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Paramyxovirus Activation and Inhibition of Innate Immune Responses

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Abstract

Paramyxoviruses represent a remarkably diverse family of enveloped nonsegmented negative-strand RNA viruses, some of which are the most ubiquitous disease-causing viruses of humans and animals. This review focuses on paramyxovirus activation of innate immune pathways, the mechanisms by which these RNA viruses counteract these pathways, and the innate response to paramyxovirus infection of dendritic cells (DC). Paramyxoviruses are potent activators of extracellular complement pathways, a first line of defense that viruses must face during natural infections. We discuss mechanisms by which these viruses activate and combat complement to delay neutralization. Once cells are infected, virus replication drives type I interferon (IFN) synthesis that has the potential to induce a large number of antiviral genes. Here we describe four approaches by which paramyxoviruses limit IFN induction: by limiting synthesis of IFN-inducing aberrant viral RNAs, through targeted inhibition of RNA sensors, by providing viral decoy substrates for cellular kinase complexes, and through direct blocking of the IFN promoter. In addition, paramyxoviruses have evolved diverse mechanisms to disrupt IFN signaling pathways. We describe three general mechanisms, including targeted proteolysis of signaling factors, sequestering cellular factors, and upregulation of cellular inhibitors. DC are exceptional cells with the capacity to generate adaptive immunity through the coupling of innate immune signals and T cell activation. We discuss the importance of innate responses in DC following paramyxovirus infection and their consequences for the ability to mount and maintain antiviral T cells.

Keywords

paramyxovirus; innate immunity; dendritic cells; interferon

Paramyxoviruses represent a remarkably diverse family of enveloped nonsegmented negative-strand RNA viruses, some of which are the most ubiquitous disease-causing viruses of humans and animals. The role of innate immunity in paramyxovirus infections has been extensively studied and has provided insight into both molecular biology and cell biology of these virus: host cell interactions and new avenues for therapeutics to combat infections. This review focuses on paramyxovirus activation of innate immune pathways, the mechanisms by which these RNA viruses counteract these pathways, and the unique capacity of dendritic cells (DC) to translate innate responses to paramyxoviruses into adaptive immunity. These focus areas are presented as part of a general overview of paramyxovirus interactions with innate immune pathways and are intended to stimulate interested readers to pursue more in-depth and detailed analyses. Prior reviews are available

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on the general structure and replication of paramyxoviruses [1] or on details of the individual family members such as Mumps virus [2] (MuV), Measles virus [3] (MeV), Parainfluenza virus 5 [4] (PIV5), Respiratory syncytial virus [5] (RSV), Metapneumovirus [6] (MPV), Sendai virus [7] (SeV), and the bioterrorism threat Nipah virus [8,9] (NiV).

This review on paramyxovirus innate immunity is organized around events that occur during a typical respiratory tract infection. After initial entry into the respiratory tract, extracellular innate immune barriers to infection can inactivate viral particles and signal for recruitment of immune cells. Here we discuss interactions of paramyxoviruses with complement (C′), a powerful system of extracellular immunity. Respiratory tract infections are typically initiated in epithelial cells, which leads to the production of immune mediators such as type I interferon (IFN-I) and proinflammatory cytokines such as tumor necrosis factor α (TNF-α).We discuss the pathways and key factors involved in IFN-I induction and signaling during paramyxovirus replication, as well as the viral factors that limit this important innate response. In addition to infection of epithelial cells, paramyxoviruses can interact with professional antigen presenting cells such as DC. We discuss the innate response of DC to paramyxovirus infection and factors that dictate the success or failure of infected DC to initiate an adaptive immune response. Many paramyxoviruses are capable of establishing persistent infections [1,3,4,7], a property that highlights the ability of these viruses to successfully limit clearance through inhibition of innate and adaptive immune responses. Throughout this review, we present major concepts in paramyxovirus interactions with and inhibition of innate responses as illustrated by specific viruses and highlight areas for future research focus.

Overview of Paramyxovirus Genome Structure and Replication

While paramyxoviruses are a highly diverse family of viruses, they all share common features of genome structure and their replication cycle. Figure 1 shows the genome structure of four main categories of paramyxoviruses, organized in this case by their strategy for coding for antagonists of antiviral responses. Paramyxovirus genomes are negative sense RNA with a 3′ proximal N gene encoding the nucleocapsid protein N that coats the viral genomic RNA, a P gene encoding the phosphoprotein subunit of the RNA-dependent RNA polymerase, and the M gene encoding the matrix protein involved in virus assembly [1]. Likewise, the genome always encodes a 5′ proximal gene encoding the large protein catalytic subunit of the viral polymerase [1]. As these viruses are enveloped, all paramyxoviruses encode a fusion protein F that, in the context of the virion particle, initiates infection through pH-independent fusion of the virion lipid bilayer with the host cell plasma membrane. A major site of diversity among paramyxovirus coding strategies is found in genes encoding the viral attachment proteins, which can be a hemagglutinin–neuraminidase (HN, e.g., MuV [2]), a hemagglutinin (H, e.g., MeV [3]), or G protein (e.g., NiV [8] and RSV [5]). Additionally, some paramyxoviruses encode a small hydrophobic protein SH whose function is not entirely clear.

After attachment and penetration, the paramyxovirus replication cycle includes three phases of RNA synthesis by the viral L–P RNA polymerase complex: (1) primary transcription of the N-encapsidated negative sense input RNA genome to produce low levels of viral mRNA, (2) replication of the genome into a complementary N-encapsidated antigenome followed by production of progeny genomes, and (3) secondary transcription of progeny genomes to produce a large burst of viral mRNA. Many products of these RNA synthesis events are strong inducers of innate immune responses, including improperly capped mRNAs and unencapsidated genomic RNA that can provide dsRNA (*d*ouble-stranded *RNA*) or pppRNA ends. Progeny nucleocapsids containing the viral genome are then assembled into viral particles through budding at the plasma membrane and capture of viral

glycoproteins. These virion-associated membrane glycoproteins can be potent inducers of extracellular innate pathways such as C′, but as discussed below, the envelope can also include membrane proteins that inhibit extracellular innate responses.

The four main paramyxovirus categories shown in Fig. 1 differ in terms of their coding strategy for antagonists of host cell innate responses. For example, Respiroviruses, Morbilliviruses, and Henipaviruses (e.g., SeV, MeV, and NiV) encode V protein and C proteins within the P/V/C gene. The Rubulaviruses (e.g., MuV and PIV5) encode V protein but no C proteins and have the addition of the SH protein gene. The Pneumovirus RSV encodes not only an SH protein but also two 3′ proximal genes NS1 and NS2, both of which counteract innate immunity. Finally, MPV does not encode V or C or NS proteins but, instead, appears to rely on the G and SH viral glycoproteins to limit host cell responses [6].

Expression of Paramyxovirus Antagonists of Innate Immunity

P/V/C gene products

With the exception of RSV and MPV, paramyxoviruses in general encode a P gene that always produces more than one polypeptide species (Fig. 1). Expression of P/V/C proteins can involve two main mechanisms, with members of a paramyxovirus genus having a characteristic combination of these expression strategies [1]. The first expression mechanism that produces the P, V, and W proteins has been termed “RNA editing” or pseudo-templated addition of nucleotides. This mechanism involves the production of mRNAs whose open reading frames (ORFs) are altered by insertion of G residues at a specific position in the mRNA. As described below, the second expression mechanism involves ribosome initiation at alternative translation codons and produces the family of C proteins. Importantly, one of the common themes that have emerged from the analysis of paramyxovirus antagonists of innate immunity is that many of these viral proteins counteract innate immune responses in two ways: by inhibiting viral RNA synthesis to reduce the level of activating products and by direct inhibition of host cell pathways.

P/V/W proteins—The P, V, and W proteins are produced as a co-N-terminal nested set of proteins (Fig. 2) from distinct mRNAs that differ only by added G nucleotides that shift the translational reading frame at a single site of insertion. During transcription, the viral RNA polymerase is directed either to make an accurate copy of the P gene template or to insert one or two G residues at a precise site in the nascent mRNA. As shown for SeV in Fig. 2, the result is that the accurate transcription product encodes the full-length P ORF, while the mRNAs with insertions of +1G and +2G have a shift in the translational ORF such that the 5′ end ORF is fused at the site of insertion in the mRNA coding sequence to a more 3′ ORF encoding V (+1G) or W (+2G).

The P protein is a heavily phosphorylated essential subunit of the viral RNA-dependent RNA polymerase that serves as a bridge to link the L catalytic subunit of the polymerase to the N-bound template [1]. For PIV5, the P protein also plays an indirect role in limiting antiviral responses [10,11]: available data suggest that P protein functions at least in part to improve the fidelity of viral RNA synthesis and limit production of dsRNA [10], a potent activator of innate responses. Consistent with this, PIV5 mutants that are defective in phosphorylation at key sites in P protein show disregulated gene expression and are strong inducers of host cell responses [12].

The V protein is an ~25- to 30-kDa polypeptide that shares an N-terminal domain with the P protein but has a distinct C-terminal V-specific region with a highly conserved histidine and cysteine motif that binds two zinc molecules [13]. For some viruses (e.g., PIV5), V protein can be found in relatively high abundance in purified virions [13]. In these cases, this virion-

associated V protein can play a role in rapid dismantling of the host cell antiviral response since it has been shown that UV-treated virus can still induce blockage of interferon signaling [14].

V protein plays a number of important roles in the virus replication cycle, as evidenced by recombinant viruses that have been engineered to disrupt expression of the V protein Cys-rich domain. These viruses often show increased levels of viral RNA synthesis and activate host cell responses. This has led to the proposal that V protein serves as a negative regulator of viral RNA synthesis, through binding of N protein to modulate encapsidation of viral RNA [1,7]. Remarkably, V protein has an independent function as an important regulator of innate immune responses, including induction of inflammatory cytokines and IFN-I signaling [3,4,7,8].

Some paramyxoviruses such as Respiroviruses, Morbilliviruses, and Henipaviruses express abundant amounts of the W protein, a third product of RNA editing (Fig. 2) in which the N-terminal shared P/V/W ORF is closed by a stop codon shortly after the editing site. As with the V protein, the W protein is thought to function as an inhibitor of viral RNA synthesis but, in some cases (e.g., NiV), can also be a strong antagonist of IFN-I pathways.

