 3, pp. 731–734, 1984.

[112] C. Schneider, S. P. Nobs, M. Kurrer, H. Rehrauer, C. Thiele, and M. Kopf, "Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages," *Nature Immunology*, vol. 15, no. 11, pp. 1026–1037, 2014.

[113] A. Chawla, "Control of macrophage activation and function by PPARs," *Circulation Research*, vol. 106, no. 10, pp. 1559–1569, 2010.

[114] C. E. Moseley, R. G. Webster, and J. R. Aldridge, "Peroxisome proliferator-activated receptor and AMP-activated protein kinase agonists protect against lethal influenza virus challenge in mice," *Influenza and Other Respiratory Viruses*, vol. 4, no. 5, pp. 307–311, 2010.

[115] S. Huang, B. Zhu, I. S. Cheon et al., "PPAR-γ in macrophages limits pulmonary inflammation and promotes host recovery following respiratory viral infection," *Journal of Virology*, vol. 93, no. 9, 2019.

[116] J. Zhou, H. K. W. Law, C. Y. Cheung, I. H. Y. Ng, J. S. M. Peiris, and Y. L. Lau, "Differential expression of chemokines and their receptors in adult and neonatal macrophages infected with human or avian influenza viruses," *The Journal of Infectious Diseases*, vol. 194, no. 1, pp. 61–70, 2006.

[117] C. Y. Cheung, L. L. M. Poon, A. S. Lau et al., "Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease?," *The Lancet*, vol. 360, no. 9348, pp. 1831–1837, 2002.

[118] J. Friesenhagen, Y. Boergeling, E. Hrincius, S. Ludwig, J. Roth, and D. Viemann, "Highly pathogenic avian influenza viruses inhibit effective immune responses of human blood-derived macrophages," *Journal of Leukocyte Biology*, vol. 92, no. 1, pp. 11–20, 2012.

[119] J. Waithman and J. D. Mintern, "Dendritic cells and influenza A virus infection," *Virulence*, vol. 3, no. 7, pp. 603–608, 2012.

[120] W. R. Heath and F. R. Carbone, "Dendritic cell subsets in primary and secondary T cell responses at body surfaces," *Nature Immunology*, vol. 10, no. 12, pp. 1237–1244, 2009.

[121] K. Shortman and Y. J. Liu, "Mouse and human dendritic cell subtypes," *Nature Reviews Immunology*, vol. 2, no. 3, pp. 151–161, 2002.

[122] Y. J. Liu, H. Kanzler, V. Soumelis, and M. Gilliet, "Dendritic cell lineage, plasticity and cross-regulation," *Nature Immunology*, vol. 2, no. 7, pp. 585–589, 2001.

[123] S.-S. J. Sung, S. M. Fu, C. E. Rose Jr., F. Gaskin, S. T. Ju, and S. R. Beaty, "A major lung CD103 (αE)-β7 integrin-positive epithelial dendritic cell population expressing Langerin and tight junction proteins," *The Journal of Immunology*, vol. 176, no. 4, pp. 2161–2172, 2006.

[124] B. N. Lambrecht and H. Hammad, "Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology," *Annual Review of Immunology*, vol. 30, no. 1, pp. 243–270, 2012.

[125] S. R. Beaty, C. E. Rose Jr., and S. S. J. Sung, "Diverse and potent chemokine production by lung CD11b^high dendritic cells in homeostasis and in allergic lung inflammation," *Journal of Immunology*, vol. 178, no. 3, pp. 1882–1895, 2007.

[126] J. Helft, F. Ginhoux, M. Bogunovic, and M. Merad, "Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice," *Immunological Reviews*, vol. 234, no. 1, pp. 55–75, 2010.

[127] C. H. GeurtsvanKessel, M. A. M. Willart, L. S. van Rijt et al., "Clearance of influenza virus from the lung depends on migratory langerin^+CD11b^- but not plasmacytoid dendritic cells," *Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1621–1634, 2008.

[128] M. Lichtner, C. M. Mastroianni, R. Rossi et al., "Severe and persistent depletion of circulating plasmacytoid dendritic cells in patients with 2009 pandemic H1N1 infection," *PLoS One*, vol. 6, no. 5, article e19872, 2011.

[129] M. A. Gill, K. Long, T. Kwon et al., "Differential recruitment of dendritic cells and monocytes to respiratory mucosal sites in children with influenza virus or respiratory syncytial virus infection," *The Journal of Infectious Diseases*, vol. 198, no. 11, pp. 1667–1676, 2008.

