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Syncytial virus respiratory infections in children – immunological aspects

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Abstract Out of all respiratory viral infections, those with human respiratory syncytial virus (HRSV) are the most frequent registered worldwide being responsible for bronchiolitis, pneumonia, and asthma in all age groups, especially in children. As children have an immature immune system, severe HRSV infection can lead to death. In this paper we will revise the most recent findings regarding the immune response in the HRSV infection focusing on children. Extensive studies indicate that viral and host factors modulate the severity of infection. The viral infection first triggers a strong innate immune response followed by a further adaptive immune antiviral response. Although HRSV infection is common, there is still no efficient therapy available. The hurdles in obtaining an efficient therapy reside in several issues: the immaturity of the immune system of infants and young children, the differences in the virulence potential of the viral strains that induce a different cytokine/chemokine host response, with the involvement of multiple molecular, still unknown pathways. Identification of antiviral immune mechanisms and the comprehensive characterization of virus-specific immune responses using new cellular and animal models would help to define the crosstalk between viral and host factors that would modulate the severity of infection. Moreover identifying the molecular mechanisms of acute airway disease would shed light also on associated long-term consequences like developing asthma in the adulthood. As HRSV is globally circulating for more than 60 years, an effective therapy remains a public health priority.

Keywords: syncytial virus, respiratory infections in children, antiviral immunity

Introduction

In children, the major disease that would encompass medical care is the respiratory tract infection (RTI). When the respiratory tract infections (RTIs) show an increased frequency, a primary immunodeficiency is suspected and that is the case for over 60% of the cases when mainly humoral immunity disorders are diagnosed. A recent retrospective study has shown that immunoglobulin deficiencies are affecting different isotypes. Thus, RTIs in children determine abnormal levels of IgG, and also of IgA and IgM (Pasternak et al., 2018). When studying cytokine secretion in RTI occurred in children decreased levels of proinflammatory cytokines were found, mainly IFN-gamma, indicating the increased risk for developing RTI.

Interestingly, the applied therapy improved the proinflammatory cytokines values and protected from new RTI (Ivanova et al., 2015). As Interferon (IFN)-stimulated genes (ISGs) sustain the antiviral immune response, the regulators of these genes were studied. Viral or bacterial infections downregulate the Nuclear Dbf2-related kinase 1 (NDR1) expression through type I IFN signaling pathway. Mechanistically, NDR1 increases the translation of the transcription factor STAT1 by directly linking to the intergenic region of miR146a. These new molecular findings show that IFN-dependent antiviral immune response, residing on NDR1 is an important molecular mechanism of viral infections control (Liu et al., 2018).

Tonsils and adenoids are secondary lymphoid organs, that have the main immunological function to facilitate the encounter of antigens/infectious agents with lymphocytes that have specific receptors triggering the specific immune response. Within these lymphoid organs a subset of T helper cells specialized to induce B-cell development, T follicular helper cells (TFH) are highly involved in the anti-pathogens response. Comparing the two secondary lymphoid organs it was shown that in resting adenoids, TFH had higher expression of CXCR5 and inducible costimulatory receptors, but lower PD-1 in comparison to the sub-population from tonsils. Moreover adenoidal B cells present a high expression of CD27 receptors. *In vitro* stimulation induced TFH and B lymphocytes from the adenoids to proliferate intensely and to secrete high levels of interleukin 21, which stimulates the differentiation of cytotoxic T cells (Tc), able to destroy the virally infected cells. All these findings prove that adenoids are better "immune guardians" then tonsils (Morris et al., 2016).

The importance of the subject relies also on the complications that can further develop due to RTIs, one of them being childhood asthma. This disease triggered by RTI resides in an increased percentage of lung mast cells (MCs). The study showed that RTI in young children induce an accumulation of alveolar MCs that would induce later asthma development (Andersson et al., 2018).

Fig. 1. Schematic representation of the immune response strenghts during childhood (adapted from Simon et al, 2015).

Infectious agents that trigger the respiratory and digestive tracts illness, are a leading cause of morbidity and mortality throughout the world in infants and young children, and T lymphocyte immune responses that develop site-specific immunity are mandatory to study for developing therapies that induce an effective immune response in a still immature immune system, like that of the children (Zens et al., 2017). Our experience regarding the persistence of virus infections, like human papilloma virus, points out that immune markers can indicate the stage of infection and orient further the therapy, even the vaccination protocols (Boda et al., 2016, 2018, Spinu et al., 2018).

