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The CD4 T cell response to respiratory syncytial virus infection

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Abstract Respiratory syncytial virus (RSV) can induce severe lower respiratory tract infections in infants and is the leading cause of bronchiolitis in children worldwide. RSV-induced inflammation is believed to contribute substantially to the severity of disease. T helper (Th)2-, Th9-, and Th17-related cytokines are all observed in infants hospitalized following a severe RSV infection. These cytokines cause an influx of inflammatory cells, resulting in mucus production and reduced lung function. Consistent with the data from RSV-infected infants, CD4 T cell production of Interleukin (IL)-9, IL-13, and IL-17 has all been shown to contribute to RSV-induced disease in a murine model of RSV infection. Conversely, murine studies indicate that the combined actions of regulatory factors such as CD4 regulatory T cells and IL-10 inhibit the inflammatory cytokine response and limit RSV-induced disease. In support of this, IL-10 polymorphisms are associated with susceptibility to severe disease in infants. Insufficient regulation and excess inflammation not only impact disease following primary RSV infection it can also have a major impact following vaccination. Prior immunization with a formalin-inactivated (FI-RSV) vaccine resulted in enhanced disease in infants following a natural RSV infection. A Th2 CD4 T cell response has been implicated to be a major contributor in mediating vaccine-enhanced disease. Thus, future RSV vaccines must induce a balanced CD4 T cell response in order to facilitate viral clearance while inducing proper regulation of the immune response.

Keywords RSV · CD4 T cell · Th2 · Treg · IL-10 · Vaccine

Introduction

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract disease in children worldwide [1], with the majority of children infected with RSV by the age of 2 [2]. An estimated 132,000–172,000 RSV-associated hospitalizations occur annually in the USA in children under the age of 5 [3]. In addition, 3–4 million children are hospitalized and an estimated 66,000–199,000 children die annually worldwide from RSV-associated acute lower respiratory infection [1].

RSV induces a localized lung infection. RSV infects the lung epithelium, resulting in a host inflammatory response that recruits immune cells required for viral clearance. Although the host inflammatory response is necessary for viral clearance, it also induces damage to the lung. The CD4 T cell response has been implicated in contributing to this immune-mediated pathology during RSV infection in children [4]. Conversely, regulation by CD4 T cells also serves to balance the immune response to RSV infection. The mouse model of RSV infection has been extensively used to examine the role of CD4 T cells in the induction

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and regulation of the immune response and their impact on disease severity.

With the high global burden of RSV-induced disease, development of an efficacious vaccine has been a high priority. However, despite continuous efforts over the last 50 years, there is currently no licensed RSV vaccine. The first candidate vaccine in the 1960s, a formalin-inactivated RSV (FI-RSV) vaccine, resulted in enhanced disease in children under 2 years of age following a natural infection. In those children that died as a result of FI-RSV vaccination prior to natural RSV infection, autopsies revealed extensive pulmonary inflammation consisting primarily of lymphocytes and granulocytes [5]. Further examination of CD4 T cell-mediated enhanced disease has been examined in the mouse model [6, 7]. Thus, a better understanding of the balance between the protective and pathogenic properties of RSV-specific CD4 T cells is needed in the ongoing efforts to create a successful RSV vaccine. This review will focus on the pathogenic and regulatory roles of CD4 T cells following acute RSV infection as well as in vaccination settings.

The role of CD4 T cell subsets in RSV-induced disease

CD4 T cell depletion in mice prior to RSV infection results in amelioration of RSV-induced disease [8]. Furthermore, RSV infection can induce mucus production and airway hyperreactivity, symptoms that are associated with CD4 T helper (Th) 2-mediated immune responses. Infants are more prone toward Th2 responses than adults as they express higher levels of Interleukin (IL)-4 receptor (R) on CD4 T cells in cord blood following in vitro RSV stimulation compared to CD4 T cells from the peripheral blood of adults [9]. In addition, compared to adults, neonatal DCs produce less IL-12, resulting in reduced interferon (IFN)-γ production by neonatal CD4 T cells [10]. Together these unique features of the immature immune system in neonates predispose infants toward Th2 responses.

