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Current views

Local cytokine response upon respiratory syncytial virus infection

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ABSTRACT

Respiratory syncytial virus (RSV) is the leading cause of childhood hospitalization and respiratory distress and has been recognized for several decades as a major health and economic burden worldwide. This virus has developed several virulence mechanisms to impair the establishment of a protective immune response to re-infection. Accordingly, inefficient immunological memory is usually generated after exposure to this pathogen. Furthermore, it has been shown that RSV can actively promote the induction of an inadequate cellular immune response at the site of infection that causes exacerbated inflammation in the respiratory tract. Such an inflammatory response is both inefficient for clearing the virus and can be responsible for detrimental symptoms, such as asthma and wheezing. Recent data suggest that RSV possesses molecular mechanisms to induce the secretion of pro-inflammatory cytokines that modulate the immune response and impair viral clearance by reducing IFN-γ production. Here, we discuss recent research leading to the identification of RSV virulence factors that are responsible of promoting a pro-inflammatory environment at the airways and their implications on pathogenicity.

1. Introduction

Over 70% of children in their first year of life and 100% of children by age 2 have been infected at least once by the respiratory syncytial virus (RSV), which is one of the leading etiological agents for lower respiratory tract infection [1]. RSV is an enveloped virus that belongs to the *Paramyxoviridae* family, harboring a genome encoding for eleven proteins [2]. The F (fusion) surface protein of the virion mediates the fusion between the virus envelope and the target cell surface and promotes later the formation of syncytia between adjacent infected epithelium cells [3]. It is thought that surface proteins F and G (attachment glycoprotein), as well as the non-structural proteins NS1 and NS2, can contribute significantly to the virus infective cycle and interfere with the immune response of the host [3,4].

Significant epidemiological studies have characterized RSV to be a relevant pathogen that causes a major health burden worldwide (World Health Organization, www.who.org) [5]. Symptoms from infection with this virus usually manifest in adults as rhinitis, however severe symptoms such as bronchiolitis and pneumonia are commonly observed in premature infants, the elderly and immunosuppressed patients [6–8]. Furthermore, increased susceptibility to recurrent allergic wheezing and asthma may result as a consequence of exposure to RSV infection early in life [5,9,10]. Clinical reports have also shown that RSV infection may cause extra-pulmonary effects at the neurological, endocrine, cardiac and hepatic level [11–16]. Although the causes leading to these extra-pulmonary symptoms have remained elusive, it is possible that both direct organ infection by RSV and damaging inflammatory responses promoted by the virus at those tissues could contribute to the observed detrimental effects [17,18].

To date, only one antiviral drug (ribavirin, a purine nucleoside analogue) is commercially available for treating severe RSV infection [19]. However, the use of this drug is controversial due to its variable efficacy and questionable cost-effectiveness [19–21]. Therefore, new pharmacological alternatives for treating RSV are required in order to diminish the adverse inflammatory response elicited by the unbalanced immune response induced by the exposure to this virus.

Here, we will discuss experimental evidence and recent findings describing the characterization of RSV molecular determinants that are recognized by host cells in the lungs and trigger the secre-

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tion of immune modulatory molecules, such as pro-inflammatory cytokines. We will also discuss the role of these molecules at promoting inflammatory damage at the airways, which is probably driven by the virus and ultimately leads to immunopathology. Based on these data, a model can be proposed involving virus components and elements of the host immune response as responsible of the RSV-induced pathology. Finally, we also review new potential therapeutic approaches to block the secretion of pathogenic cytokines induced by RSV infection.