[130] M. A. Gill, A. K. Palucka, T. Barton et al., "Mobilization of plasmacytoid and myeloid dendritic cells to mucosal sites in children with respiratory syncytial virus and other viral respiratory infections," *The Journal of Infectious Diseases*, vol. 191, no. 7, pp. 1105–1115, 2005.

[131] A. W. S. Ho, N. Prabhu, R. J. Betts et al., "Lung CD103^+ dendritic cells efficiently transport influenza virus to the lymph node and load viral antigen onto MHC class I for presentation to CD8 T cells," *The Journal of Immunology*, vol. 187, no. 11, pp. 6011–6021, 2011.

[132] J. Helft, B. Manicassamy, P. Guermonprez et al., "Cross-presenting CD103^+ dendritic cells are protected from influenza virus infection," *Journal of Clinical Investigation*, vol. 122, no. 11, pp. 4037–4047, 2012.

[133] M.-L. del Rio, J. I. Rodriguez-Barbosa, E. Kremmer, and R. Förster, "CD103^- and CD103^+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4^+ and CD8^+ T cells," *The Journal of Immunology*, vol. 178, no. 11, pp. 6861–6866, 2007.

[134] B. Moltedo, W. Li, J. S. Yount, and T. M. Moran, "Unique type I interferon responses determine the functional fate of migratory lung dendritic cells during influenza virus infection," *PLoS Pathogens*, vol. 7, no. 11, article e1002345, 2011.

[135] T. S. Kim and T. J. Braciale, "Respiratory dendritic cell subsets differ in their capacity to support the induction of virus-specific cytotoxic CD8^+ T cell responses," *PLoS One*, vol. 4, no. 1, article e4204, 2009.

[136] Z. Mikhak, J. P. Strassner, and A. D. Luster, "Lung dendritic cells imprint T cell lung homing and promote lung immunity through the chemokine receptor CCR4," *Journal of Experimental Medicine*, vol. 210, no. 9, pp. 1855–1869, 2013.

[137] A. Iparraguirre, J. W. Tobias, S. E. Hensley et al., "Two distinct activation states of plasmacytoid dendritic cells induced by influenza virus and CpG 1826 oligonucleotide," *Journal of Leukocyte Biology*, vol. 83, no. 3, pp. 610–620, 2008.

[138] A. I. Wolf, D. Buehler, S. E. Hensley et al., "Plasmacytoid dendritic cells are dispensable during primary influenza virus infection," *Journal of Immunology*, vol. 182, no. 2, pp. 871–879, 2009.

[139] A. Thitithanyanont, A. Engering, P. Ekchariyawat et al., "High susceptibility of human dendritic cells to avian influenza H5N1 virus infection and protection by IFN-α and TLR ligands," *Journal of Immunology*, vol. 179, no. 8, pp. 5220–5227, 2007.

[140] S. Vangeti, J. Gertow, M. Yu et al., "Human blood and tonsil plasmacytoid dendritic cells display similar gene expression profiles but exhibit differential type I IFN responses to influenza A virus infection," *Journal of Immunology*, vol. 202, no. 7, pp. 2069–2081, 2019.

[141] G. Lui, O. Manches, J. Angel, J. P. Molens, L. Chaperot, and J. Plumas, "Plasmacytoid dendritic cells capture and cross-present viral antigens from influenza-virus exposed cells," *PLoS One*, vol. 4, no. 9, article e7111, 2009.

[142] G. Jego, A. K. Palucka, J. P. Blanck, C. Chalouni, V. Pascual, and J. Banchereau, "Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6," *Immunity*, vol. 19, no. 2, pp. 225–234, 2003.

[143] L. A. Doorley, K. S. LeMessurier, A. R. Iverson, M. Palipane, and A. E. Samarasinghe, "Humoral immune responses during asthma and influenza co-morbidity in mice," *Immunobiology*, vol. 222, no. 12, pp. 1064–1073, 2017.

[144] M. R. Sandbulte, A. C. M. Boon, R. J. Webby, and J. M. Riberdy, "Analysis of cytokine secretion from human plasmacytoid dendritic cells infected with H5N1 or low-pathogenicity influenza viruses," *Virology*, vol. 381, no. 1, pp. 22–28, 2008.

[145] J.-F. Fonteneau, M. Gilliet, M. Larsson et al., "Activation of influenza virus-specific CD4^+ and CD8^+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity," *Blood*, vol. 101, no. 9, pp. 3520–3526, 2003.