Conflicting reports exist regarding the presence of Th2-associated cytokines in RSV-infected infants. While higher levels of IFN-γ than IL-4 appear to be present in nasal washes of RSV-infected infants [11], the ratio of IL-4 to IFN-γ is increased with more severe disease [12]. Furthermore, Murai et al. [13] observed higher levels of IFN-γ than IL-4 in nasal washes but only IL-4 exhibited a significant increase in cytokine levels compared to uninfected controls. Similar results are found following stimulation of peripheral blood mononuclear cells (PBMCs) from RSV-infected infants [14–16]. Furthermore, increased severity of RSV-induced disease is associated with higher cortisol levels, which is known to inhibit IFN-γ responses [15]. This data suggest that both Th1 and Th2 CD4 T cells are present in both the lung and blood during RSV infection but more severe disease is associated with a relative increase in Th2 responses. However, a recent study examining over 450 RSV-infected infants under 1 year of

Table 1 Compilation of CD4 T cell cytokines produced during RSV infection in infants

Th subset	Cytokine	Detection in humans[a]
Th1	IFN-γ	Nasal wash
		6.4–39[b] ($n = 86$) [11]
		15.7–56.8 ($n = 28$) [12]
		15[c] ($n = 70$) [13]
		ND[d] ($n = 478$, LOD[e] = 5[b]) [17]
		Tracheal aspirate
		r = .862[f] ($n = 18$) [11]
Th2	IL-4	Nasal wash
		14.5–74.5 ($n = 86$) [11]
		0.71–1.1 ($n = 28$) [12]
		6[c] ($n = 69$) [13]
		ND ($n = 478$, LOD = 5) [17]
		Tracheal aspirate
		r = .957f ($n = 18$) [11]
	IL-5	Nasal wash
		1.4–3.4 ($n = 32$) [11],
		ND ($n = 478$, LOD = 3) [17]
		Tracheal aspirate:
		ND ($n = 18$) [11]
	IL-13	Nasal wash
		3.6–9.8 ($n = 40$) [11],
		ND ($n = 478$, LOD = 5) [17]
		Tracheal aspirate
		ND ($n = 18$) [11]
Th9	IL-9	Tracheal aspirate
		8,500 (n = 78) [25]
Th17	IL-17	Nasal wash
		ND ($n = 478$, LOD = 16) [17]
		Tracheal aspirate
		55 ($n = 11$) [28]
Treg	IL-10	Nasal wash
		202–465 ($n = 28$) [12]
		20[c] ($n = 49$) [13]
		26.2 ($n = 49$) [49]
		ND ($n = 478$, LOD = 5) [17]
		Tracheal aspirate
		32 ($n = 31$) [49]

[a] All data complied from infants under 2 years of age

[b] Cytokine values are expressed in pg/ml

[c] Data inferred from graphical representation

[d] *ND* not detected

[e] *LOD* limit of detection

[f] Correlation to nasal wash

age collected over 5 years with a range of disease from mild to severe did not find detectable levels of either Th1- (i.e., IFN-γ, TNF-α) or Th2- (i.e., IL-4, IL-5, IL-13) associated cytokines in nasal washes [17]. However, the limit of detection in their assay appears to have been above those levels detected by other groups for some cytokines (Table 1). This also highlights the minimal levels of Th2 cytokines detected in RSV-infected infants, indicating that these cytokines may not play a critical role in disease. Furthermore, Th2 cytokines are only detectable in the upper respiratory tract as measured in nasal wash samples, but in the same infants only IL-4 is detected in the lower respiratory tract from tracheal aspirates (Table 1). This indicates that Th2 cytokines may not be strongly associated with severe lower respiratory tract disease following a primary RSV infection. Thus, the contribution of Th2 cells in contributing to the severity of RSV-induced disease in infants remains unclear.