Out of all respiratory virus infections, human respiratory syncytial virus (HRSV) infections are the most frequent registered worldwide being responsible for bronchiolitis, pneumonia, and asthma at all age groups but especially in children. Worldwide HRSV infection is the leading cause of viral bronchiolitis and pneumonia in children.

70% of children are infected in their first year of life, and in the second year all children will be infected with this virus (Bueno et al., 2008).

The infection will lead to mild upper respiratory tract symptoms, and up to 40% lower respiratory tract infections, mainly bronchiolitis (McNamara and Smyth 2002).

As children have an immature immune system, severe HRSV infection can lead to death. Around 3% of cases will reach Intensive Care Unit (ICU) where the associated mortality will reach up to 10% (Hervás et al., 2012). At the development of HRSV infection, the immune response maturity of the host is crucial and due to the fact that it does not induce a long lasting adaptive immunity, HRSV infection becomes recurrent and impedes vaccine development (Farrag and Almajhdi 2016).

Within our paper we will revise the most recent findings about HRSV infection and host immune response, focusing on children's immunity.

Immune system development in children

The immune system undergoes major changes throughout life. There are over 1600 genes that are activated/suppressed during activation/control of innate

and adaptive immune responses (Abbas et al., 2005). The genes that encode immune-related elements and the transcriptomic apparatus that regulates these genes have to continuously adapt to changes in the external and internal environment. For humans, the immune system is immature at birth and hence will evolve during childhood, as being exposed to various new antigens (Simon et al., 2015). In Figure 1 a schematic representation of the immune response strenghts during childhood is presented. At birth and several months after, the immune effectors are represented mainly by the maternal antibodies (natural passive immunity). Afterwards, as the immune system is becoming more mature, the innate and the adaptive arms of immunity evolve and maintain an increased strength up to around 15 years where it enters in a plateau phase that will decline afterwards in old ages.

In early childhood the immune system will have to cope with a large variety of antigens /pathogens. The immune system is immature, and, in early life, the functionality of T lymphocytes, is highly involved in anti-infectious response (Zens et al., 2017).

Neonatal colonization of the airways with different respiratory pathogens are responsible for the increased risk of lower respiratory infections (LRI) in the early childhood. Vissing and coworkers (2016) realized an interesting study, starting from the hypothesis that children developing LRI have an aberrant immune response to bacterial pathogens in infancy. Therefore, they performed on over 400 children up to 3 years of age (born of mothers with asthma); they surveyed them by 6-monthly planned visits and visits at acute respiratory episodes. In the same time, the authors realized an *in vitro* study of the systemic immune response by stimulation of the peripheral blood mononuclear cells (isolated at age 6 months from 291 infants) exposed to common respiratory pathogens (e.g. *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae*), the response being characterized by the production of TNF-α, IFN-γ, IL-2, IL-5, IL-10, IL-13 and IL-17. This study proved that when children were diagnosed with LRI, TNF-α and IL-5 were found mainly downregulated, but did not explain entirely the LRI susceptibility. These findings show that if an alteration of a systemic immune response occurs in early life after exposure to common airway pathogens the children will have a high risk for future LRI (Vissing et al., 2016).

Recent reports have shown that the T cell mediated immune response that is related to lung offers protection to respiratory infections. Local *versus* systemic immune response was studied in youngsters aged less then 4 years with severe respiratory failure upon viral respiratory tract infections. Although total $CD3^+$ T lymphocytes did not reveal the presence of an infection, the CD8:CD4 T cell ratio was significantly increased in infected infants with acute lung infection. T lymphocytes that were identified in the airways were phenotypically and functionally different from the ones identifyed in the peripheral blood having an activated/memory phenotype and an increased cytotoxic activity (Connors et al., 2016). In infants with bronchitis, bronchopneumonia, and bronchiolitis different results were obtained in terms of T cell response. Thus, lower numbers of $CD3^+$ and $CD3^+CD8^+$ T cells were reported in comparison to control groups and therefore a significantly higher CD4/CD8 ratio. In severe bronchopneumonia, the reduction of T cells was correlated with the increase in B cells. In the bronchiolitis group a significantly higher number of Th cells, and thus a significantly higher CD4:CD8 ratio was found. All the disease groups had a higher number of B cells and lower number of natural killer cells (NK) in comparison to controls (Jia et al., 2016).