Nested set of C proteins—In addition to RNA editing, some (but not all) paramyxoviruses use alternative translation initiation to express the C proteins from the P mRNA. As shown in Fig. 2, the SeV C′, C, Y1, and Y2 proteins are expressed by independent initiation of translation at alternative start codons, but translation is terminated at the same downstream stop codon, and thus, these proteins share a common C-terminus. The C′ and C proteins are translated by a leaky scanning mechanism, whereas translation of the Y1 and Y2 proteins involves a ribosome shunting mechanism on the RNA segment [15]. Different paramyxoviruses express a characteristic profile of C proteins ranging from none (e.g., PIV5) to one C protein (e.g., MeV and NiV), to expression of all four C, C′, Y1, and Y2 polypeptides (e.g., SeV).

C proteins are small abundantly expressed basic polypeptides that are involved in the control of viral RNA synthesis and counteracting host cell antiviral pathways. The C proteins have been shown to inhibit RNA synthesis in a promoter-specific manner [16,17], which correlates with the ability to bind to the L subunit of the viral polymerase [18]. C proteins can also directly block antiviral responses. For SeV, the C′, C, Y1, and Y2 proteins can all antagonize IFN pathways to some degree [19], and some naturally occurring SeV C protein mutant viruses (phenylalanine 170 to serine) that are defective in blocking IFN signaling [20] are attenuated for growth in mice.

Nonstructural 1 (NS1) and nonstructural 2 (NS2) proteins

The RSV genome is unique among paramyxoviruses in that it encodes 3′ proximal genes for the nonstructural 1 (NS1; 139 amino acids) and nonstructural 2 (NS2; 124 amino acids) proteins. These abundantly expressed proteins interact together to form a hetero-dimeric complex [5,21]. Viruses with deletions in the NS1 or NS2 genes have shown that these proteins are nonessential for virus growth in tissue culture cells or in the respiratory tract of chimpanzees [22]. However, both of these NS proteins play a role in suppression of antiviral responses, as evidenced by the finding that deletion of NS1 or NS2 converts RSV from a poor inducer of cytokines to a potent activator of IFN-I and proinflammatory cytokine expression [23].

As with other paramyxovirus antagonists of host cell responses (e.g., V protein, C protein), the RSV NS1 and NS2 proteins also appear to play roles in control of viral RNA synthesis, although the mechanism of suppression is currently unknown. In minigenome reconstitution

experiments using plasmid-expressed proteins, NS1 had a strong inhibitory effect on RSV genomic and antigenomic RNA synthesis [24]. NS2 also inhibited RSV RNA synthesis, but only at very high concentrations [5].

Small hydrophobic protein SH

Three of the four categories of paramyxovirus genomes (Fig. 1 above) code for a small hydrophobic protein (SH), but the location within the genome, the overall structure, and the function of these SH proteins differ significantly between viruses. The PIV5 SH protein is a 44-residue type II integral membrane protein that is expressed at the plasma membrane and is packaged in small amounts into virions [25]. PIV5 containing a deletion of the SH gene (PIV5deltaSH) grows, as well as wild-type (WT) virus in tissue culture cells, but the virus is attenuated for growth and pathogenesis in an immunodeficient mouse model system [26]. The related MuV SH protein is a 57-residue integral membrane protein, which due to sequence variability has been used as marker to identify MuV isolates [27]. MuV lacking an SH gene induced levels of cytokine responses higher than WT and was attenuated in an animal model system [28], although the effect of the SH gene deletion on the gradient of viral gene transcription also likely contributes to these results [29].

The available evidence indicates that the SH proteins of PIV5 and MuV function to block TNF-α signaling and induction of apoptosis [28,30]. Support for this proposal comes from the findings of enhanced TNF-α synthesis from cells infected with PIV5deltaSH and from SH-mediated inhibition of TNF-α signaling in reporter gene assays [30]. However, this conclusion is at odds with the finding that WT PIV5 is not able to block TNF-α signaling when infected human cell lines are treated with exogenous TNF-α [31]. These discordant findings may reflect the use of different cell lines or cell types from different animal species since the effect of SH deletion on PIV5 replication is most profound when assayed in mouse or MDCK cells [26] as opposed to human cells. The ability of paramyxoviruses to prevent TNF-α signaling can have profound effects on the function of infected cells. This was evident from our recent findings that primary human macrophages infected in culture with an SH-deficient MuV strain produce TNF-α, and this was both necessary and sufficient to restrict their subsequent migration toward a chemokine gradient [32].

RSV and MPV also encode an SH gene (Fig. 1), but the importance of SH for virus replication and pathogenesis differs from that seen with PIV5 and MuV. The RSV SH protein is a 64-residue type II integral membrane protein [5] expressed as four polypeptide species that differ by the processing of carbohydrate residues [33]. Similar to PIV5, the RSV SH protein appears to block TNF-α signaling [5]. However, this function is not clearly apparent in current animal model systems since RSV lacking the SH had only minor alterations in virus growth properties in the respiratory tract of mice [34]. In addition, biophysical studies suggest that the RSV SH protein may function as an oligomeric viroporin, which modulates ion flux across membranes [35]. MPV encodes a 179-residue SH protein that is similar to the RSV SH protein in being heavily glycosylated and nonessential for virus growth in cell culture or in animals [36]. Together, these data indicate that, despite the conservation of an SH gene across three of the four paramyxovirus groups shown in Fig. 1, no common function for this protein in growth or pathogenesis has emerged from studies so far.

Paramyxovirus Interactions with Extracellular Antiviral Pathways

There are a number of important extracellular defense mechanisms that can influence paramyxovirus infections, including surfactants, lectins such as galectin-1, and antimicrobial peptides. Among these, complement (C′) is a powerful first line of extracellular defense that viruses must face during animal infections [37]. This includes initial infections within the

respiratory tract where C′ factors exist in relatively low steady-state levels at the mucosal surface. However, during cellular stress and injury associated with infection, the concentration and profile of C′ factors can change dramatically [38], either through new gene expression from infected epithelial cells or through recruited immune cells. The C′ system is composed of both serum-associated and cell-associated components that are activated when they recognize foreign surfaces on virus particles. This initiates a cascade of proteolytic events leading to inactivation of virus and a linking of innate and adaptive immune responses. As with many other innate responses, our understanding of paramyxovirus activation and inhibition of C′ pathways is incomplete.

The C′ cascade can be initiated through three main pathways: the classical pathway, lectin pathway, or alternative pathway [37], all of which converge on a central component C3.When activated, C3 is cleaved into C3a and C3b. C3a serves as a potent anaphylatoxin to promote inflammation. C3b can bind covalently to viral components to aid in opsonization, aggregation, and phagocytosis or to propagate the signal to further downstream components such as C5 through C9 to form the membrane attack complex that is capable of lysing virus particles or infected cells. Thus, virus particles can be neutralized by direct binding or aggregation by the upstream components (e.g., C3) or by virion lysis after the cascade has propagated and terminated at the membrane attack complex stage.

The paramyxovirus particle contains strong C′-activating signatures, largely derived from the viral spike glycoproteins that subsequently are targets for C′-mediated neutralization. Conversely, the viral envelope also contains strong inhibitors of C′ pathways, which act to uncouple the activation of the pathway by pathogenic surfaces from the downstream C′ effectors that act to neutralize virus particles. As described below, we have demonstrated that changes in the balance of these virion-associated C′-activating and C′-inhibiting factors can be critical determinants of resistance of paramyxoviruses to neutralization by innate pathways [39,40].

Complement activation

Figure 3a shows an electron micrograph of a PIV5 particle, displaying spike glycoproteins HN and F that radiate from the viron surface. While the exact structure of C′-activating signatures on virus particles is largely unknown, it is clear that paramyxovirus glycoproteins themselves [41,42] and their carbohydrate modifications can play a key role in recognition as a foreign surface. Those viruses that encode an HN protein with neuraminidase activity typically activate the alternative pathway [43]. The extent of activation is inversely related to sialic acid concentrations on either the particle or the infected cells due to the presence of HN neuraminidase activity [44]. C′ activation can also depend on the cell type from which the viral glycoproteins are derived, indicating that carbohydrate and protein structures other than the presence or absence of sialic acid provide signatures driving C′ activation. This is clearly shown in the case of Newcastle disease virus (NDV) that, although it encodes an HN protein with neuraminidase activity, can activate all three C′ pathways [45]. As viruses such as MeV and NDV are in development as therapeutic vectors, future work will be necessary to better understand these C′-activating motifs and signatures.

While the attachment protein can in some cases contribute to C′ activation, the mechanisms by which F protein activate C′ are less well understood. F protein can be a target of C′ pathways since expression of the MeV F protein on cell surfaces by transfection approaches leads to activation of the alternative pathway and C3 can be found conjugated to F protein [41]. The strength of C′ activation can be dependent on F protein fusion activity. This is evident from our finding that PIV5 particles containing a hyperfusogenic F protein differ from WT PIV5 in C′-mediated neutralization by human sera due to enhanced binding of naturally -occurring antibodies in sera [46]. These findings led us to speculate that there may

selective immune pressure to limit the emergence of hyperfusogenic viruses that may be more easily neutralized by C′-dependent mechanisms.

Complement inhibition

Paramyxoviruses are sensitive to neutralization by C′ pathways and, therefore, must inhibit or delay C′ activities to survive in nature. Given their small coding capacity, it is assumed that none of the viral proteins has direct anti-C′ activity. Instead, paramyxoviruses take advantage of normal cellular pathways that function to block C′ pathways as a means to prevent inappropriate activation and potential cellular damage [47]. Among these regulators, CD46 plays an important role by combining with cellular protease factor I to mediate cleavage of C3b, thereby arresting propagation of the pathway. Similarly, CD55 (decay accelerating factor) is a glycosyl-phosphatidylinositol-linked membrane protein that blocks propagation of C′ pathways by either inhibiting C3 complex formation or promoting its disassembly. It is important to note that most of these C′ regulators act in a species-specific manner [38,45], for example, with regulators from human cells only inhibiting human C′ pathways. Thus, paramyxoviruses can take advantage of these two key regulators for C′ inhibition and this may contribute to species-specific restrictions on growth.

We have shown that PIV5 and MuV incorporate membrane-bound hostCD46 andCD55 into the virion envelope during the budding process, and this renders these viruses resistant to C′-mediated neutralization [39,40]. As shown in the immunogold labeling of purified PIV5 in Fig. 3b, virion-associated inhibitors CD46 and CD55 typically localize to opposing faces of the virus particle [40]. This may reflect incorporation of these host cell proteins into budding particles through association with distinct plasma membrane microdomains. It is important to note that virion-associated CD55 and CD46 only delay but do not render virus completely resistant to neutralization [40]. This may be in part due to partitioning of viral glycoproteins that are the targets of C′ binding into different microdomains in the virion than those that harbor the C′-inactivating CD46 and CD55 proteins [48]. A greater understanding of mechanisms for virus assembly with C′ inhibitors could allow development of more effective vectors that achieve a balance between survival in a host long enough to have a therapeutic effect and safety concerns that arise from enhancing resistance to neutralization.