The magnitude of Th2 responses following RSV infection in mice appears to be dependent on the viral strain. The line 19 and 2–20 strains of RSV induce significantly greater amounts of the Th2-associated cytokine IL-13 in the lung than the A2 and Long strains, resulting in increased mucus production and airway hyperreactivity [18, 19]. Blockade of IL-13 reduces mucus production and disease [18, 20]. In RSV strains where minimal Th2 responses are observed, disruption of the immune response can result in an increased Th2 response. For example, Treg depletion and NK cell depletion have both been shown to increase the pro-inflammatory CD4 T cell response, including an increased Th1 as well as Th2 response, resulting in enhanced disease [21, 22]. Thus, data from murine studies indicate that an increased pro-inflammatory CD4 T cell response, including IL-13, results in an increase in RSV-induced disease severity.

A newly identified CD4 T cell subset, Th9, has also been implicated in contributing to disease through IL-9 production during RSV infection. IL-9 is increased in asthmatic patients and was initially categorized as a Th2 cytokine. IL-9 has been shown to stimulate mucus production in humans and mice [23]. IL-9 levels are increased in both human tracheal samples and mouse bronchial alveolar lavage (BAL) and lung samples following RSV infection (Table 1) [24, 25]. Furthermore, IL-9 polymorphisms in humans are associated with increased RSV disease severity [26]. Taken together, these findings indicate that Th9 cells may contribute to severe lower respiratory tract disease following RSV infection.

The Th17 subset of CD4 T cells has been shown to induce airway hyperreactivity during asthma [27]. IL-17 is increased in the tracheal aspirates of RSV-infected infants compared to controls (Table 1) [28]. However, IL-17 was not detected in nasal washes, indicating that IL-17, unlike the Th2 cytokines, may play a more significant role in the lower respiratory tract rather than in the upper airways (Table 1). In addition, IL-17, but not the Th2-associated cytokines IL-4 and IL-13, was shown to stimulate human primary tracheobronchial epithelial cells cultured in an air liquid interface to produce the mucus-related mRNA transcripts, muc5b and muc5ac [29, 30]. Following RSV infection in mice, IL-17 neutralizing antibody treatment resulted in reduced mucus production as measured by both mucus-associated mRNA transcripts and histology [28]. Thus, Th17 cells may play a more critical role than Th2 cells in the induction mucus production and airway hyperreactivity in the lower respiratory tract during RSV infection.

It is clear that RSV infection results in pro-inflammatory responses resulting in mucus production and airway hyperreactivity. However, the cause of these symptoms is less certain. In addition to CD4 T cells, multiple cell types in the lung are capable of producing IL-4, IL-5, IL-13 and IL-17, including eosinophils, mast cells, type 2 innate lymphoid cells (ILC2) and macrophages [31–34]. However, IL-4, IL-5, IL-13 and IL-17 cytokine production by these cells has not been examined in RSV infection. Furthermore, the symptoms and cytokine profile observed during RSV infection are similar to what is seen during some asthmatic responses of which allergen-specific CD4 T cells are known to play a major role. Owing to the similarities with asthma observed in humans and the pro-inflammatory role that CD4 T cells play during murine RSV infection, CD4 T cells may be a major contributor to immune-mediated disease during RSV infections in infants. No matter the cell type responsible, the presence of the pro-inflammatory environment created during RSV infection indicates that regulation of the immune response is vital to prevent excessive damage to host tissue.

Immune regulation by CD4 T cells

The lung utilizes multiple mechanisms of immune regulation to maintain homeostasis. While CD4 T cells have been shown to exhibit deleterious effects during RSV infection, they also play a vital role in immune regulation. Regulatory T cells (Tregs) represent a subset of CD4 T cells that inhibit the immune response and play a critical role in maintaining the equilibrium of the immune system in the lung. Tregs are classified as either naturally occurring (nTregs) following development in the thymus in response to self antigen, or as inducible (iTreg) following stimulation of naïve CD4 T cells by foreign antigen in the periphery. Both iTregs and nTregs have been shown to mediate suppressive effects on the immune system. In addition, IL-10 is an important regulatory cytokine that can

be produced by both conventional and regulatory CD4 T cells. Both Tregs and IL-10 appear to be critical regulators of the immune response in the lung during RSV infection.