2. Detection of RSV by pattern recognition receptors

The respiratory epithelium is the first site of encounter between the virus and host cells. As a result of this initial interaction, an early innate immune response is promoted at the site of infection. RSV attachment to epithelial cells leads to the detection of viral components by means of pattern recognition receptors, such as toll-like receptors (TLRs) and the retinoic acid-inducible gene I-like receptor (RIG-I). Engagement of these receptors promotes the initiation of an immune response against the virus [22,23]. For instance, TLR3 expressed by respiratory epithelial cells contributes at recognizing RSV during infection by binding to viral RNA [22,24,25]. TLR3- and RIG-I-derived signaling promotes nuclear factor-κB (NF-κB) activation and cytokine secretion in response to RSV infection (see below). In agreement, TLR3 signaling induced by RSV infection is preceded by an IFN-β loop, which is regulated by RIG-I upon recognition of single stranded RNA [26,27]. Together, these findings underscore distinct temporal roles for RIG-I and TLR3 at mediating RSV-induced innate immunity, which are coupled to distinct pathways controlling NF-κB activation. Further, respiratory epithelial cells also express TLR2 and TLR6, which can recognize molecular patterns present on pathogens such as hepatitis C virus, herpes simplex virus and human cytomegalovirus. These two receptors have been recently shown to be involved in the control of RSV replication [28]. TLR2 and TLR6 contribute to the production of TNF-α, IL-6, CCL2 and RANTES by leukocytes, as well as to neutrophil migration and dendritic cell activation in the lungs [28]. All these events reduce RSV replication and dissemination.

However, upon infection with RSV, substantial changes in TLR expression can be observed. An alteration of TLR expression pattern is likely to play an important role on the clinical outcome of the infected individual [29]. For instance, it has been described that expression of TLR4 is significantly increased in epithelial cells after RSV challenge and during the inflammatory response induced by the virus [30]. Consistent with this observation, neutrophils recovered from bronchoalveolar lavages of RSV-infected preterm infants expressed significantly higher levels of TLR4 than did healthy infants, suggesting an increased inflammatory potential in those patients [31]. Furthermore, a recent study has also shown that RSV-infected human bronchial epithelial cells secrete the heat shock protein HSP72, which binds to TLR4 on neutrophils and leads to an increase on IL-8 and TNF-α production [32]. Taken together, these findings suggest that RSV might alter the expression of TLR4 to modulate the signaling pathway associated with this receptor and enhance the expression of pro-inflammatory molecules by infected cells.

On the other hand, the RSV glycoprotein G has been shown to inhibit TLR3/4-mediated activation of interferon-stimulated response elements (ISREs) and block IFN-β production [33]. This observation is consistent with the fact that the RSV G protein can modulate the expression of IFN-stimulated gene (ISG)-15 and the suppressors of cytokine signaling (SOCS), the latter linked to TLR signaling [34–36]. Negative modulation of TLR signaling by the G glycoprotein might serve to interfere with the induction of type I IFNs secretion through TLR4 activation and signaling by the RSV F protein [35]. These data support the notion that exposure to RSV might condition lung tissues to a complex interplay between enhanced inflammatory stimuli and IFN shutdown that could manifest during subsequent infections with virus or bacteria [37,38].

Although TLR7 expression is up-regulated on lung epithelial cells as early as 1 h after infection with RSV, the participation of this receptor on cytokine secretion and modulation of RSV pathology has been only poorly evaluated [39]. It was not until recently that RSV and the measles virus were described as the first viruses capable of blocking IFN secretion through TLR7 and TLR9 signaling [40]. In agreement with this observation, deletion of TLR7 was recently shown to worsen RSV-induced pathology with increased expression of IL-4, IL-13, and IL-17 in the lungs [41]. However, some TLR7 agonists have been shown to enhance disease severity in RSV infected mice [42]. Taken together, these data suggest that in response to a variety of TLR stimuli, type I IFN expression can be inhibited by host cell infection by RSV. Such an inhibitory mechanism is due probably to the capacity of RSV to simultaneously target convergent signaling pathways downstream of several activating receptors, such as the STAT proteins discussed below [43,44].

Due to the pivotal role played by the cytokines produced in the lungs in response to infection by pathogens such as RSV [45], it is important to further assess the relative contribution of TLRs and pattern recognition receptors to the anti-viral response, lung inflammation, immune cell recruitment and viral clearance. Defining the contribution of each individual TLR to RSV-mediated pathogenesis could be extremely useful for the development of new therapeutic strategies for modulating the signaling cascades induced by these receptors. It is likely that targeting the viral motifs recognized by these receptors could help to attenuate the detrimental secretion of pro-inflammatory cytokines at the infected mucosa.