Paramyxovirus Interactions with Intracellular RNA-Sensing Pathways

Paramyxovirus inhibition of interferon synthesis

Overview of IFN-I induction—RNA virus infections are detected by host cells to activate IFN synthesis through a range of RNA-sensing pattern recognition receptors. In this review, we focus on those pathways and receptors that have been described as important for response to paramyxovirus infection [49]. One of the best characterized systems includes the two cytoplasmic RNA helicase proteins RIG-I (*r*etinoic acid-*i*nducible *g*ene-*1*) and MDA-5 (*m*elanoma *d*ifferentiation-*a*ssociated gene-*5*). As shown schematically in Fig. 4, these two cytoplasmic recognition receptors react to signatures contained within viral RNA such as dsRNA structures (MDA-5) or improperly capped 5′-pppRNA structures (RIG-I). In combination with other specific signals, this leads to activation of the mitochondria-associated factor MAVS (*m*itochondrial *a*nti*v*iral *s*ignaling), which in turn leads to activation of a kinase complex composed in part of TBK-1 and IKK-ε (Fig. 4, right side). This kinase complex phosphorylates a latent cytoplasmic transcription factor interferon regulatory factor-3 (IRF-3). After homo-dimerization, IRF-3 translocates to the nucleus where it associates with nuclear factor κB (NF-κB) and activating transcription factor 2 (ATF2/c-jun) to drive IFN-β gene transcription.

In some cell types such as plasmacytoid DC (pDC), paramyxoviruses can induce IFN-α expression through activation of Toll-like receptor 7 (TLR-7), an endosome-localized sensor of viral single-stranded RNA (left side, Fig. 4). TLR-7 signaling leads to activation of a kinase complex consisting in part of IKK-α, myeloid differentiation primary response gene-88 (MYD88), and the E3-ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF6). This kinase complex phosphorylates and promotes hetero-dimerization of IRF-3 and IRF-7, which along with other transcription factors can upregulate synthesis of IFN-α.

Paramyxoviruses have evolved a diverse set of mechanisms to prevent activation of IFN synthesis (Fig. 4). As detailed below, these can be divided into four general mechanisms: (1) controlling aberrant viral RNA synthesis, (2) inhibiting cellular RNA sensors, (3) deceiving signaling kinases, and (4) directly suppressing the IFN promoter.

Control of RNA synthesis as a mechanism to limit innate immune responses—As shown in Fig. 4, by-products of RNA synthesis from the viral nucleocapsid can include dsRNA or 5′ uncapped mRNA, two potent inducers of innate antiviral responses. As such, one mechanism by which paramyxoviruses can limit innate responses is by limiting the synthesis of aberrant RNAs. Depending on the virus, this involves actions by the P, V, C, or NS1/NS2 proteins.

Evidence in support of a link between control of viral RNA synthesis and innate responses comes from many reports that individual paramyxoviruses harboring alterations to the function of auxiliary proteins such as V, C, or NS1/NS2 proteins not only show higher-than-WT levels of innate responses as would be expected but also display elevated or disregulated viral RNA synthesis. These altered RNA synthesis profiles can be seen in the case of viral V, C, or NS mutants that have changes in the synthesis of viral mRNA, genomic RNA, or both types of RNA products.

Direct evidence supporting a role for control of viral RNA synthesis in limiting antiviral responses emerged from our recent finding that point mutations in the 3′ end non-coding leader promoter region convert WT PIV5 that is a poor inducer of host cell responses into a virus that overexpresses viral RNAs and induces IFN-I and proinflammatory cytokines [50]. Importantly, these responses occur even in the context of a WT version of the IFN antagonist V protein (see the next section). Similar findings were found for a PIV5 P/V gene mutant [51]. In support of aberrant RNA synthesis as a driving factor, innate responses to the PIV5 leader mutant and to the P/V mutant can be suppressed by either of two mechanisms: (1) engineered expression of a foreign protein that binds and sequesters dsRNA [11,50] or (2) expression of the WT version of the polymerase subunit P protein [10]. The latter result indicates that the phenotype of the WT PIV5 polymerase is dominant over that of the mutant, a finding that may reflect proper protein–protein interactions between components of the viral RNA polymerase including the L protein, P protein, soluble N protein, and nucleocapsid template. Similar findings have been made for MeV and SeV harboring mutant C proteins that lead to defects in regulation of RNA synthesis, and these mutants are potent inducers of IFN [52,53].

The replication of many paramyxoviruses involves the expression of abundant levels of viral proteins without inducing a shut-off of host protein synthesis [1]. One of the mechanisms by which host shut-off is avoided involves the control of viral RNA synthesis by viral auxiliary proteins. For PIV5 [10,11], MeV [52], and SeV [53], viral mutants that are altered in either their V or C proteins show aberrant viral RNA synthesis, the activation of the host dsRNA-activated kinase PKR, and the subsequent shut-off of translation through phosphorylation of eIF-2. PKR-mediated translational shut-off can be reversed by expression of WT auxiliary

proteins or of a foreign protein that specifically binds and sequesters dsRNA [11]. Thus, while the V and C auxiliary proteins do not appear to actively target PKR for inhibition, they indirectly limit translational shut-off through their effect on the fidelity of the viral polymerase.

These findings show that an important and often overlooked aspect of paramyxovirus control of antiviral responses lies in the fidelity of viral RNA synthesis. Mutants engineered to be defective in control of viral RNA synthesis could serve as excellent therapeutic vectors since they would be attenuated for growth and could be strong inducers of innate and adaptive immunity. As such, an important focus of future research will be understanding of how auxiliary proteins modify the fidelity of viral polymerase activity.

Inhibition of cellular RNA sensors—A widely shared mechanism for inhibition of IFN-β induction involves the targeted inhibition of cellular MDA-5 by the V protein (Fig. 4). This was first discovered by Andrejeva *et al*. and Poole *et al*. who showed that the V protein expressed in transfected cells was able to block induction of the IFN-β promoter by exogenously added dsRNA [54,55]. This was consistent with results from PIV5 mutants in which the V protein was altered by point mutations [51] or by deletion of the V-specific C-terminal region [56]. The cysteine-rich zinc-binding domain at the C-terminus of V protein is sufficient to blockMDA-5 activation. Mechanistically, it is proposed that V protein binds MDA-5 directly through the unique cysteine-rich domain and inhibits activation through a competition with dsRNA for MDA-5 binding and an inhibition of MDA-5 multimerization into the active form [54,57]. Recently, elegant data from crystallography studies [58] have shown that the V protein disrupts MDA-5 function by inducing an unfolding of an MDA-5 ATP hydrolysis domain that is required for assembly of MDA-5 into signaling filaments.

As an additional strategy, some paramyxoviruses target RIG-I for inhibition through either direct or indirect mechanisms. In the case of RSV, the NS2 protein binds to RIG-I to prevent interactions with MAVS and downstream signaling to IRF-3 [59]. Additional regulation of the RIG-I pathway by NS proteins occurs through formation of a large “degradasome”, which targets destruction of multiple signaling factors including MAVS and IRFs [60]. It has been reported that the SeV C protein prevents RIG-I activity [61], although these results should be interpreted with caution as they were based on the use of recombinant viruses that were engineered to express high amounts of dsRNAs.

An intriguing question has been why some paramyxoviruses directly target MDA-5 (e.g., PIV5) while others (e.g., RSV) directly target RIG-I. This may reflect the types of viral RNA produced by a particular virus during replication (e.g., more dsRNA or more pppRNA) or may reflect the specific pathways that may be dominant in certain cell types. Although available data indicate that V protein does not directly bind or inhibit RIG-I, V protein does bind to the cellular protein LGP2 (*l*aboratory of *g*enetics and *p*hysiology *2*), a related cellular protein that in turn complexes with RIG-I to block recognition of viral RNA [62].

Viral decoys that block kinase complexes—In addition to targeting MDA-5, the multifunctional V protein can disrupt kinase complexes that are involved in propagating IFN-I induction signals, either through acting as a decoy substrate for these kinases or through inhibition of IRF modification (Fig. 4). For example, the MeV V protein binds to IKK-α and is phosphorylated by the IKK-α/MYD88/TRAF6 kinase complex at the expense of the normal substrate IRF-7, thus attenuating TLR-7 signaling that would otherwise activate IFN-α synthesis [63]. This finding could account for the profound suppression of IFN-α production from MeV-infected pDC [64]. It is note-worthy that some paramyxoviruses fail to inhibit TLR-7-mediated IFN-α production during infection of pDC [65].

The PIV5 and MuV V proteins are reported to inhibit the (TANK)-binding kinase 1 (TBK-1)/inhibitor of κB kinase ε (IKK-ε) kinase complex, through a mechanism involving V protein acting as an alternative substrate that mimics steps in IRF-3 phosphorylation [66]. While intriguing, this would be inconsistent with previous work showing that PIV5 is unable to block signaling through Toll-like receptors [65,67] or RIG-I [68]. These disparate findings may reflect differences in the targeting of TBK-1 pathways in different cell types, including cell lines *versus* primary cells. If this widespread inhibition of multiple signaling pathways occurs *in vivo*, it would have significant impact on a wide range of cell types, including epithelial cells, macrophages, and DC.

Recent evidence supports a different mechanism for HPIV2 to block the activity of kinase complexes downstream of TLR-7 signaling. Using transfection approaches, the HPIV2 V protein was shown to bind to TRAF6 through the conserved Cys-rich C-terminal domain [69], resulting in a block in downstream polyubiquitination of IRF-7. Thus, these four viruses (MeV, MuV, PIV5, and HPIV2) achieve suppression of multiple signaling pathways through targeting kinase complexes. While this has strong implications for pDC antiviral responses, it is noteworthy that PIV5 fails to inhibit TLR-7-mediated IFN-α production during infection of pDC [65], indicating that not all V proteins are equally potent at inhibiting all antiviral pathways.