To study these specific CD8+ lymphocytes it was reported that CD8α chain of the dimeric CD8 protein is the most importnat coreceptor of cytotoxic T cells. This protein is encoded by the gene CD8A that encompasses a missense mutation in children with increased susceptibility to recurrent respiratory infections. This mutation does not affect the TCR-mediated proliferation of T cells, but gives a hint for the children sub-populations that are proned to respiratory infections (Dumontet et al., 2015).

Syncitial virus respiratory infections in children

HRSV is an enveloped virus from the Pneumoviridae family, Orthopneumovirus genus. This virus is the most important pathological agent that causes severe acute lower respiratory tract infection, especially in infants, children and in the immuno-compromised adults. HRSV can cause bronchiolitis and pneumonia and is associated with the development of recurrent wheezing and asthma (Bohmwald et al., 2017). The lung pathology is due not only to the virus infection *per se* but also to the exacerbated host immune response, where immune cells infiltrate the lungs. HRSV has a non-segmented genome and a single RNA that has 10 genes encoding for 11 proteins including Fusion protein (F), the Glycoprotein (G), and the Small Hydrophobic (SH) protein, all these proteins being on the virus surface. Inside the virion, there is another group of proteins: Nucleoprotein (N), Phosphoprotein (P) large polymerase protein (L) part of the RNA-dependent RNA polymerase complex, M2-1 protein (transcription elongation factor), M2-2 protein (regulator of viral transcription) and (M) protein. HRSV genome encodes also for the non-structural 1 and 2 proteins (NS1 and NS2). All these proteins take part in the immune evasion mechanisms, as virulence factors and inhibitors for IFN-related mechanisms. NS-1 and NS-2 proteins can inhibit type I IFN response. HRSV nucleoprotein inhibits the immunological synapsis, meaning the physical contact between DCs and T cells during infection and activation of the cellular specific immune response. This inhibition induces an inefficient T

cell activation and evasion of the virus (Canedo-Marroquín et al., 2017).

When HRSV attaches itself to the airway epithelia, the host's immune system response is triggered, starting with innate immune response and further with adaptive immune response. Infection of the alveolar epithelial cells induces the recruitment and activation of several leukocyte populations. Macrophages, NK cells, eosinophils, dendritic cells, and neutrophils are activated through cytokines, chemokines, and other immune mediators. All this enhanced activation of the innate immunity besides clearance of the virus induces bronchiolitis and asthma (Farrag and Almajhdi 2016).

The complex immunological pathways triggered by HRSV leads also to the therapeutical issues. A specific therapy for this viral infection is not currently available. In a recent published study it was shown that an exchange protein that is directly activated by cyclic AMP (EPAC) could be a future therapy target. Using an EPAC inhibitor (ESI-09), in *in vitro* studies it was shown that HRSV replication was inhibited along with an induction of proinflammatory cytokine/chemokines. Moreover ESI-09 suppressed the RSV-activated transcriptional factors from NF-κB and IRF families. Thus this EPAC inhibitor can hinder HRSV replication and enhance innate inflammatory responses (Choi et al., 2018).

From the therapeutical point of view HRSV infection had dramatic turns when in 1967, infants and toddlers were immunized with an inactivated form of HRSV as a vaccine and when encountering the wild-type HRSV they had an exacerbated disease with high fever, bronchopneumonia, and wheezing and two immunized toddlers died. After decades of years of research, the immunological explanation came: the immunization induced production of a pathogenic Th2 memory lymphocyte, that upon encounter with the wild-type HRSV determined an enhanced number of eosinophils and immune complex deposition in the lungs, instead of an effective antiviral response (Acosta et al., 2016).

Innate immunity in HRSV infection

The first line of defence in HRSV infection is the important unspecific immune cells: monocytes, macrophages (MΦ), and dendritic cells (DCs). All these populations of immune cells contribute to the acute inflammation that is developed during HRSV-induced bronchiolitis and asthma. HRSV will encounter first the respiratory epithelial cells, alveolar macrophages (AMs), DCs, and monocytes that are resident in the airways. In this niche the main sentinel-cells are AMs so these cells will be the first ones which recognize an invader of the tissue and will develop the early immune response against HRSV. These cells will start to induce the viral clearance (Bohmwald et al., 2017).