3. Early immune response induced by RSV infection

Upon activation of pattern recognition receptors, NF-κB is translocated to the nucleus, promoting the transcription of several pro-inflammatory genes (Fig. 1) [24,26,46,47,48]. This event leads to the production and secretion of cytokines and chemokines that promote the recruitment of inflammatory cells, such as neutrophils, eosinophils, natural killer (NK) cells and $CD4^+$ T cells to the infected lungs [49]. As a result of RSV infection, there is an increased expression of molecules inducing local inflammation, antigen processing and chemoattraction of inflammatory cells to the lung epithelium [50]. Interestingly, it has been suggested induce early NF-κB dependent-responses through a non-canonical activation of the NF-κB pathway [51]. Such an alternative pathway involves the activity of NF-κB-inducing kinase/IκB kinase alpha (NIK/IKK-α) and the NF-κB2 complex, prior to the activation of the more potent canonical pathway (Fig. 2) [51]. In addition, it has been suggested that RSV infection can also induce the nuclear translocation of NF-κB through the canonical pathway due to the production of reactive oxygen species (ROS) [52] and induction of RelA phosphorylation by mitogen- and stress-activated protein kinase (MSK1) [53]. Furthermore, RSV proteins NS2 and M2-1 have also been identified to be activators of NF-κB, either indirectly or directly by binding to RelA [54,55]. Finally, the retinoic acid-inducible gene I (RIG-I) was recently identified as a major intracellular RSV sensor upstream of both NF-κB canonical and non-canonical pathways (Fig. 2) [48,52].

As a result of RSV infection and NF-κB activation, respiratory epithelial cells are induced to secrete specific cytokines and chemokines, such as type I interferons, CXCL10 and CCL5 [56–61] (Fig. 1). The secretion of these molecules by RSV-infected epithelia promotes the recruitment of neutrophils, eosinophils, monocytes, regulatory and memory T cells from the peripheral blood into