[146] S. Davidson, S. Crotta, T. M. McCabe, and A. Wack, "Pathogenic potential of interferon αβ in acute influenza infection," *Nature Communications*, vol. 5, no. 1, 2014.

[147] K. Boonnak, L. Vogel, F. Feldmann, H. Feldmann, K. L. Legge, and K. Subbarao, "Lymphopenia associated with highly virulent H5N1 virus infection due to plasmacytoid dendritic cell-mediated apoptosis of T cells," *Journal of Immunology*, vol. 192, no. 12, pp. 5906–5912, 2014.

[148] R. A. Langlois and K. L. Legge, "Plasmacytoid dendritic cells enhance mortality during lethal influenza infections by eliminating virus-specific CD8 T cells," *Journal of Immunology*, vol. 184, no. 8, pp. 4440–4446, 2010.

[149] P. Waring and A. Mullbacher, "Cell death induced by the Fas/Fas ligand pathway and its role in pathology," *Immunology & Cell Biology*, vol. 77, no. 4, pp. 312–317, 1999.

[150] A. C. Soloff, H. K. Weirback, T. M. Ross, and S. M. Barratt-Boyes, "Plasmacytoid dendritic cell depletion leads to an enhanced mononuclear phagocyte response in lungs of mice with lethal influenza virus infection," *Comparative Immunology, Microbiology and Infectious Diseases*, vol. 35, no. 4, pp. 309–317, 2012.

[151] A. M. Bramley, S. Dasgupta, J. Skarbinski et al., "Intensive care unit patients with 2009 pandemic influenza A (H1N1pdm09) virus infection - United States, 2009," *Influenza and Other Respiratory Viruses*, vol. 6, no. 6, pp. e134–e142, 2012.

[152] J. K. Louie, M. Acosta, K. Winter et al., "Factors associated with death or hospitalization due to pandemic 2009 influenza A (H1N1) infection in California," *JAMA*, vol. 302, no. 17, pp. 1896–1902, 2009.

[153] L. Vaillant, G. la Ruche, A. Tarantola, P. Barboza, and epidemic intelligence team, "Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009," *Euro Surveillance*, vol. 14, no. 33, 2009.

[154] M. Terai, T. Honda, S. Yamamoto et al., "Early induction of interleukin-5 and peripheral eosinophilia in acute pneumonia in Japanese children infected by pandemic 2009 influenza A in the Tokyo area," *Microbiology and Immunology*, vol. 55, no. 5, pp. 341–346, 2011.

[155] S. A. Gorski, Y. S. Hahn, and T. J. Braciale, "Group 2 innate lymphoid cell production of IL-5 is regulated by NKT cells during influenza virus infection," *PLoS Pathogens*, vol. 9, no. 9, article e1003615, 2013.

[156] A. DiPiazza, A. Nogales, N. Poulton, P. C. Wilson, L. Martínez-Sobrido, and A. J. Sant, "Pandemic 2009 H1N1 influenza Venus reporter virus reveals broad diversity of MHC class II-positive antigen-bearing cells following infection in vivo," *Scientific Reports*, vol. 7, no. 1, article 10857, 2017.

[157] A. E. Samarasinghe, S. N. Woolard, K. L. Boyd, S. A. Hoselton, J. M. Schuh, and J. A. McCullers, "The immune profile associated with acute allergic asthma accelerates clearance of influenza virus," *Immunology and Cell Biology*, vol. 92, no. 5, pp. 449–459, 2014.

[158] R. C. Furze, T. Hussell, and M. E. Selkirk, "Amelioration of influenza-induced pathology in mice by coinfection with Trichinella spiralis," *Infection and Immunity*, vol. 74, no. 3, pp. 1924–1932, 2006.

[159] J. J. Lee, E. A. Jacobsen, M. P. McGarry, R. P. Schleimer, and N. A. Lee, "Eosinophils in health and disease: the LIAR hypothesis," *Clinical and Experimental Allergy*, vol. 40, no. 4, pp. 563–575, 2010.

[160] A. E. Samarasinghe, R. C. N. Melo, S. Duan et al., "Eosinophils promote antiviral immunity in mice infected with influenza A virus," *The Journal of Immunology*, vol. 198, no. 8, pp. 3214–3226, 2017.

[161] L. A. Spencer, C. T. Szela, S. A. C. Perez et al., "Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially," *Journal of Leukocyte Biology*, vol. 85, no. 1, pp. 117–123, 2009.

[162] J. A. Cañas, B. Sastre, C. Mazzeo et al., "Exosomes from eosinophils autoregulate and promote eosinophil functions," *Journal of Leukocyte Biology*, vol. 101, no. 5, pp. 1191–1199, 2017.