When the immune response is triggered, neutrophils are recruited as well. The mechanisms of NETosis triggered by neutrophils upon viral infection was shown by Muraro et al, 2018. The study showed that HRSV infection triggered reactive oxygen species (ROS) - dependent NETosis in human neutrophils and the virus was trapped in the extracellular DNA lattices.

Intracellular signaling activation was induced through PI3K/AKT, ERK and p38 MAPK pathways. Upon infection of alveolar epithelial cells or lung fibroblasts NET-DNA is released by neutrophils, pointing out that neutrophils can identify HRSV-infected cells and respond to infection by releasing NETs. This recently shown mechanism can lead to designing of new therapeutic approaches in HRSV infection (Muraro et al., 2018).

HRSV induces in airway epithelial cells an inflammation triggered by epidermal growth factor receptor (EGFR). Kalinowski and coworkers (2018) have shown that inhibiting EGFR the infection can be significantly reduced. Moreover it was demonstrated that infection suppresses IFN regulatory factor (IRF) 1-induced IFN-λ production. If EGFR inhibition is applied during respiratory infection, an augmentation of IRF1, IFN-λ, and a decrement of HRSV titers were obtained. This report suggests that the antiviral action of the immune – related molecule IFN is mediated by a direct action of the virus upon a receptor that is specific to the airway epithelial cells. By these findings a new mechanism for antiviral therapy action can be elucidated (Kalinowski et al., 2018).

During severe HSVR bronchiolitis, it was shown that children display in the bronchoalveolar lavage (BAL) and peripheral blood various unspecific immune cells (or cells of the innate immunity). Thus, in BAL classic DCs (cDCs), NKT cells, NK cells were accumulating. Additional plasmacytoid DCs (pDCs) and T cells were identified along with high levels of proinflammatory cytokines. In their peripheral blood cDCs exhibit increased activation markers. Interesting findings were reported when these immune cell populations where correlated with the children's age. Preterm babies and under 4 months infants had fewer BAL pDCs in comparison to term born babies and older then 4 months infants. During HSRV infection, cDCs accumulated in the lower airways in the bronchiolitis phase, become activated inducing further activation of T cells, NKT cells and NK cells, the entire cells' orchestra inducing the inflammatory pattern of the disease. The immaturity of the immune system proven by the low pDCs cell population in preterm and under 4 months infants can explain the weak antiviral responses (Kerrin et al., 2017).

In infected children in their peripheral blood mononuclear cells (PBMCs) increased levels of miR-26b and lower TLR4 mRNA were found. This study predicted that miR-26b probably has as target TLR4. When verifying *in vitro* this hypothesis, miR-26b significantly down-regulated TLR4 mRNA and its protein expression along with IFNβ concentrations. It is possible that HRSV infection to inhibited TLR4 signaling by up-regulation miR-26b, that could thus become a novel therapeutic target for preventing the infection (Liu et al., 2015).

Adaptive immunity in HRSV infection

In the adaptive immunity against HRSV infection, two subsets of T lymphocytes are involved: T helper 17 cells (Th17) and the regulatory T-cells (Treg), and the ratio between the two sets. During the infection, these cells have different functions (Mangodt et al., 2015), as summarized in Table 1.

In the serum or nasopharyngeal fluids of HRSV infected children it was demonstrated a ***Th2 predominance*** indicated by an increased IL-4 concentration. In the same biological fluids it was demonstrated a decreased Th1 response, associated with decreased levels of IFN-γ (Legg et al., 2003; Hassan et al., 2008; Dulek et al., 2014; Qin et al., 2014).

The severity of the infection was correlated with an increased IL-4/IFN-γ ratio (Hassan et al., 2008). This Th1/Th2 balance is not an absolute marker for HSVR infection because there were also reported studies with Th2 predominance (Pinto et al., 2006) or decreased Th1 response (Benten et al., 2003). These results show in fact that the mechanisms that are involved in the immunity during severe HSVR infections are more complex (Mangodt et al., 2015).

With almost 100% of children infected with HSVR in the first 2 years of life, just 3% develop a severe case of respiratory infection. Thus the assessment of gene expression encoding adaptive immune system elements can bring additional data. Children with severe HSVR infection had over-expression of genes from pathways related to JAK/STAT, prolactin, and IL-9 signaling pathway genes (Mariani et al., 2017).