Suppression of IFN promoter activity—In addition to the above mentioned mechanisms of inhibiting the IFN-I signaling pathway, some paramyxoviruses directly block activation of the IFN-β promoter downstream of RNA sensors, kinases, and IRFs. For example, activation of the IFN-β promoter by exogenous dsRNA and infection is inhibited by the NiV W protein but not the closely related V protein [70]. This inhibitory property is thought to depend on the unique nuclear targeting of the W protein. Similarly, the MeV C protein localizes to the nucleus, where it inhibits activation of the IFN-β promoter at a point downstream of IRF modification [71]. Interestingly, some MeV vaccine strains are defective in C-mediated suppression of the IFN promoter, and this phenotype corresponds to defects in the C protein nuclear localization sequences. Thus, MeV pathogenesis may be linked to C protein trafficking and its capacity to limit activation of the IFN promoter.

Paramyxovirus inhibition of interferon signaling

Overview of IFN-I signaling—IFNs are potent cytokines that can induce a wide range of cellular genes, many of which can inhibit virus replication. As shown in Fig. 5, IFN-I binding to the extracellular domain of the dimeric IFN-I receptor induces assembly of two latent cytoplasmic transcription factors STAT1 (*s*ignal *t*ransducer and *a*ctivator of *t*ranscription *1*) and STAT2 into a hetero-dimeric complex that is phosphorylated at tyrosine residues by receptor-associated kinases Jak1 and Tyk2 (reviewed in Ref. [72]). Phosphorylated STAT1–STAT2 dimers combine with interferon regulatory factor-9 (IRF-9), forming the interferon stimulatory gene factor 3 (ISGF3) complex. ISGF3 binds to and can activate promoters containing an interferon stimulatory response element (ISRE), resulting in expression of interferon-stimulated genes (ISGs). The IFN signaling pathway can also result in further induction of IFN-I through a positive feedback loop that activates synthesis of IFN-α [49].

All of the ISGs that are important for suppression of paramyxovirus replication have not been completely identified. In the case of PIV5, studies with mutant viruses that cannot block IFN-I responses have shown that IFN signaling altered the pattern of viral gene expression, consistent with an IFN-induced change in the gradient of viral transcription [73]. Individually, the ISGs MxA, PKR, and the oligo A synthetase/RNase L did not show dramatic effects on PIV5 replication. However, recent work has shown that restricted PIV5

replication is due in large part to ISG56 [74], a gene product limiting replication of viruses that produce mRNA with undermethylated 5′ cap structures. Interestingly, the expression pattern of the PIV5 M gene was most affected by IFN, suggesting that there are gene-specific restrictions that ISGs instill to limit virus production.

Restricted virus growth by ISGs can in some cases involve physical changes to the paramyxovirus genomic RNA. This was evident from the finding that the IFN-α-induced APOBEC3G (*apo*lipoprotein *B* mRNA-editing *e*nzyme, *c*atalytic polypeptide *3G*) was associated with MeV, MuV, and RSV genomes [75]. The presence of APOBEC3G affected several steps in viral RNA synthesis, resulting in impaired viral transcription and increased genome mutation frequency.

As paramyxovirus particles contain a lipid bilayer, it is not surprising that some IFN-mediated changes in lipid metabolism can also be an important aspect of antiviral immunity. This is evident by the recent discovery of a role for IFN-induced cholesterol-25-hydroxylase (CH25H) in restriction of a number of RNA viruses including NiV [76]. IFN-induced CH25H modifies cellular membranes such that viral entry is altered at the fusion stage and cell–cell fusion is blocked [76]. Consistent with IFN-induced alterations to cellular membranes, RSV is sensitive to the actions of the IFN-inducible viperin [77], an endoplasmic-reticulum-localized protein that modulates cholesterol, isoprenoid biosynthesis, and lipid raft formation. A detailed understanding of the important host ISG products and their mechanisms of action against paramyxovirus replication is an important topic of future research.

Mechanisms of paramyxovirus inhibition of IFN signaling—As highlighted in Fig. 5, paramyxoviruses have evolved a range of diverse mechanisms to block IFN signaling (reviewed in Ref. [78]), and many of these mechanisms have the potential to impact on species-specific virus replication. In general, these inhibitory mechanisms include (1) targeted degradation of signaling proteins, (2) sequestration of signaling factors, or (3) upregulation of cellular inhibitory molecules.

Some paramyxoviruses block IFN signaling by inducing degradation of cellular factors—Randall and colleagues were the first to make the remarkable discovery that paramyxoviruses can block IFN signaling through selective degradation of cellular factors [14,79,80]. These seminal findings were initially made through studies with PIV5, and to date, this virus system is the most extensively characterized for this particular mechanism of restricting IFN signaling. The PIV5 V protein is both necessary and sufficient by itself for blocking IFN-I signaling through a mechanism of targeting STAT1 for proteolytic degradation, while STAT2 and other factors in the pathway appear to be unaffected (left side, Fig. 5). While the unique cysteine-rich C-terminal zinc-binding domain of V protein is necessary for this function, additional determinants are located in the shared P/V region [51,56,81,82].

There has been intense interest in the mechanism by which the PIV5 V protein can mediate the assembly of a cellular complex that selectively targets STAT1 for degradation [78,83]. V-directed STAT1 degradation is very efficient and can be driven in a catalytic mechanism by the V protein contained within the incoming virion [84]. The identified cellular components in the PIV5 V protein degradation complex include bothSTAT1 and STAT2, as well as the cellular UV-damaged DNA binding protein (DDB1) and Cullin 4a [83,85], two components of a cellular E3-ubiquitin ligase complex that directs polyubiquitylation proteosome-mediated degradation of targeted polypeptides. While STAT2 is not targeted for degradation in PIV5-infected cells, V-mediated STAT1 degradation requires the presence of

STAT2 [83,86]. This requirement likely reflects the binding of V protein to STAT2, which is linked to STAT1 through the DDB1 component of the degradation complex [85].

PIV5 V-mediated degradation of STAT1 is very efficient in cells from a wide range of animals; however, this does not apply to infections of murine cells [87], where STAT1 remains intact and infected cells are capable of an IFN-I-induced antiviral state. Based on the fact that murine and human STAT2 differ significantly at the amino acid level, Parisien *et al*. showed that PIV5 was capable of blocking IFN signaling and V protein could target STAT1 degradation in mouse cells that were engineered to express human STAT2 [88]. Compared to WT mice, PIV5 grew to higher titers in the respiratory tract of transgenic mice that were engineered to constitutively express human STAT2 [89]. Taken together, these elegant studies indicate that species-specific sequences in STAT2 and the ability of V protein to assemble a complex for STAT1 degradation are intracellular determinants of PIV5 host range restriction.

Conversely, the PIV5 V protein can also be a determinant of host range restriction. This is evident from the finding of a PIV5 variant with a single asparagine-to-aspartate change at residue 100 of the P and V proteins (N100D). This change was sufficient to confer on the V protein the ability to target murine STAT1 for degradation and block IFN signaling in transfected murine cells [87]. As expected, a recombinant PIV5 harboring this N100D substitution grew to higher titers in mouse tissue culture cells. Interestingly, this effect of the N100D mutation on enhanced virus growth was not evident in the mouse respiratory tract [90], suggesting that there are likely additional factors that contribute to restriction of PIV5 growth in mice. The concept of V protein function contributing to host range restriction has also been reported for NDV [91].

HPIV2 V protein also assembles cellular degradation complexes [85] but induces degradation of STAT2 and STAT1 that is left intact [92]. As with PIV5, MuV V protein targets STAT1 degradation, but MuV is unique by having the additional capacity to block IL-6 signaling through targeted STAT3 degradation [93]. The differences between STAT targets for these closely related viruses (PIV5, HPIV2, and MuV) highlight the versatility of V protein interactions with multiple partners and innate immune pathways.

RSV lacks expression of a V protein but is fully capable of dismantling IFN-I signaling pathways. Similar to V-mediated degradation of STAT1 (PIV5) or STAT2 (HPIV2), the RSV NS2 protein has been shown to induce proteolytic degradation of STAT2 [94,95]. Available evidence indicates that NS1 enhances the function of NS2 in STAT2 inhibition but does not, by itself, have inhibitory activity. In sharp contrast, the related MPV does not alter levels of STAT proteins but, instead, is reported to decrease cell surface levels of Jak1 and Tyk2, through proteasomal degradation and decreased gene transcription [96].

Some paramyxoviruses block IFN signaling by sequestering cellular factors— As an alternative to targeted protein degradation, paramyxovirus P/V/W gene products can act to inhibit IFN-I signaling through sequestration or changes in cellular protein localization. For example, the NiV V and W proteins alter the location of IFN-I signaling molecules through the use of nuclear export or localization sequences, respectively. The NiV V protein induces the formation of cytoplasm high-molecular-weight complexes (Fig. 5) consisting of non-phosphorylated STAT1 and STAT2 [97]. This localization is due to a nuclear export signal in V that shifts steady-state levels of STAT1-containing complexes out of the nucleus and into the cytoplasm where they are incapable of signaling [98]. Conversely, the NiV W protein accumulates in the nucleus due to a nuclear localization sequence in the short W-specific C-terminal region [99,100]. This function coupled to

STAT1 binding leads to trapping of STAT1-containing complexes in the nucleus in a nonfunctional complex.

By a different mechanism that does not involve redistribution of factors, the measles virus V protein prevents IFN signaling by blocking translocation of both STAT1 and STAT2 into the nucleus [101,102], and cytoplasmic aggregates can be detected where STATs co-localize with nucleic acids and viral N protein. The MeV V protein can bind to STAT2 through interactions with the C-terminal zinc-binding domain [103]. Remarkably, the measles virus P protein also has inhibitory functions in IFN signaling. In this case, MeV P protein binds to STAT1 to prevent its phosphorylation [104], through interactions involving a specific tyrosine residue contained in both P and V.

A systematic analysis of recombinant SeVs that were deficient in expression of either the V protein, subsets of the four C proteins, or all four C proteins (C′, C, Y1, Y2) demonstrated that the mutant lacking all four C proteins had completely lost the ability to suppress the establishment of an antiviral state and ISG expression [105]. C protein can be detected in complex with STAT1; however, the inhibition of IFN signaling correlates with a block in STAT2 phosphorylation [19] that likely reflects the use of STAT1 as a scaffold for modulating STAT2 events. Others have reported that C protein can induce ubiquitination and degradation of STAT1 in some cell types [106]. Thus, the mechanisms by which the SeV C proteins block IFN signaling are not completely understood and may differ depending on a particular cell type and virus strain.