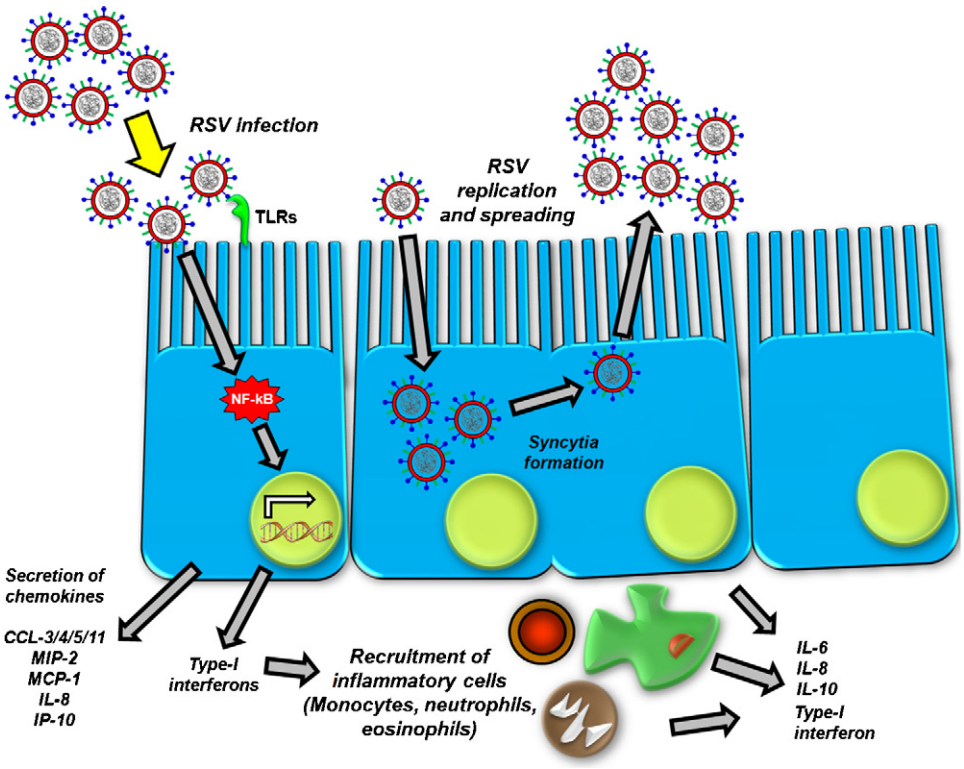

Fig. 1. RSV infection modifies the inflammatory environment in the airways. The first event after RSV enters the body involves the infection of airway epithelial cells at the alveoli. As a result, a defense mechanism against viral spreading is induced in infected cells that promotes the activation of NF-κB by RSV-derived molecular patterns (dsRNA), followed by secretion of IFNα/β. Simultaneously, engagement of surface TLR4 by RSV induces the secretion of several chemokines and cytokines, including MIP-1β/CCL4, MIP-1α/CCL3, MIP-2, IP-10/CXCL10, IL-8/CXCL8, eotaxin-1/CCL11, MCP-1 and RANTES/CCL5. On the other hand, viral replication inside cells leads to the formation of syncytia, which promotes virus replication and spreading. Later on during infection, viral replication is accompanied by massive infiltration of inflammatory cells into the lungs in response to a chemokine- and cytokine-rich environment.

infected tissues [60,62,63]. Then, inflammatory cytokine secretion is further enhanced at the site of infection by those recruited immune cells [60,64,65]. However, certain T cell subsets that infiltrate the infected lungs have been shown to secrete also anti-inflammatory cytokines in the lungs, such as IL-10 [66–68]. It is thought that T cells secrete this molecule in an attempt to reduce the airway inflammation caused by innate immune cells. This notion is in agreement with the observation that increased IL-10 expression correlates with reduced eosinophilia in a murine model of RSV-enhanced disease [69–71]. Nevertheless, IL-10 combined with particular Th2-signature cytokines, such as IL-4, can in turn promote detrimental immune responses that fail to clear RSV infection [72–75]. Accordingly, it has been shown that increased levels of IL-10 together with soluble ICAM-1 in nasopharyngeal secretions correlate with a more severe pathology in infected children [71]. Thus, differential rates and combinations of pro- and anti-inflammatory molecules are likely to influence in a significant manner the outcome of RSV pathogenesis. Furthermore, these findings suggest that the combined secretion of IL-10 in the lungs with Th1-promoting cytokines might reduce to some extent the lung-damage caused by the virus, while the same cytokine in combination with other molecules could contribute to enhancing the pathology [67,72,76,77].

In addition, it is thought that the early response to RSV promoted by NF-κB activation could shape the damaging inflammatory responses observed in the lungs of infected individuals. Accordingly, down-regulation of NF-κB activity has been shown to decrease lung inflammation upon infection [78]. This notion is supported by a recent study suggesting that the susceptibility to display an asthma-like syndrome after RSV infection could be due to differential expression of NF-κB subunits in the lungs [79]. For instance, expression of the NF-κB p50-subunit was increased in the lungs before and after RSV-infection in susceptible mice [79]. In contrast, animals that fully recovered displayed predominant expression of the NF-κB p65-subunit prior to infection, which shifted only moderately to the usage of the p50-subunit after infection (Fig. 2) [79]. Along these lines, rat strains susceptible to long-term chronic airway disease expressed higher levels of the NF-κB p50-subunit in the lungs upon viral challenge as compared to strains that fully recover from infection [79]. On the other hand, animals suffering less severe disease were shown to predominantly express the NF-κB p65 subunit in the airways, which temporarily shifted to the p50 subunit upon virus infection [78].