Experimental models of HSVR infection aided in this case also the elucidation of adaptive immunity involvement and moreover brought new data for the development of a HSVR vaccine.

Table 1. Treg and Th17 lymphocyte action in HRSV infections

Treg cells	Th17 cells
Recruit $CD8^+$ cytotoxic T-cells in the lungs	Decreases effector $CD8^+$ T-cell responses
Control inefficient Th2 immune responses	Increases Th2 cytokine production
Control innate immune response of neutrophils and NK cells	Increases neutrophils infiltration in the lungs
Control RSV-specific $CD4^+$ and $CD8^+$ T-cell responses	-

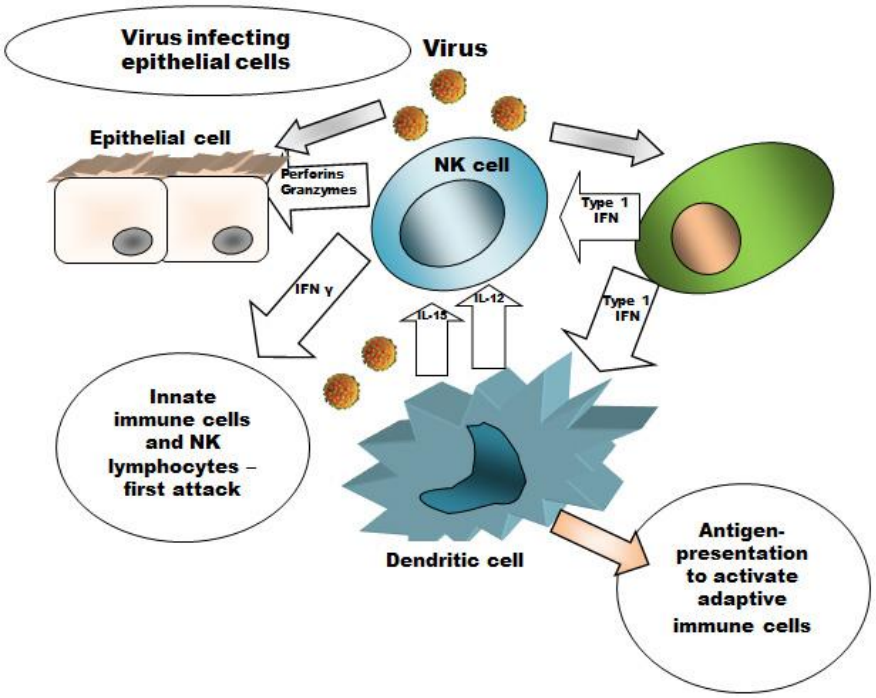

Fig. 2. Cellular immune mechanisms triggered by HSVR infection. Epithelial cells are infected with the virus and trigger NK cell activation that leads to perforines and granzymes production. Dendritic cells upon infection will activate through cytokine production innate immunity and will trigger the activation of adaptive immune cells.

Li and coworkers (2017) have shown in juvenile naïve AGMs monkeys that when infected with HSVR the viral loads at nasopharyngeal surfaces and in the lung have the highest values on day 5 post- infection, and decreases on

day 10. The infection induced $CD8^+$, and low values of $CD4^+$ T cells in the peripheral blood. Virus-specific $CD8^+$ T lymphocytes were 10 fold higher in lavage (BAL) compared to peripheral blood. These lymphocytes had effector memory phenotype with $CD95^+CD28^-$ pattern or tissue resident memory with the $CD69^+CD103^+$ phenotype. Declining viral titers were correlated with virus-specific $CD8^+$ T cells from peripheral blood and BAL. This finding suggests that cellular immune response, that is virus-specific, contributes to the virus clearance. Interesting is that infected monkeys were protected from subsequent exposure to infection, this bringing new important data for future therapy development (Li et al., 2017).

The main cellular immune mechanisms that are generated upon HSRV infection are presented in Figure 2.