Upregulation of cellular inhibitors of IFN signaling pathways—A relatively new finding has emerged for some paramyxoviruses, whereby virus infection can lead to upregulated expression of cellular inhibitors of IFN pathways. These inhibitors are temporally expressed by cells following IFN induction, as a means to dampen cytokine expression and function. An example of this mechanism can be found with RSV, where infection increases the expression of SOCS1 (*s*uppressor *o*f *c*ytokine *s*ignaling *1*) [107], a cellular protein that acts to inhibit Jak/STAT signaling. These responses could be a normal nonspecific downregulation of excessive cytokine production or alternatively cellular gene expression that is specifically targeted by viral factors. In support of the latter mechanism, expression of specific viral proteins is associated with increased expression of cellular SOCS1 [108] and A20 [109], an inducible inhibitor of cellular signaling pathways.

Interactions of Paramyxoviruses with DC

DC in the lung

DC sit at the crossroads of the innate and adaptive immune response. As with epithelial cells, DC possess an array of innate sensors to detect the presence of viral pathogens, including TLRs, RIG-I, MDA-5, and nod-like receptors (NLR) [110,111]. Activation of these innate pathways during virus infection is an essential driver of DC maturation, a process by which immature DC are converted into a cell type that is now competent for T cell activation. Thus, DC are exceptional cells with the capacity to generate adaptive immunity through the coupling of innate immune signals generated during infection with the presentation of viral antigens.

DC efficiently survey for the presence of pathogens by establishing a network that extends throughout the body, including the lung. As paramyxovirus entry occurs predominantly through the respiratory tract, the ability of DC to effectively sense virus at this site is critical for host survival [112]. The resident population of DC in the lung can be divided into three major types: airway DC, parenchymal DC, and pDC [112]. The former two appear to be primarily involved in linking innate responses to activation of the adaptive immune response

and thus will be discussed here. In the lung, airway and parenchymal DC can be distinguished by differential expression of cell surface markers (Fig. 6). DC lining the airway are identified by the expression of the αE integrin CD103, while parenchymal DC, by the expression of CD11b [113].

In addition to differences in location, airway and parenchymal DC also express a divergent array of innate receptor molecules available for sensing virus infection. For example, a divergence in TLR expression has been observed in human [114] and mouse lung DC: mouse $CD103^+$ airway DC express predominantly TLR-3, while parenchymal DC principally express TLR-2 and TLR-7 [115]. This suggests that these two DC types could show differential response to and inhibition by individual viruses. The capacity for negative regulation of TLR signaling has been reported for a number of paramyxoviruses. For example, monocyte-derived DC (moDC) show suppressed responsiveness to poly IC following infection with RSV and MPV[116]. Thus, viruses that target one innate signaling pathway *versus* the other could result in promotion of selective maturation of distinct DC subsets in the lung.

As a third difference between these lung resident DC subsets, recent data suggest that airway and parenchymal DC have divergent functions in the generation of an adaptive immune response. In general, it is largely thought that airway DC play a pivotal role in the activation of $CD8^+$ T cells following respiratory infection, while parenchymal DC are preferential activators of $CD4^+$ T cells [117–119]. $CD103^+$ DC have been shown to be critical contributors to the generation of the T cell response against SeV [118]; however, information on the relative roles of parenchymal $CD11b^+$ *versus* airway$CD103^+$ DC in $CD8^+$ T cell responses to other paramyxoviruses is limited. The enhanced capacity of $CD103^+$ DC to activate $CD8^+$ T cells is due in part to their ability to cross-present exogenously acquired viral antigen [115,117]. In addition, these cells appear to be the exclusive population of DC that take up and present antigen that is derived from apoptotic cells [115], a property likely to be particularly important in the case paramyxoviruses that cause cytopathic infections, such as PIV5 [67] and HPIV3 [120]. Importantly, the anatomic location of $CD103^+$ DC with dendrites that can extend into the airway allows for efficient access to viral antigen.

At steady state, DC in the lung exist in an immature state in which they are minimally stimulatory for T cell activation. Instead, they are highly efficient at uptake of environmental pathogens and are at their most susceptible to virus infection. In concert with activation of innate pathways, immature DC undergo changes in gene expression, which confers the capacity to migrate to the lung draining lymph node (LN) where they can activate T cells (Fig. 6).

Maturation of DC during paramyxovirus infection

The processes whereby immature DC undergo maturation involve a number of changes such that they are now competent for activation of naïve T cells. These maturation changes include (1) increased cell surface expression of chemokine receptors, (2) upregulation of costimulatory molecules, and (3) the production of cytokines (Fig. 7).

The majority of pulmonary antigen reaching the LN is trafficked by lung resident DC [121–123]. This can come in the form of antigen uptake (from dying infected cells or virus proteins/defective particles) or as a result of direct infection. At this time, our understanding of the relative contribution of these two mechanisms of antigen acquisition by lung DC *in vivo* following paramyxovirus infection is highly limited as this question is relatively unexplored. The efficient migration of DC to the draining lymph node (Fig. 6) requires expression of the chemokine receptor CCR7 [124], one of the critical changes that occur

during the maturation process. CCR7 expression allows DC to respond to CCL19 and CCL21 that are constitutively produced by cells in the lymph node. Not surprisingly, a number of paramyxoviruses have developed strategies to inhibit this critical step in elicitation of the adaptive immune response. In tissue culture, exposure of human moDC to either RSV or MPV did not lead to increased expression of CCR7 [125]. This was likely due to a failure to induce as opposed to active inhibition of CCR7 expression since subsequent exposure to LPS resulted in efficient upregulation of this molecule [125]. While RSV and MPV are ineffective inducers of CCR7, other paramyxoviruses (e.g., HPIV3) can drive high-level expression of CCR7 and efficient migration in response to CCL19 [125].

Numerous maturation molecules have been identified that can contribute to priming, growth, and/or survival of DC. However, the most highly studied costimulatory molecules are CD80 and CD86, which bind to CD28 molecules constitutively expressed on T cells [126]. DC maturation is often considered as an all-or-nothing process in which the innate response drives cells from no expression to a full expression of maturation signature. However, there are clear examples of maturation states that result in distinct patterns of a subset of costimulatory markers and cytokine expression [127,128]. For example, our studies with PIV5 revealed a phenotype that we have designated partial maturation [127]. Exposure of murine DC to low multiplicity of infection with PIV5 resulted in the upregulation of CD86, while CD80 levels remained relatively unchanged [127]. In contrast, a higher PIV5 multiplicity of infection resulted in robust upregulation of both molecules. Interestingly, this increased expression of both costimulatory molecules was coupled with conversion from an abortive to productive viral infection. Partial maturation (as evidenced by selective upregulation of CD86) had significant repercussions with regard to DC function as these cells were impaired in their capacity for naïve T cell activation [129]. At a mechanistic level, we found that the failure to express CD80 on the stimulatory DC resulted in diminished expression of CD25 (indicative of the high-affinity IL-2R) on responding T cells [129]. A similar selective upregulation of CD86 was reported in SeV-infected mouse lung DC [128], but how this phenotype related to the permissiveness of infection was not investigated. The molecular mechanism by which DC undergo partial maturation remains to be determined. However, these findings show that the interaction of paramyxoviruses with DC can result in varied innate responses (e.g., CD80 and CD86 expression) and competencies for adaptive immune function, perhaps depending upon the level of virus to which they are exposed.

In contrast to the above mentioned findings of partial DC maturation, other paramyxoviruses induce expression of both CD80 and CD86. For example, RSV, MPV, and some strains of MeV induce bothCD80 and CD86 expression in human DC [130–133]. Finally, there are examples of paramyxovirus infections that fail to induce any costimulatory molecules, for example, PIV5 infection of human moDC [134]. While PIV5 infection of human DC was associated with significant cytopathic effects, this was not the underlying cause of the failure to undergo maturation [67] since infected DC failed to undergo maturation even when death was blocked with inhibitors.

It is interesting that DC can exhibit divergence in their response to infection, even in the context of the seemingly homogenous population of moDC. HPIV3 exposure results in high rates of infection and viral replication in human moDC that induces apoptosis in the majority of cells within 72 h [120]. Surprisingly, however, the surviving DC undergo robust maturation as measured by upregulation of costimulatory molecules [120]. Whether the divergence in response reflects heterogeneity within the moDC culture was not investigated. One possibility is variability in baseline maturation, which is always present within these cultures. This may impact the response to virus, that is, DC that have higher levels of baseline costimulatory molecule expression may have increased resistance to cytopathic

effects. Certainly, the maturation state can impact how permissive DC are to infection [135,136].

A third critical aspect of DC maturation is the capacity to secrete cytokines necessary for T cell differentiation and acquisition of effector function. IFN-I and IL-12 are known to provide signals that directly impact $CD8^+$ T cells [137,138]. In addition, however, IFN-I can contribute to T cell activation through an indirect mechanism involving the promotion of DC maturation [139].While it is clear that paramyxoviruses have a number of effective strategies to limit IFN-I production (as described above), in most cases, DC exposed to virus produce detectable amounts of IFN-α/β [116,120,140,141] that can serve as an effective signal for T cell differentiation. Interestingly, in some cases, for example, with SeV strain Cantell, production of IFN-I depends on the presence of defective interfering particles within the inoculum [142]. In these cases, facilitation of immune activation may occur when IFN-I is supplied by a bystander cell. There are also examples where IFN-I induction from infected DC appears to be very effectively blocked, including studies reporting the failure of RSV to induce IFN-β from infected human DC [116,141,143].

In addition to IFN-I, paramyxovirus infection often results in the production of IL-12 [116,120,127,131], but this is highly dependent on the particular virus examined. For example, infection of human moDC with MPV induced expression of IFN-I but failed to result in IL-12 production [116]. A similar dissociation between production of these two cytokine signals occurs with RSV infection where IFN-I synthesis is inhibited; IL-12 is efficiently produced [116]. These findings suggest that although viruses have developed mechanisms to inhibit the immune response, the host is adept at circumventing these in order to allow generation of T cells.

Recent data have shown that DC are also an important contributor to the maintenance of effector cells following their entry into the lung [144,145]. DC-mediated signals that result in survival of effectors come from surface-bound IL-15 together with antigen [144].DC subsets can differ in their capacity to provide survival signals to effector T cells that have entered the lung to aid in viral clearance. Initial studies identified $CD8\alpha^+$ DC and pDC as important mediators of this effect [144], although a recent study found that, at later times following infection, $CD11b^+$ DC can also contribute [146]. The ability of these DC to maintain effector cells in the lung is dependent on the production of IL-15, as well as the costimulatory molecule CD70 [145,146]. These findings suggest that paramyxovirus-mediated regulation of innate DC function has the potential to impact adaptive immune effectors at multiple levels, that is, survival in the tissue and generation in the lymph node.