The secretion of other inflammatory molecules, such as TNF-α and RANTES, is also tightly regulated by the differential expression of NF-κB subunits (i.e. p50 vs. p65), suggesting an important role for the regulation of these transcription factors at shaping inflammation in the lungs [79]. TNF-α is a major pro-inflammatory cytokine that can cause chronic inflammatory disease when secreted in large quantities [80] and has been linked to eosinophilia upon RSV-infection [67]. Consistently, a recent study has associated RSV-induced asthma with TNF-α polymorphisms, suggesting that genetically mediated up-regulation of this cytokine could contribute to the excessive airway inflammation and more severe RSV pathology linked to the onset of asthma (Fig. 2) [80]. Similarly, a point mutation in the RANTES promoter, modulates NF-κB activity has been associated with increased susceptibility to severe bronchiolitis and recurrent wheezing after RSV infection (Fig. 2) [52,81]. These findings suggest that secretion of chemokines by epithelial cells at the airways and infiltrating immune cells after RSV infection can be detrimental to the host by promoting immunopathology and tissue damage with no

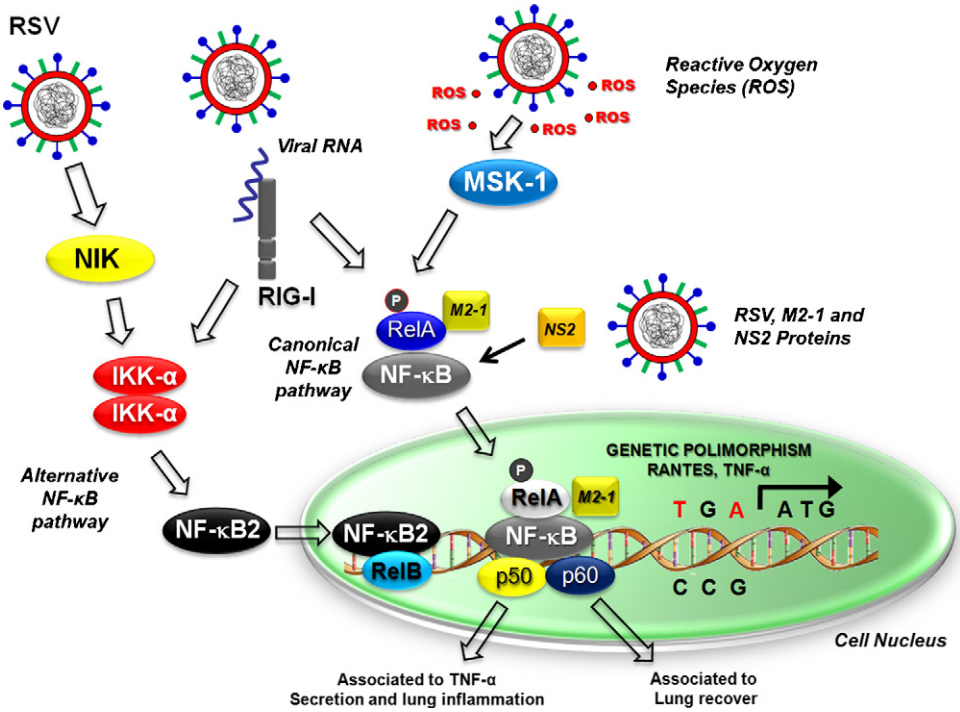

Fig. 2. NF-κB activation induced by RSV. Early upon infection, RSV induces NF-κB activation through a non-canonical activation pathway involving the NIK/IKK-α and the NF-κB2 complex prior to the more potent canonical pathway. On the other hand, RSV produces reactive oxygen species (ROS), which induce MSK1-dependent RelA phosphorylation leading to NF-κB translocation to the nucleus through the canonical pathway. The retinoic acid-inducible gene I (RIG-I) has been recently identified as a major intracellular RSV sensor upstream of the canonical and non-canonical pathways, promoting NF-κB translocation to the nucleus. Also, RSV proteins NS2 and M2-1 have been shown to be activators of NF-κB either indirectly or directly by binding to RelA. Another study revealed that expression of distinct NF-κB subunits in the lungs may determine susceptibility to an asthma-like clinical syndrome after RSV infection. Expression of the NF-κB p50-subunit is associated with a developing RSV-infection. Contrarily, lungs fully recovered from infection with RSV predominantly display expression of the NF-κB p65-subunit. Finally, genetic polymorphisms on crucial cytokine genes involved in RSV pathology, such as CCL5/RANTES and TNF-α have been recently linked to the onset of asthma and increased susceptibility to severe RSV-induced bronchiolitis.

significant contribution at controlling virus replication and dissemination.