Immune regulation by Tregs

Tregs have been shown to play a vital role in preventing immune-mediated pathology during RSV infection in mice [35]. Tregs, identified by expression of the forkhead box P3 (Foxp3) transcription factor, expand in the lung and BAL following RSV infection in mice [35, 36]. This expansion coincides with conventional CD4 and CD8 T cell expansion. RSV-specific Tregs have been identified in C57BL/6 mice by MHC class II tetramer staining following RSV infection, indicating that at least some Tregs are virus specific [37]. However, following RSV infection, it is unclear if all Tregs are virus-specific or if other signals can trigger Treg activation and expansion.

Treg depletion by either anti-CD25 antibody treatment or diphtheria toxin treatment in Foxp3-diphtheria toxin receptor mice results in a significant increase in the inflammatory response to RSV infection. Treg depletion during RSV infection results in an increased influx of innate immune cells including NK cells, eosinophils, and neutrophils into the lung after day 6 post-infection coinciding with when Tregs induce their suppressive effect [22, 35, 36, 38]. In addition, there is an increase in the number of conventional CD4 and CD8 T cells following Treg depletion. This increased inflammatory environment in the absence of Tregs results in enhanced weight loss and increased mucus production following RSV infection [22, 35, 36, 38]. These data indicate that Tregs play a vital role in limiting inflammation during RSV infection in mice.

Tregs regulate the immune response through both direct interactions as well as by cytokine production. Following RSV infection in mice, Tregs upregulate the inhibitory molecule CTLA-4 [35]. CTLA-4 has been shown to directly interact with both CD80 and CD86 to inhibit DC maturation and function [39]. In addition, Tregs have been shown to express and release granzyme B following RSV infection in mice [38]. This indicates that Tregs may be able to regulate the immune response by directly killing activated CD4 and CD8 T cells. Tregs, in addition to conventional CD4 T cells, can also broadly downregulate the immune system through the production of IL-10 during RSV infection [40–42]. These inhibitory actions by Tregs make this CD4 T cell subset vital in limiting the severity RSV-induced disease.

In humans, the role of Tregs during RSV infection is less clear. Activated conventional human CD4 T cells can transiently express Foxp3, requiring the use of additional markers to identify Tregs [43]. In addition, Tregs have not been examined in the airways during RSV infection in humans. Further study of the Treg response following mild versus severe RSV infection in humans would increase our understanding of the role of Tregs in modulating RSV-mediated disease.

Immune regulation by IL-10

Extensive studies in mice have examined the role of the inhibitory cytokine IL-10 during RSV infection. Following RSV infection, IL-10 is produced, regulating the adaptive immune response and limiting immunopathology [41, 42]. Rag2-deficient mice exhibit significantly reduced IL-10 protein in the lungs indicating that T cells are the primary source of IL-10 following RSV infection [42]. Furthermore, the use of an IL-10-eGFP reporter mouse strain demonstrates T cells are the main cell population responsible for the in vivo production of IL-10 following RSV infection [40, 42]. Both CD8 and CD4 T cells are capable of producing IL-10 in vivo as well as following in vitro stimulation of cells from RSV-infected lungs [41, 42]. However, using anti-CD4 antibody-mediated depletion in vivo, Weiss et al. [40] demonstrated that CD4 T cells account for the majority of IL-10 protein production in the lung following RSV infection.

There is a clear role for IL-10 in suppressing RSV-induced immunopathology. RSV-induced disease severity is increased either in the absence of IL-10 or following IL-10 receptor blockade [40–42]. Increased weight loss with decreased lung function is observed following RSV infection in both IL-10-deficient and anti-IL-10 receptor antibody-treated mice as compared to controls [40–42]. Furthermore, increased RSV-induced pathogenesis is associated with increased levels of pro-inflammatory cytokines and chemokines in the lungs [40–42]. Interestingly, the increase in pro-inflammatory cytokines and chemokines observed following Treg depletion are very similar to observations in IL-10-deficient mice following RSV infection with significantly increased IL-6, IFN-γ, TNF-α, MIP-1α and MCP-1 as compared to controls [36, 40, 41, 44]. This suggests that Tregs and IL-10 may play an overlapping role in inhibiting the pro-inflammatory environment induced following RSV infection.