The above mentioned results provide significant insights into the ability of innate signals present during paramyxovirus infection to promote DC that are competent for generation of an adaptive immune response. For many paramyxoviruses of clinical interest, human DC are arguably the most relevant cell for study. This is supported by examples of differential regulation of the IFN pathway [87,147], DC maturation [127,134], and cytopathic effects [127,134] in mouse-derived *versus* human-derived cells. Human studies have employed DC generated from peripheral blood monocytes, in order to overcome this species limitation, since lung derived human DC are not readily available for study. However, the extent to which the response in these cells reflects lung DC infected *in vivo* and the contribution of species-specific factors are open questions, as significant differences have been observed when assessing *in vitro* and *in vivo* derived DC [119,135,148].

This review has highlighted progress in our understanding of paramyxovirus interactions with the innate immune system, including extracellular pathways (e.g., C′), intracellular RNA-sensing pathways (e.g., IFN-I), and the unique role of DC in converting innate signals

from paramyxovirus infections into adaptive immunity. Great progress has been made in these areas using tissue culture cells and mouse model systems, but future work is needed on the innate interactions that occur *in vivo*. While mice are a highly tractable model system, direct translation of results from such studies must be tempered by a detailed understanding of how species-specific differences impact permissivity, innate responses, and the ability of a virus to counteract host responses. Other attractive non-murine animals for model development include chinchilla [149], ferret [150], and nonhuman primates since many of these animals share greater similarity with humans with regard to innate sensing [151]. As emphasized in recent reviews [152,153], there can be substantial differences in virus replication, which cell types are infected, the extent of innate responses, and the landscape of host cell innate sensing molecules depending on which animal model is employed for virus: host immunity studies [154]. While such experiments in animals such as nonhuman primates are not without limitations, they may prove a more valid mimic of the human system recognition and response to paramyxovirus infection.

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Abbreviations used

DC	dendritic cells
ORF	open reading frame
WT	wild type
ISG	interferon-stimulated gene
pDC	plasmacytoid DC
moDC	monocyte-derived DC

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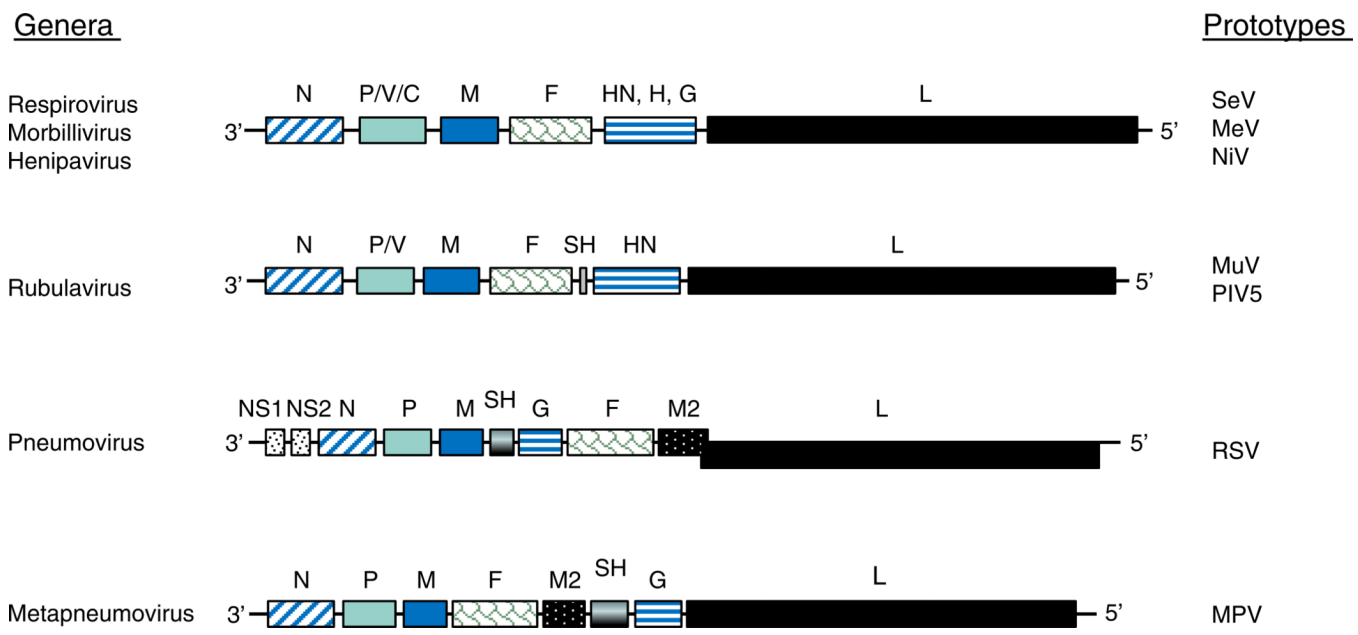

Fig. 1.

Schematic diagram of representative paramyxovirus genomes. Four paramyxovirus genome structures are categorized by the differential coding strategies for viral antagonists of innate immune responses, including V protein, C proteins, SH protein, and NS proteins. Genomes are shown schematically in a 3′-to-5′ orientation with boxes indicating the approximate size of ORFs for viral proteins and thin lines indicating intergenic regions. Prototype members of the paramyxovirus genera are listed on the right.

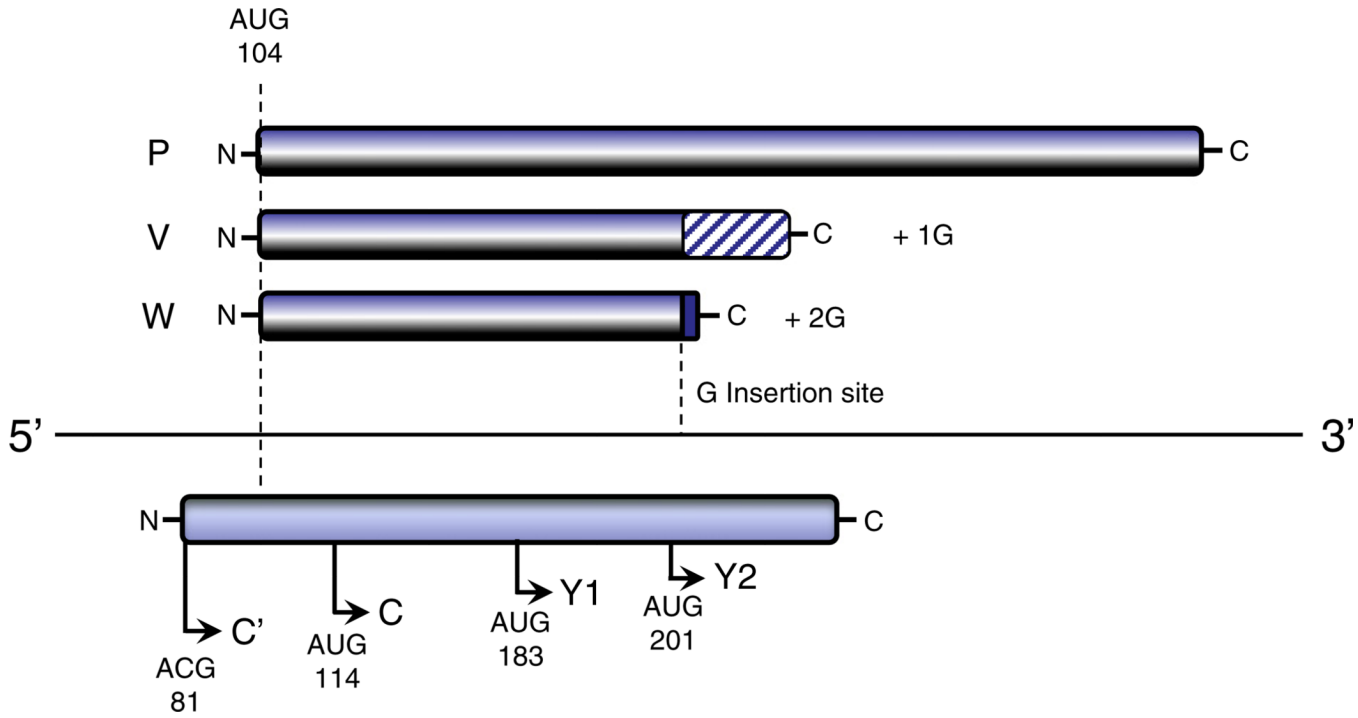

Fig. 2.

Schematic diagram of coding strategy in the SeV P/V/C gene. The position of the common initiating AUG codon for the P, V, and W ORFs at base 104 is shown above a line indicating the viral mRNA. Hatched and black boxes indicate the V protein Cys-rich C-terminal domain that is fused to the shared P N-terminal domain by addition of a G residue during viral transcription and the short W domain that is accessed by insertion of two G residues, respectively. The four in-frame initiation codons for the COOH-terminal nested set of C′, C, Y1, and Y2 ORFs are shown as rightward arrows below the P gene mRNA.