4. Role of type I IFNs on RSV immunopathogenesis

Another set of important molecules that are secreted by immune and non-immune cells upon viral infection are type I interferons (IFNs). These cytokines are produced by infected cells to counteract viral replication and persistence (Fig. 1) [82]. These molecules bind to surface receptors belonging to the type I IFNAR complex, which in turn activate several intracellular signaling pathways that promote the activation of anti-viral responses [83,84]. Such cellular responses are characterized by the expression of interferon-stimulated genes (ISGs) [52,55,72,77,78,81,84,85] that trigger effector functions which include the activation of ribonuclease L for RNA degradation [86], proliferation and activation of NK cells [87]. In the lungs, production of type I IFNs derives mainly from plasmacytoid dendritic cells (pDCs), epithelial cells and macrophages [88,89].

Several studies have suggested that RSV can actively prevent type I IFN production. The mechanism of inhibition seems to rely on RSV NS1 and NS2 proteins, which are required for suppressing type I IFN secretion by epithelial cells [90]. Co-immunoprecipitation studies have shown that NS1 and NS2 associate as heterodimers in the infected cells [91]. Apparently, these two RSV proteins work in a coordinate fashion by selectively promoting proteasomal degradation of STAT-2, a transcription factor essential for the expression of IFN-induced antiviral genes [92]. As a result, several cellular responses promoted by the activity of STAT-2 are suppressed. NS1 and NS2 have also been shown to cooperatively dampen the activation and nuclear translocation of IRF-3, a transcription factor inducing the expression of type I IFNs [43]. Consistent with this notion, a recent report has provided evidence suggesting that NS1 reduces IKKε expression, which phosphorylates and activates IFN regulatory factor 3 [91]. In the same study, NS2 was shown to decrease the levels of STAT-2. Acting together and by a mechanism independent of proteasomal activity, NS1 and NS2 also decrease TRAF3 levels, a molecule required for integrating multiple signals leading to the induction of IFN [91]. Moreover, NS2 was recently shown to inhibit the transcription of IFN induced by either the RIG-I or the TLR3 pathways [46]. It is thought that NS2 can bind to the N-terminal CARD domain of RIG-I and block its interaction with the downstream component MAVS [46]. Thus, the NS2 protein can also act as an IFN antagonist that downmodulates both IFN induction and IFN signaling pathways [46].

In agreement with the relevant role of type-I IFN production by RSV are the data obtained from mice deficient on STAT-1 and -2 proteins. These two molecules work as signaling elements required for the response to IFN α/β and IFN-γ. These studies have shown that animals deficient on these signaling molecules display severe inflammation, enhanced eosinophil airway infiltration and increased Th2 cytokine secretion in the lungs after infection with RSV [93]. Nevertheless, secretion of IFN-α/β and IFN-γ during the innate immune response can also contribute to the recruitment of additional inflammatory cells to the airways after RSV infection [94]. While lung eosinophilia could be observed in mice lacking IFNα/β and IFNγ receptors ($IFN\alpha\beta\gamma R^{-/-}$) after RSV infection, these animals showed reduced lymphocyte infiltration in the lungs [94]. On the other hand, mice lacking only the IFN-γ receptor, displayed moderate eosinophilia [94]. Taken together, these data suggest that type I IFNs might be critical for the recruitment of inflammatory cells to the lungs during RSV infection. It is likely

that RSV replication in infected tissues is enhanced as a result of down-regulation of the type I IFN response. In addition, these data suggest that RSV can reduce type I IFN secretion within infected tissues while promoting bystander recruitment of inflammatory cells to the airways, further contributing to lung damage caused by viral infection.