[163] K. S. LeMessurier and A. E. Samarasinghe, "Eosinophils: nemeses of pulmonary pathogens?," *Current Allergy and Asthma Reports*, vol. 19, no. 8, p. 36, 2019.

[164] M. J. G. van Helden, D. M. W. Zaiss, and A. J. A. M. Sijts, "CCR2 defines a distinct population of NK cells and mediates their migration during influenza virus infection in mice," *PLoS One*, vol. 7, no. 12, article e52027, 2012.

[165] P. Kumar, M. S. Thakar, W. Ouyang, and S. Malarkannan, "IL-22 from conventional NK cells is epithelial regenerative and inflammation protective during influenza infection," *Mucosal Immunology*, vol. 6, no. 1, pp. 69–82, 2013.

[166] R. Gazit, R. Gruda, M. Elboim et al., "Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1," *Nature Immunology*, vol. 7, no. 5, pp. 517–523, 2006.

[167] L. E. Carlin, E. A. Hemann, Z. R. Zacharias, J. W. Heusel, and K. L. Legge, "Natural killer cell recruitment to the lung during influenza A virus infection is dependent on CXCR3, CCR5, and virus exposure dose," *Frontiers in Immunology*, vol. 9, p. 781, 2018.

[168] H. Forberg, A. G. Hauge, M. Valheim et al., "Early responses of natural killer cells in pigs experimentally infected with 2009 pandemic H1N1 influenza A virus," *PLoS One*, vol. 9, no. 6, article e100619, 2014.

[169] C. Grégoire, L. Chasson, C. Luci et al., "The trafficking of natural killer cells," *Immunological Reviews*, vol. 220, no. 1, pp. 169–182, 2007.

[170] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[171] H. Mao, W. Tu, G. Qin et al., "Influenza virus directly infects human natural killer cells and induces cell apoptosis," *Journal of Virology*, vol. 83, no. 18, pp. 9215–9222, 2009.

[172] T. I. Arnon, H. Achdout, N. Lieberman et al., "The mechanisms controlling the recognition of tumor- and virus-infected cells by NKp46," *Blood*, vol. 103, no. 2, pp. 664–672, 2004.

[173] O. Mandelboim, N. Lieberman, M. Lev et al., "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells," *Nature*, vol. 409, no. 6823, pp. 1055–1060, 2001.

[174] N. Du, J. Zhou, X. Lin et al., "Differential activation of NK cells by influenza A pseudotype H5N1 and 1918 and 2009 pandemic H1N1 viruses," *Journal of Virology*, vol. 84, no. 15, pp. 7822–7831, 2010.

[175] Y. Zheng, P. A. Valdez, D. M. Danilenko et al., "Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens," *Nature Medicine*, vol. 14, no. 3, pp. 282–289, 2008.

[176] W. L. Kok, L. Denney, K. Benam et al., "Pivotal advance: invariant NKT cells reduce accumulation of inflammatory monocytes in the lungs and decrease immune-pathology during severe influenza A virus infection," *Journal of Leukocyte Biology*, vol. 91, no. 3, pp. 357–368, 2012.

[177] C. De Santo, M. Salio, S. H. Masri et al., "Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans," *The Journal of Clinical Investigation*, vol. 118, no. 12, pp. 4036–4048, 2008.

[178] C. Paget, S. Ivanov, J. Fontaine et al., "Interleukin-22 is produced by invariant natural killer T lymphocytes during influenza A virus infection: potential role in protection against lung epithelial damages," *Journal of Biological Chemistry*, vol. 287, no. 12, pp. 8816–8829, 2012.

[179] E. Vivier, D. Artis, M. Colonna et al., "Innate lymphoid cells: 10 years on," *Cell*, vol. 174, no. 5, pp. 1054–1066, 2018.

[180] D. Artis and H. Spits, "The biology of innate lymphoid cells," *Nature*, vol. 517, no. 7534, pp. 293–301, 2015.

[181] G. Eberl, M. Colonna, J. P. di Santo, and A. N. J. McKenzie, "Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology," *Science*, vol. 348, no. 6237, article aaa6566, 2015.

[182] H. Spits, D. Artis, M. Colonna et al., "Innate lymphoid cells — a proposal for uniform nomenclature," *Nature Reviews Immunology*, vol. 13, no. 2, pp. 145–149, 2013.