Experimental models of HRSV infection

In vitro experimental models

Cellular models of infections are used for the evaluation of mechanisms underlying infection and for depicting future therapy targets. Using ***lung mucoepidermoid cells* (NCI-H292)** and DNA microarrays technology the genome-wide transcriptional anti-infectious response was studied in an experimental HSRV infection. A number of 330 differentially expressed genes were identified during the time course of infection. Out of the total genes, around 60% were found to be up-regulated after 2-3 days post-infection and reversed to normal starting from the 4th day post-infection. The genes that were found over-expressed encoded for proteins involved in interferon signaling, antigen processing and presentation, double-stranded RNA binding and chemokine activity. The highest expression was detected for genes encoding IFIT1, IFI44, MX1, CXCL11 and OAS1. The second wave of genes that were up-regulated encoded for proteins involved in cell cycle division and microtubule cytoskeleton organization. The experimental infection induces in the first 3 days an early innate immune response and thereafter host cell recovery is activated (Ampuero et al., 2018).

In ***airway epithelial cells*** (***A549 and small airway epithelial cells***) experimentaly infected with the virus, Hosakote and coworkers (2016) have studied the High-mobility group box 1 (HMGB1); the HMGB1 is a nuclear protein which interacts with nucleosomes and regulates transpription process. It is released from activated immune cells, being involved in inflammation and in pathogenesis induced by various infectious agents. Upon infection, HMGB1 is translocated from nucleus to the cytoplasm, being subsequently released into the extracellular space. HMGB1 that is released by airway epithelial cells upon infection will increase the activation of epithelial cells and induces the over-activation of monocytes in the airways, acting as a cytokine mediator of inflammation, bindind to Toll-like receptors (TLR) - TLR2 and TLR4 and mediating the release of other cytokines. Moreover it appears that the oxidative stress response is involved in the pathogenesis of HRSV infection, and when some anti-oxidants are used the release of HMGB1 is reduced. Besides the fact that HMGB1 is a DNA regulatory protein it serves as an extracellular cytokine promoting airway inflammation and acting as damage-associated molecular pattern (DAMP). These results open the way for new future therapy targets in HSVR infection (Hosakote et al., 2016).

As exosomes carry biologically active molecules, viral infections induce profound changes in exosome pattern and morover can be involved in viral transmission. A549 cells infected with HSRV were used also for studying the exosomes pattern upon infection. After infection RNA and protein signatures of the virus could be identified in exosomes. Although these exosomes have not been able to infect uninfected cells in an *in vitro* model, they could activate the production of cytokines and chemokines by the innate immune cells (monocytes), that establishing the role of subsequent proinflammatory players (Chahar et al., 2018).

In a 3D cellular model with differentiated pediatric primary bronchial epithelial cell cultures (**WD-PBECs**) the effect of HRSV was studied. In these cell IFN-λ1 and IL-29 was induced, but not type I IFNs. These effects mimicked the ones that HRSV induced in lower airway clinical samples from infected infants. When IL-29 was applied on these cells a prophylactic, but not therapeutic, effect was obtained against infection, probably due to the antagonism of IFN-mediated antiviral effect. Actually IL-29 is a type III IFN, and has the potential to prevent a HRSV infection (Villenave et al., 2015).

In vivo experimental models

Animal models are used mainly to explore new therapy targets and to explain the failure of classical vaccination ones.

Experimental models using CD169-diphtheria toxin receptor (DTR) transgenic mice have shown that alveolar macrophages (AMs) accumulate in infected lungs (Kerrin et al., 2017). After administration of diphtheria toxin to this type of mouse strain AM depletion and reduced recruitment of monocytes were observed and also the depletion of $CD169^+$ cells, reduced IFN-β, IL-6, and TNF-α levels, in BAL during HRSV infection but did not affect proinflammatory chemokines. The depletion of $CD169^+$ cells decreased the effector $CD8^+$ T lymphocytes recruitment to the lungs upon infection (Oh et al., 2017).

In fact, $CD169^+$ cells are macrophages and antigen presenting cells (APCs) that are redistributed upon immune activation in the lymphoid organs. These cells are involved in B lymphocyte activation and facilitate activation of invariant natural killer T (iNKT) cells. There are also data that show that $CD169^+$ macrophages can activate $CD8^+$ T cells transferring antigen to DCs in the spleen. Recent findings have shown that $CD169^+$ cells

as new therapeutic strategy in viral respiratory infections (Martinez-Pomares and Gordon 2012; Oh et al., 2017).