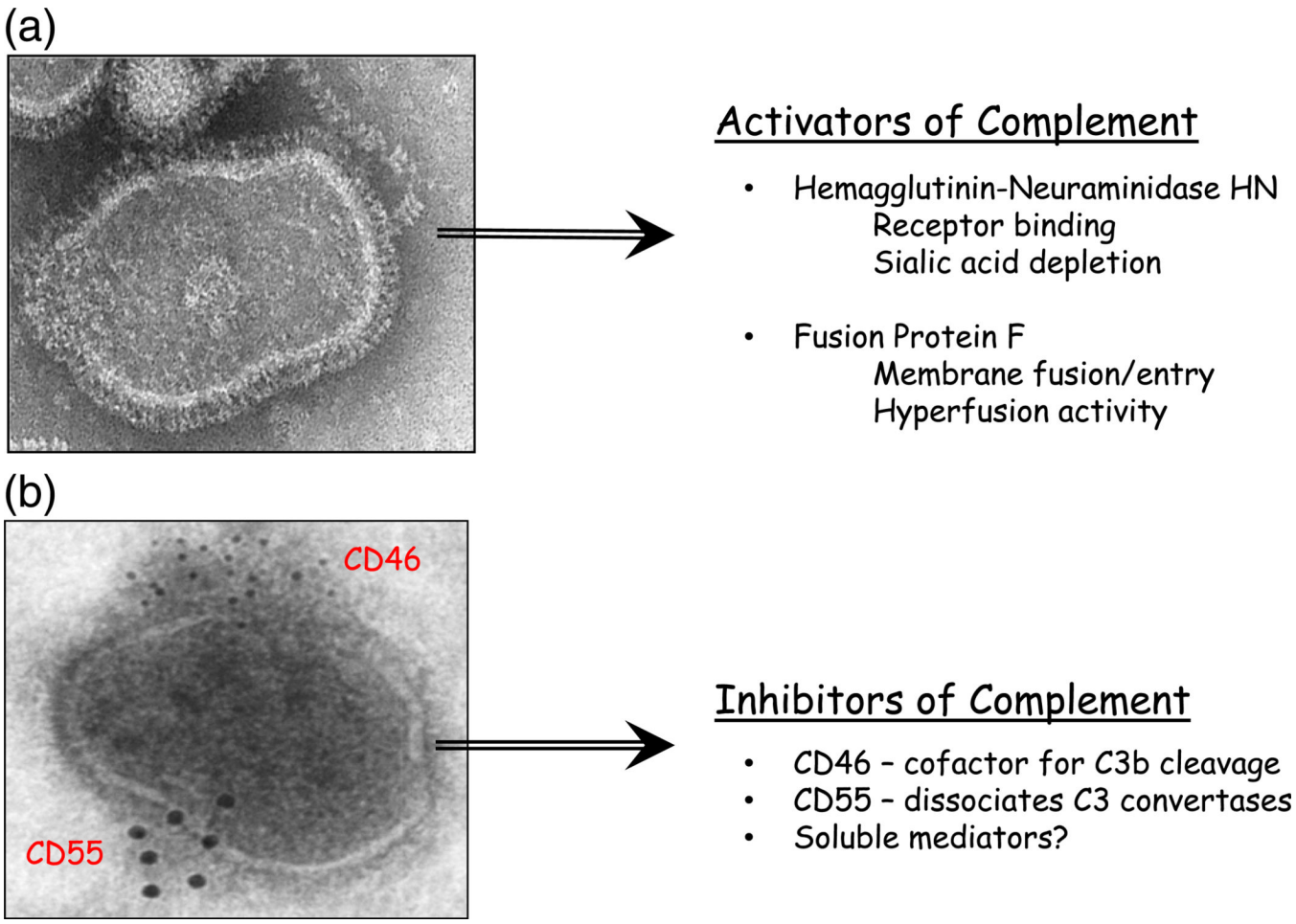

Fig. 3.

Activation and inhibition of complement by paramyxovirus envelope proteins. Electron micrographs of purified PIV5 are shown (~55,000×) to illustrate the spike glycoproteins HN and F that radiate from the virion surface and are potent activators of C′ pathways (a). An electron micrograph of purified PIV5 that has been immunogold labeled with anti-CD46 and anti-CD55 antibodies is shown (b) to illustrate the apparent polarized distribution of C′ regulators in the virion envelope.

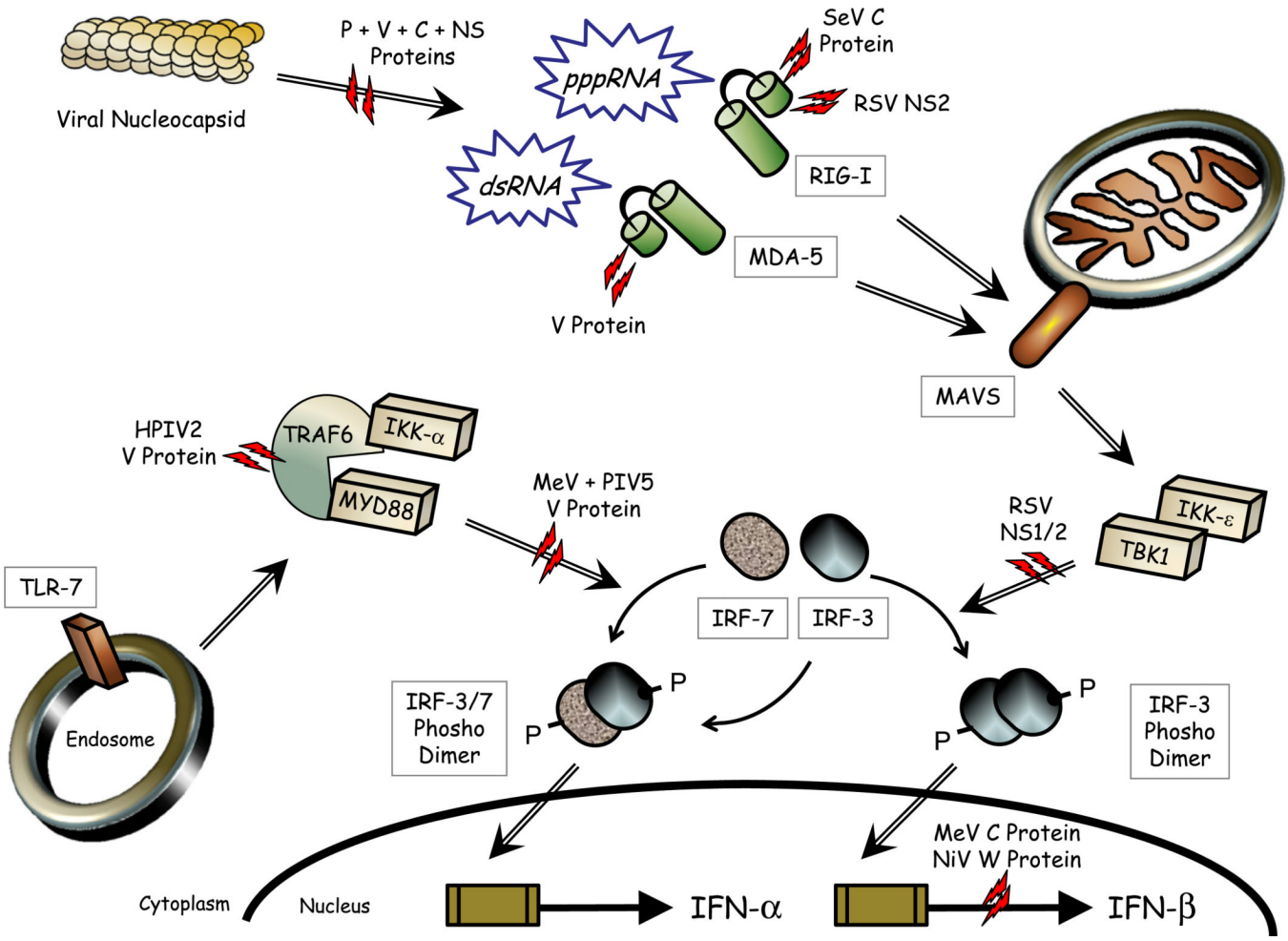

Fig. 4.

Activation and inhibition of IFN-β and IFN-α synthesis pathways during paramyxovirus replication. The viral nucleocapsid template is shown on the upper left with RNA products such as dsRNA and pppRNA that can activate latent factors MDA-5 and RIG-I. This in turn signals through MAVS to activate a cytoplasmic kinase complex composed in part of TBK-1 and IKK-ε. Alternatively, in some cell types, viral single-stranded RNA can activate endosomal TLR-7, leading to signaling to a complex composed in part by TRAF6/MYD88/IKK-α. Latent transcription factors IRF-3 and IRF-7 are phosphorylated and assembled into homo-dimers (IRF-3) or hetero-dimers (IRF-3 and IRF-7) that function with other transcription factors to activate the IFN-β or IFN-α promoters, respectively. Steps in the signaling pathways that are blocked by select paramyxovirus proteins are indicated by dual red lightning bolts.

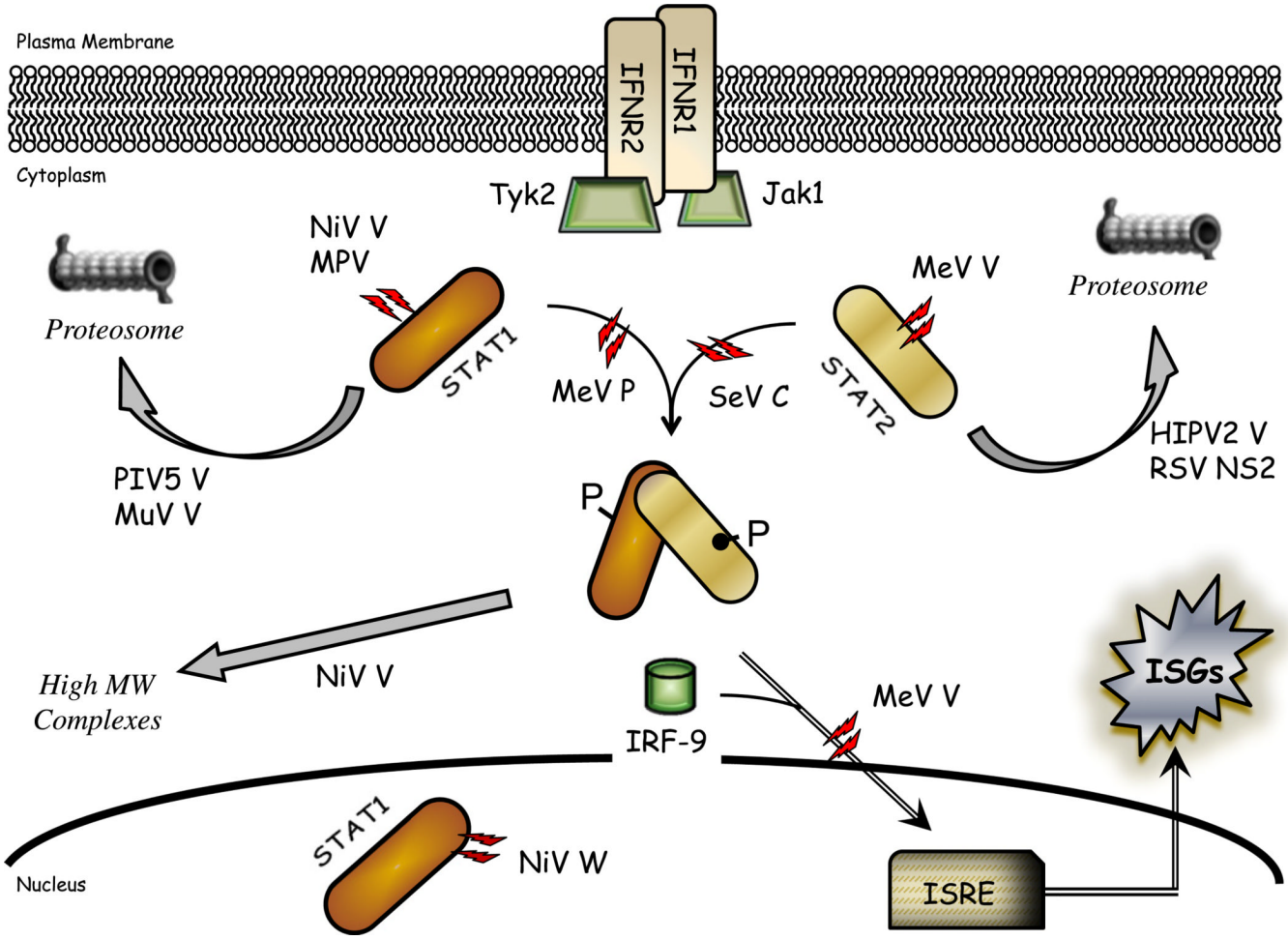

Fig. 5.