[183] B. W. S. Li, M. J. W. de Bruijn, M. Lukkes et al., "T cells and ILC2s are major effector cells in influenza-induced

exacerbation of allergic airway inflammation in mice," *European Journal of Immunology*, vol. 49, no. 1, pp. 144–156, 2019.

[184] K. M. Robinson, K. Ramanan, M. E. Clay, K. J. McHugh, H. E. Rich, and J. F. Alcorn, "Novel protective mechanism for interleukin-33 at the mucosal barrier during influenza-associated bacterial superinfection," *Mucosal Immunology*, vol. 11, no. 1, pp. 199–208, 2018.

[185] A. S. Mirchandani, R. J. Salmond, and F. Y. Liew, "Interleukin-33 and the function of innate lymphoid cells," *Trends in Immunology*, vol. 33, no. 8, pp. 389–396, 2012.

[186] R. Le Goffic, M. I. Arshad, M. Rauch et al., "Infection with influenza virus induces IL-33 in murine lungs," *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 6, pp. 1125–1132, 2011.

[187] Y. J. Chang, H. Y. Kim, L. A. Albacker et al., "Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity," *Nature Immunology*, vol. 12, no. 7, pp. 631–638, 2011.

[188] L. A. Monticelli, G. F. Sonnenberg, M. C. Abt et al., "Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus," *Nature Immunology*, vol. 12, no. 11, pp. 1045–1054, 2011.

[189] D. Califano, Y. Furuya, S. Roberts, D. Avram, A. N. J. McKenzie, and D. W. Metzger, "IFN-γ increases susceptibility to influenza A infection through suppression of group II innate lymphoid cells," *Mucosal Immunology*, vol. 11, no. 1, pp. 209–219, 2018.

[190] Y. H. Chien, C. Meyer, and M. Bonneville, "$\gamma\delta$ T cells: first line of defense and beyond," *Annual Review of Immunology*, vol. 32, no. 1, pp. 121–155, 2014.

[191] W. Tu, J. Zheng, Y. Liu et al., "The aminobisphosphonate pamidronate controls influenza pathogenesis by expanding a $\gamma\delta$ T cell population in humanized mice," *Journal of Experimental Medicine*, vol. 208, no. 7, pp. 1511–1522, 2011.

[192] G. Qin, Y. Liu, J. Zheng et al., "Type 1 responses of human Vγ9Vδ2 T cells to influenza A viruses," *Journal of Virology*, vol. 85, no. 19, pp. 10109–10116, 2011.

[193] A. Glatzel, D. Wesch, F. Schiemann, E. Brandt, O. Janssen, and D. Kabelitz, "Patterns of chemokine receptor expression on peripheral blood $\gamma\delta$ T lymphocytes: strong expression of CCR5 is a selective feature of Vδ2/Vγ9 $\gamma\delta$ T cells," *The Journal of Immunology*, vol. 168, no. 10, pp. 4920–4929, 2002.

[194] H. Li, Z. Xiang, T. Feng et al., "Human Vγ9Vδ2-T cells efficiently kill influenza virus-infected lung alveolar epithelial cells," *Cellular & Molecular Immunology*, vol. 10, no. 2, pp. 159–164, 2013.

[195] G. Qin, H. Mao, J. Zheng et al., "Phosphoantigen-expanded human $\gamma\delta$ T cells display potent cytotoxicity against monocyte-derived macrophages infected with human and avian influenza viruses," *The Journal of Infectious Diseases*, vol. 200, no. 6, pp. 858–865, 2009.

[196] J. M. Jameson, J. Cruz, A. Costanzo, M. Terajima, and F. A. Ennis, "A role for the mevalonate pathway in the induction of subtype cross-reactive immunity to influenza A virus by human $\gamma\delta$ T lymphocytes," *Cellular Immunology*, vol. 264, no. 1, pp. 71–77, 2010.

[197] P. Dong, X. Ju, Y. Yan et al., "$\gamma\delta$ T cells provide protective function in highly pathogenic avian H5N1 influenza A virus infection," *Frontiers in Immunology*, vol. 9, p. 2812, 2018.

[198] Y. Lu, Z. Li, C. Ma et al., "The interaction of influenza H5N1 viral hemagglutinin with sialic acid receptors leads to the activation of human $\gamma\delta$ T cells," *Cellular & Molecular Immunology*, vol. 10, no. 6, pp. 463–470, 2013.

[199] X. J. Guo, P. Dash, J. C. Crawford et al., "Lung $\gamma\delta$ T cells mediate protective responses during neonatal influenza infection that are associated with type 2 immunity," *Immunity*, vol. 49, no. 3, pp. 531–544.e6, 2018.