DCs and macrophages are the main sources of type I interferons (IFNs) production and proinflammatory cytokines in HRSV infection, but actually the pattern recognition receptors (PRRs) involved in HRSV sensing in these cells remain unclear. In a IFN-β/YFP reporter mouse model it was recently shown that myeloid differentiation primary response 88 protein (MyD88), but not Toll-like receptor 7 (TLR7) are recognizing HRSV and through these receptors is induced the production of type I IFN and proinflammatory cytokines in DCs and macrophages. This study shows that molecular receptors on unspecific cells are involved in HRSV infection and in the antiviral cytokines production (Oh et al., 2019).

As indoleamine-2,3-dioxygenase (IDO) was found upregulated upon HRSV infection, it was firstly studied *ex vivo* in A549 cells and then in animal models, these studies aiming for the development of vaccines against HRSV infections. Rajan and coworkers (2017) found that up-regulation of IDO is IFN-γ-dependent and involved in viral inhibition (Rajan et al., 2017).

Another experimental model developed in order to elucidate therapeutic targets was done by Ren and coworkers (2016) investigating immune-responsive gene-1 (IRG1) expression during HRSV infection. In both *in vitro* models, as well as in infected mice the IRG1 overexpression was correlated with ROS production, while knocking down IRG1 genes blocked ROS production and moreover proinflammatory cytokines' gene expression. IRG1 gene suppression reduced immune cell infiltration in lungs and hence the damage that this immune overreaction could induce. Therefore this study shed some light on the possibilities to therapeutically reduce post-infection lung damage (Ren et al., 2016). In light of these findings, these damages can be the foundation of future development of other pathologies. Studies reported in 2019 have shown that type 2 immune response, HRSV-dependent can be linked to asthma development. As previously shown in this review HMGB1 promotes type 2 immune responses in HRSV infection. In mouse experimental models, type 2 cytokines are increased in the later stages of infections, and these are clearly diminished when anti-HMGB1 antibodies are administered. Indeed, in nasopharyngeal aspirates from children with HRSV bronchiolitis HMGB1 levels are 10 times higher compared to children without lower respiratory tract infections and the HMGB1 level is correlated with clinical severity. Moreover these findings can pinpoint long-term consequences for infected children to develop asthma in their adulthood (Chen et al., 2019).

Innate immune arm will induce the activation of the adaptive immunity in infectious diseases. HSRV infection would lead to the production of the B cell activating factor (BAFF). In a murine model of infection it was shown that the highest N gene viral expression was seen at day 4 post-infection, while BAFF peaked at day 2 and 7. B cell chemokines (CXCL12, CXCL13, CCL19 and CCL21) had various levels of expression. Thus, CXCL13 was elevated at days 1, 2 and 7, while for CXCL12, CCL19 and CCL21 no significant differences were found compared to controls. The study shows that BAFF and CXCL13 are localized in the lymphoid aggregates, proving that there is a specific kinetics of chemoattractants for adaptive immune cells as well in infection (Alturaiki et al., 2018).

In under 2 years of age infected children the infection severity was related to the development of T follicular helper cells and that the antiviral inflammatory pattern was due to the high activation of BCL6; the acute phase of infection showed an increased expression of BCL6 (B cell lymphoma 6) gene, that mediates the development of T follicular helper cells and regulates Th2 immune responses (Do et al., 2018).

Knowing the vaccination issue that rose in the 60th (Acosta et al., 2016) in BALB/c and C57BL/6 (B6) mice the impact of alum adjuvant effects was evaluated after HRSV immunization in comparison with live HRSV reinfections. Viral clearance was induced, but live HRSV reinfections induced important pulmonary inflammation due to increased CD4 and CD8 T cells with pehotypes IFN-γ^+IL4$^-$, IFN-γ^-TNF-α^+, IFN-γ^+TNF-α^-. Alum adjuvant was found responsible for inducing a high percentage of CD4$^+$ T cells with IFN-γ-IL4$^+$, IFN-γ-TNF-α^+, correlated with proinflammatory cytokines IL-6 and IL-4 and B220$^+$ plasmacytoid and CD4$^+$ dendritic cells. Actually it is one of the first experimental studies that shows that alum adjuvant in HRSV vaccines that although induces viral clearance, it is also correlated with an atypical T helper CD4$^+$ T cells and inflammatory DC subset response that leads to the enhanced pathology upon HRSV infection (Kim et al., 2015).