Paramyxovirus inhibition of IFN signaling pathways. IFN binding to the extracellular domain of the plasma-membrane-localized IFN receptor complex results in phosphorylation of intracellular STAT1 and STAT2 by the Tyk2/Jak1 kinase and hetero-dimerization. Following association with IRF-9, the complex is translocated to the nucleus to activate transcription of ISGs containing an interferon-stimulated response element (ISRE). Steps and targets in the signaling pathway that are blocked by select paramyxovirus proteins are indicated by dual red lightning bolts.

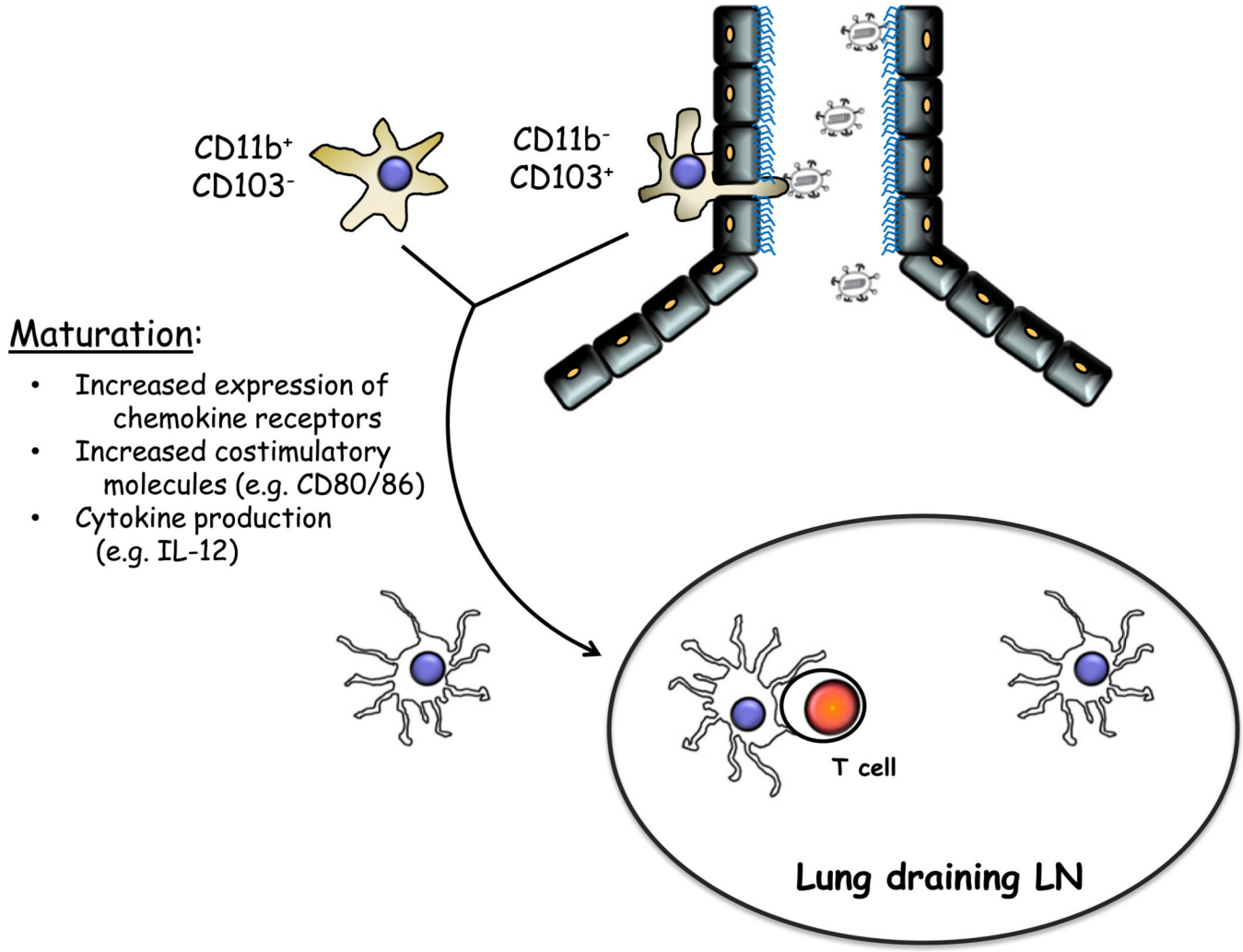

Fig. 6.

DC survey the lung airway and parenchyma for the presence of viral pathogens. DC in the lung are a heterogenous population that includes two major DC types important for innate sensing, antigen acquisition, and subsequent T cell activation. These two subtypes can be differentiated by the distinct anatomic niches in which they reside, the airway *versus* the parenchyma, and by the cell surface molecules they express. Airway DC are marked by the expression of CD103, while parenchymal DC express CD11b. Following innate immune signals that occur as a result of paramyxovirus infection or encounter with virus components, DC undergo maturation, leave the lung, and migrate to the draining lymph node. There they engage naïve T cells to induce activation and differentiation into effector cells.

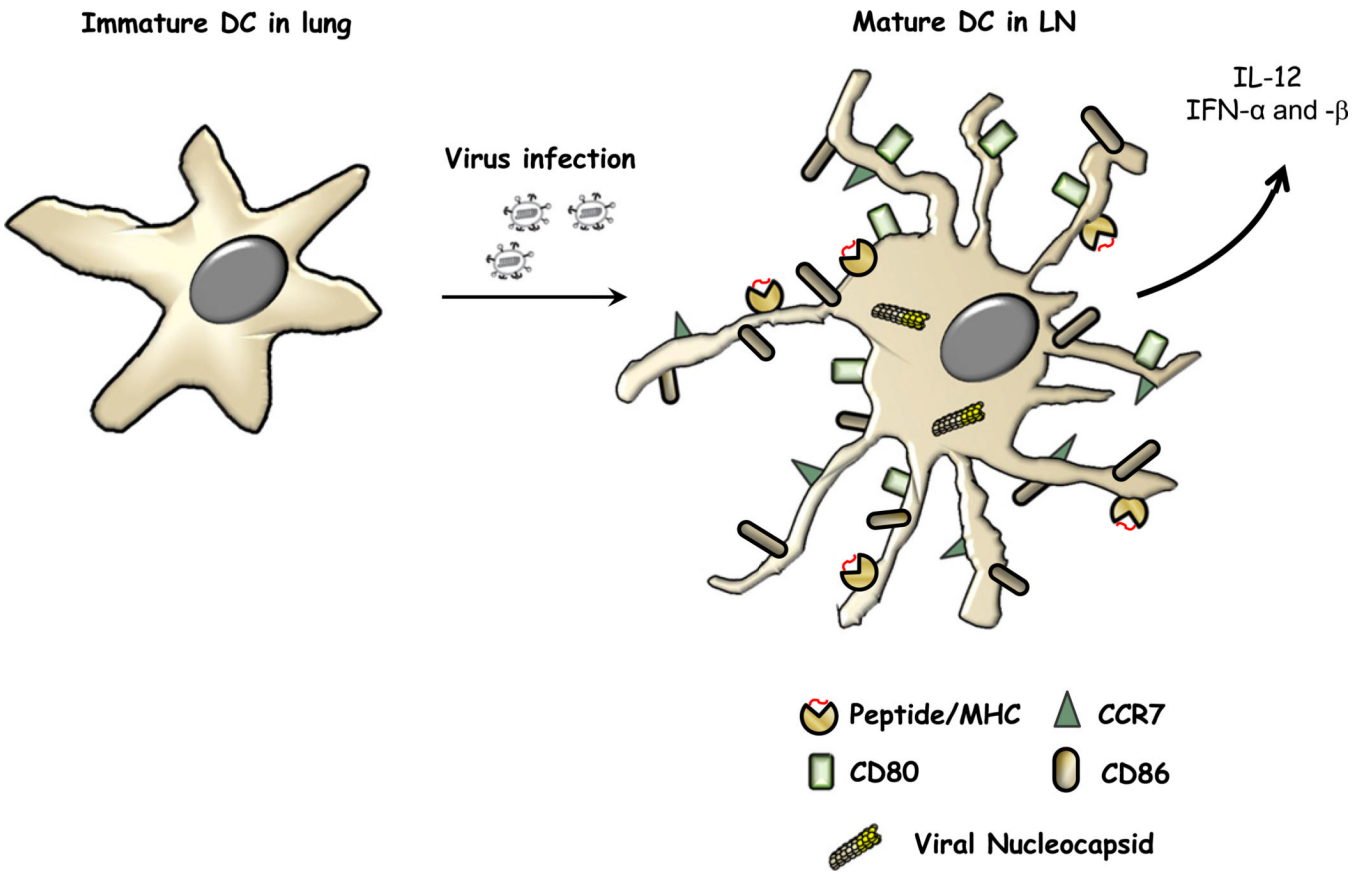

Fig. 7.

DC maturation results in upregulation of costimulatory markers and chemokine receptors together with the production of cytokines. DC (left side) reside in the lung in an immature state. As a result of virus infection or exposure to viral constituents, DC undergo an innate immune response termed maturation. This involves upregulated expression of the chemokine receptor CCR7, which promotes efficient trafficking to the lymph node, as well as costimulatory molecules (e.g., CD80 and CD86) that facilitate T cell interactions, expansion, and survival. In addition, mature DC produce cytokines that promote the acquisition of effector function in activated T cells (IL-12 and IFN-α/β). In the context of these innate responses, mature DC present antigen (red lines) in the context of surface complexes (yellow circles).