5. Viral and host elements that influence RSV-induced cytokine secretion

Unlike other respiratory viruses, such as rhinovirus [95] and influenza [96], RSV shows little antigenic variability during outbreaks. However, RSV outbreaks fluctuate between two well characterized subgroups defined based on the variability of the attachment glycoprotein G (subgroups A and B) [97–100]. The G protein harbors most of the virus variability, reaching up to 12% of aminoacid sequence diversity, within a defined subgroup and up to 47% across subgroups, both at the amino acid level [100,102]. It is thought that the diversity that accumulates within this protein could play an important role in virulence, because during outbreaks a reduced but consistent level of selection of strains expressing G protein variants takes place [101,103]. Although no particular strains are considered significantly more virulent than others, disease severity has been correlated with certain specific viral genotypes, which generally map to the gene of the G protein [104]. On the other hand, the amino acid sequence of the surface fusion F protein is 4-fold less variable than the G protein [105,106], which has allowed the development of neutralizing antibodies for clinical treatments against all the circulating RSV strains [107]. However, to date there are practically no experimental studies assessing the contribution of polymorphisms on these viral proteins to RSV-induced secretion of pro-inflammatory cytokines. It is very likely that polymorphisms in viral proteins could significantly alter their recognition by the immune system (such as pattern recognition receptors). Thus, such polymorphisms could modulate downstream signaling cascades leading to the nature of the immune response triggered by the virus in the host.

Previous studies in mice have suggested an important contribution of the host genetic background to the susceptibility of developing severe symptoms after RSV infection [108,109]. These studies have allowed characterizing certain mouse strains as more permissive than others for establishing a model for RSV infection [87,108]. Several relevant pathology markers related to RSV infection in humans, such as lung infiltration and lung resistance, can be also observed in most mouse strains that are used as models for RSV infection [34,110]. Furthermore, the availability of knock-out and transgenic mouse strains in the genetic backgrounds that show RSV-induced symptoms will contribute to defining some of the host immunological factors that modulate the virus-induced pathology.

Several genetic markers that predispose to severe RSV pathology have been identified in humans, such as RANTES and TNF-α as described above (Fig. 2) [50,80,81,111]. However, a contribution of the polymorphisms initially identified in TLR4, has been ruled out [112,113]. In addition, single nucleotide polymorphisms (SNPs) have been mapped in other innate immune system-related genes, such as VDR (vitamin D receptor), JUN (a protein that forms part of the transcription factor AP-1), IFNA5 (interferon alpha 5) and NOS2 (radical formation and proinflammatory response) [50]. Along these lines, a study has identified SNPs within the IL-4 gene and a risk haplotype across IL13 CNS-1 and IL-4 (haplotype at the IL13-IL4 locus) that seems to associate with increased IL-13 production and an elevated risk to develop severe primary RSV bronchiolitis in early infancy [114]. More recently, a SNP polymorphism was identified in the IL-9 gene, which surprisingly had opposite effects on the susceptibility to severe pathology depending on whether the polymorphism was expressed in boys or girls, the former being more susceptible [115]. Another study also has associated the major histocompatibility complex (MHC) haplotype as a relevant determinant affecting the outcome of neonatal RSV infection [116]. Despite the existence of several studies identifying host polymorphisms associated with the severity of RSV pathology, they usually lack functional assessment. Hopefully, the development of new haplotype-based analysis tools facilitated by advances in biostatistics and bioinformatics could promote the identification of specific relevant loci across the genome, which contribute significantly to disease susceptibility during RSV infection.

Strong correlations have also implicated age as another important parameter defining severity of the disease caused by RSV infection. It has been reported that a detrimental phenotype in response to RSV is limited to infants less than 6 months of age [114,117]. Consistent with this notion, experiments in mice reveal increased pathology in neonatal individuals as compared to adults, despite similar viral kinetics [117,118]. In fact, newborn mice display increased pathology as manifested by augmented inflammatory cell recruitment to the lungs with reduced or delayed IFN-γ secretion [118]. Furthermore, differential immune outcomes can be observed in response to subsequent re-infections when comparing animals infected during neonatal life with those infected at weaning, the former group showing more severe and damaging symptoms [114]. It has been suggested that age-related responsiveness to viral infection and lung injury could be explained by lung maturity at the time of infection. Immature lungs from preterm or newborn babies usually display decreased alveolarization and gas exchange efficiency, as well as differential synthesis and release of surfactant phospholipids and apoproteins as compared to adults [119]. Furthermore, maturation status of the lungs can determine the quantity of inflammatory cell infiltrate, subepithelial basement membrane thickening and fibrosis, goblet cell hyperplasia and smooth muscle hypertrophy, in response to tissue insults, such as epithelial desquamation [120,121]. Thus, it is likely that the nature of the inflammatory response and the patient outcome after viral infection will significantly depend on the maturation level of the lungs.