Immunological hurdles in HSRV infection prevention and therapy

In spite of the abundance of data regarding HRSV genetics, structure, transmission route, replication, there are still no antiviral treatments on the market (Xing and Proesmans 2019).

HSRV vaccination was one of the medical failures of the 20th century due to the fact that it induced a vaccine-enhanced respiratory disease after imposing in the years 60s the vaccination of infants and children with formalin-inactivated HRSV (FI-HRSV) in alum formulation (Acosta et al., 2016). Although intense studies were performed on *in vitro* and *in vivo* models and lately in clinical trials there is still no therapy to shorten the disease or hinder symptoms in children. That is because there are some real hurdles in the drug development pathways. Therefore probably the most important is that during replication the virus HRSV suffers genetic changes (e.g. SNPs), that would make unefficient any vaccine (Agoti et al., 2014; Griffiths et al., 2017). Another hurdle is the fact that infants and children have

an immature immune system and on clinical trial basis is a group that is more difficult to enroll. There are several clases of molecules used in clinics and/or still in the clinical phases. Thus the major drugs represented by ***immunoglobulins*** (***immunotherapy***): 1) ALX-0171 targets F protein from HRSV and it is developed for acute HRSV infection; 2) RI-001 is an intravenous immunoglobulin obtained from pooled human plasma that has anti-HRSV antibodies; it targets epitopes G, F, and SH on HRSV surface and has also antiinflammatory effects. Other class of molecules used in clinics are: ***nucleoside analogues*** which are synthetic nucleosides designed to enter in the viral DNA or RNA, inducing viral enzymes and viral replication inhibition; ***fusion inhibitors*** which are molecules that inhibit viral F proteins that are critical for the viral envelope to fusion with host-cell plasma membrane: 1) MDT-637 can reduce HRSV replication, but the intimate pathways are still unknown (Douglas et al., 2003); 2) JNJ-53718678 binds to a symmetric central cavity inside the pre-fusion F protein; the conformational folding of the viral protein is hindered and no cell fusion takes place. It was shown that oral treatment with JNJ-53718678 decreased viral titers and reduced lung inflammation (Roymans et al., 2017).

In 2018 a clinical phase 3 trial that evaluated suptavumab (REGN2222), an antibody anti - F protein, was halted as did not prevent HRSV infections in preterm infants (Regeneron Pharmaceuticals, 2017). Despite these drawbacks, a commercial monoclonal antibody against F protein (palivizumab) is used. ***G protein***, is another viral target and specific monoclonal antibodies have been shown to have a potent antiviral effect in animal models. It is very likely that in 2019 this possible drug will enter the clinical trials (Tripp et al., 2018).

Production of a safe, effective vaccine against HRSV remains a public health priority in order to limit viral replication while inducing controlled immune responses. This vaccine should prove the balance between antigenicity and safety (Whelan et al., 2016). Table 2 summarizes clinical therapies used in the clinics for managing HRSV infection in children.

Table 2. Summary of therapeutical approaches in HSRV infection

Therapy	Prophylaxis	Efficacy
Bronchodilatators Corticosteroids Ribavirin	Vaccines	Not conclusive
Fluid intake Oxygen	Palivizumab	Yes
Antivirals DNase Surfactant	New monoclonal antibodies New vaccines	In research

Conclusion

Respiratory syncytial virus is the major cause of respiratory infection in infants and children, but as well as in high-risk adults, elderly, and immunocompromised individuals. The viral infection triggeres first a strong innate immunity response and further an adaptive immune antiviral response. The respiratory infection can be further clinically complicated with bronchiolitis, pneumonia, and asthma exacerbations. Although it is a common viral infection, there is still no efficient therapy. The hurdles in obtaining an efficient therapy reside in several issues: the immaturity of the immune system of infants and young children, the different virulence potential of clinical strains that trigger a different cytokine/chemokine response and the involvement of multiple molecular, still unknown pathways (Levitz et al., 2017). The identification of antiviral immune mechanisms and a comprehensive characterization of virus-specific immune responses, using new cellular and animal models would help to define new aspects of the viral - host crosstalk that would modulate the severity of infection. Moreover identifying the molecular mechanisms of acute airway disease would bring light also on associated long-term consequences, like developing asthma in the adulthood. As syncytial respiratory virus is globally circulating for more than 60 years, an effective therapy remains a public health priority.

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