In the absence of IL-10 signaling, CD4 and CD8 T cells also exhibit increased effector functions [40–42]. Moreover, T cell production of IL-10 provides a mechanism for their self-regulation. Transgenic mice that do not express the IL-10 receptor on the surface of T cells exhibit similar pathology following RSV infection as compared to both IL-10-deficient and anti-IL-10 receptor antibody-treated mice [42]. Together these data highlight the critical role T cells play in the production of IL-10 leading to their autocrine regulation and amelioration of RSV-induced pathogenesis.

The increased disease caused by the heightened inflammatory response in the lungs of IL-10-deficient mice as compared to controls is consistent with the notion that RSV pathogenesis is the result of immunopathology. In

addition, the increased levels of cytokines such as IL-6, TNF-α, CXCL9 and MCP-1 are observed in the nasal washes of infants with severe RSV-induced disease compared to those with mild disease, which is similar to the upregulation in cytokines observed between wild-type (WT) and IL-10-deficient mice following RSV infection [17, 40]. In conjunction with the increased pro-inflammatory environment, there is an increase in lung cellular infiltration comprised of primarily neutrophils as well as monocytes and lymphocytes either in the absence of IL-10 or following IL-10 receptor blockade [41, 42]. Neutrophils and monocytes are the primary cell populations found in the airways of infants with severe RSV-induced disease [45]. These increased cell populations in the absence of IL-10 signaling in RSV-infected mice further support the belief that RSV pathogenesis is immune-mediated [45–47].

In humans, IL-10 protein is variably detected in the nasal washes of infants with severe RSV-induced disease [12, 13, 17, 48, 49]. Thus, there is no clear correlation between IL-10 protein levels and RSV-induced disease severity in humans. Therefore, studies with larger numbers of infants will be necessary to further elucidate the role of IL-10 in RSV pathogenesis. However, there are multiple single-nucleotide polymorphisms (SNPs) in the human IL-10 gene that have been correlated to increased RSV-induced disease in infants [50, 51]. Hoebee et al. [50] reported an association between the *IL10* −592C SNP and RSV-induced bronchiolitis in infants <6 months of age. In contrast, Wilson et al. [51] did not observe the same association between the −592C (referred to as −627C in their manuscript) and RSV-induced bronchiolitis. In contrast, Wilson et al. [51] found significant independent associations between both the *IL10* −1117G and −3585A SNPs and the need for mechanical ventilation in infants <12 months of age hospitalized for RSV infection. However, a relatively small number of RSV-infected infants were analyzed for both of these studies, highlighting the need for an analysis of a larger population size to clearly assess the relationship between *IL10* SNPs and RSV pathogenesis. Moreover, a relationship between *IL10* SNPs and RSV-induced disease may in part contribute to race being a risk factor for severe disease in humans, since certain *IL10* SNPs are associated with specific races [52]. Thus, IL-10 has been shown to play an important role in limiting RSV-induced disease in animal models, and IL-10 polymorphisms provide an important indicator of disease susceptibility in humans.

Pathogenesis of CD4 T cell memory associated with RSV vaccination

The failure of the FI-RSV vaccine has led to significant changes in how new RSV vaccines are evaluated. An increased frequency of morbidity among vaccinees was observed following natural RSV infections in all four cohorts of the FI-RSV vaccine trials [53–56]. In one study, 80 % of the FI-RSV-immunized children required medical care, and two vaccinees died as compared to only a 5 % hospitalization rate in the parainfluenza vaccine control group [53]. This would suggest that the FI-RSV immunization either altered the immune response or enhanced viral replication that led to increased disease severity following a natural RSV infection. In agreement with an altered immune response, a significant number of vaccinated infants exhibited increased eosinophil numbers in their peripheral blood as compared to the control group [56]. Furthermore, autopsy reports of the two children that died during the FI-RSV vaccine trial revealed a significant increase in many leukocytic populations in the lung including eosinophils, neutrophils, lymphocytes, and monocytes [5]. Taken together, these studies indicated that an altered immune response in FI-RSV-immunized children mediated enhanced immunopathology upon a natural RSV infection.