Taken together, specific factors from the virus such as virus diversity, as well as features of the host, such as genetic polymorphisms and age at infection (lung maturation) are likely to significantly influence the polarization and cytokine profiles of immune response against the virus. Thus, these factors require further research to better understand RSV-induced pathology.

6. Potential strategies for treating RSV-induced cytokine pathology

As for many other viruses, treatment against RSV infection is limited and deficient, consisting mainly of approaches aimed at alleviating infection-associated symptoms [122–124]. As cytokines have been shown to play an important role during RSV pathology, they could be considered as potential pharmacological targets for preventing disease in severe cases of infection or predictors for prognosis [125–126]. In fact, blockade of certain cytokine/chemokines could be used to reduce lung pathology caused by RSV. Such an approach consists in the administration of antibodies that block the activity of certain cytokines *in vivo*, as RANTES and CCL20 [127,128]. These treatments have been shown to significantly decrease airway hyperreactivity in mice and prevent excessive production of mucus by epithelial cells after RSV infection [129]. Another effective approach has been the treatment with Met-RANTES, an antagonistic competitor for the RANTES receptor, which reduced the recruitment of inflammatory cells into the lungs of infected mice [60]. Furthermore, *in vitro* treatment with

RANTES/CCL5 has been shown to reduce RSV infection of HEp-2 cells, probably by blocking the interaction between the F protein of RSV and proteins on the surface of epithelial cells [128]. In addition, treatment with an antibody that blocks CCL11 can diminish lung eosinophilia and disease severity in mice [130]. Such a treatment also inhibited infiltration of $CD4^+$ T cells into the lungs, without preventing infiltration of $CD8^+$ T cells [130]. These data are consistent with observations in MIP-1α knock-out mice challenged with RSV, which showed a significant reduction in lung histopathology and inflammation, as compared to wild-type animals. However, when lung viral titers were measured, equivalent viral loads were observed for MIP-1α knock-out and WT mice [131]. These results suggest that blockade of specific independent cytokines could serve as an effective strategy to treat severe cases of RSV infection. Moreover, it is likely that combinations of blocking agents targeting multiple cytokines may achieve better results. However, whether the blockade of these inflammatory cytokines could be detrimental for the clearance of other viruses affecting infants remains to be ruled out.

7. Concluding remarks

Accumulative data generated throughout the last decades, suggest an important role for cytokines in lung damage induced upon infection with RSV. The lack of a strong IFN response, together with excessive secretion of pro-inflammatory cytokines has led to the notion that RSV is likely to produce immunopathology in the host, characterized by immune infiltration and inflammatory cytokine secretion. Thus, the immune system can contribute significantly to lung damage, a major hallmark of this virus. Importantly, specific molecular patterns within viral proteins can produce *per se* severe immune responses and contribute to the establishment of an inadequate immune response in infected patients. These findings have proven to be extremely relevant for the rational design of current and future prophylactic and therapeutic strategies. RSV has proven to be a complex virus with multiple molecular mechanisms that contribute to immunopathology and interfere with the establishment of adequate immunity. It is noteworthy to mention that understanding the role of cytokines within a populated network of redundant, synergistic and antagonistic molecules is an extremely difficult task. In fact, this issue is an important limiting step for determining the effective contribution of individual cytokines, which would be diminished using breakthrough bioinformatics. Undoubtedly, comprehension of the dynamics of cytokines in complex modulatory networks will significantly contribute to the understanding of specific cytokine profiles arising during infection with respiratory viruses, such as RSV. This knowledge will contribute to identifying key targets for pathology treatment and to generate optimal approaches for the induction of efficient immunity against this pathogen.

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