A follow-up study by Kim et al. [5] of blood leukocytes from vaccinated, but uninfected, children revealed significantly increased proliferation by lymphocytes upon stimulation with RSV-derived antigens as compared to lymphocytes from the parainfluenza vaccine control group. This suggested that a heightened cellular immune response may have contributed to the enhanced disease severity associated with FI-RSV immunization. Further development of small rodent animal models including cotton rats and BALB/c mice has allowed for more precise evaluation of the immune response associated with FI-RSV immunization [57, 58]. Both rodent models elicit increased histopathology and increased pulmonary cellular infiltration in FI-RSV-immunized hosts following RSV infection [57, 58]. It remains unclear what specific immunological mechanism(s) mediates the enhanced disease associated with FI-RSV immunization. However, in the BALB/c murine model, it was demonstrated by Connors et al. [59] that CD4 T cells were necessary to mediate the enhanced histopathology. In addition, simultaneous in vivo neutralization of both the Th2-associated cytokine IL-4 and the regulatory cytokine IL-10 led to significantly reduced pulmonary histopathology in FI-RSV-immunized mice following RSV challenge [7]. Furthermore, an alternate study demonstrated that inhibition of IL-4 and IL-13 signaling significantly reduced bronchovascular and interstitial disease parameters in FI-RSV-immunized mice [60]. Together these data implicate Th2-associated cytokines as mediators of RSV vaccine-enhanced disease. Consistent with this idea, shifting the CD4 T cell helper response from Th2-biased to Th1 with TLR4 or TLR9 agonists has also been demonstrated to limit the severe pulmonary

histopathology and weight loss associated with FI-RSV immunization [61, 62]. Further evaluation of the CD4 T cell response associated with FI-RSV immunization is necessary to determine precisely how CD4 T cells mediate the enhanced pulmonary immunopathology.

The enhanced disease associated with the memory CD4 T cell response following FI-RSV immunization may also be due to defective regulation of the host immune response. A recent study by Loebbermann et al. [63] indicates that poor regulation of the CD4 T cell response by Tregs contributes to the augmented disease associated with FI-RSV immunization. Depletion of the Tregs in FI-RSV-immunized mice did not potentiate the weight loss in mice as the Treg response was already significantly reduced as compared to the controls. However, increased recruitment of Tregs to the lung via intranasal supplementation with chemokines CCL17 and CCL22 led to significantly reduced weight loss in FI-RSV immunized mice [63]. This suggests that FI-RSV immunization leads to an impaired Treg response upon RSV challenge that fails to properly regulate the pathogenic properties of the conventional memory CD4 T cell response.

Pathogenesis associated with the memory CD4 T cell response has also been demonstrated in mice immunized with a recombinant vaccinia virus engineered to express the RSV G protein (VACV-G) [64]. Immunization with VACV-G induces both Th1 CD4 T cell responses as well as Th2 responses that are associated with the induction of pulmonary eosinophilia following RSV infection [64–66]. Approximately half of the CD4 T cells utilize a single Vβ gene Vβ14, in the lungs of VACV-G-immunized mice following RSV challenge [67]. Depletion of CD4 T cells that express the Vβ14 T cell receptor ameliorates clinical illness and weight loss associated with VACV-G immunization [67]. These studies indicate that induction of CD4 T cell memory specific to the G glycoprotein of RSV can mediate pathogenic immune responses following an RSV infection.

Similar to FI-RSV immunization, inhibition of IL-4 and IL-13 signaling in VACV-G-immunized mice significantly reduces both bronchovascular and interstitial histopathology following RSV challenge [60]. These data suggest that a Th2-biased immune response mediates the enhanced pulmonary inflammation associated with VACV-G immunization. However, it has also been demonstrated that eosinophils and STAT6 signaling, a transcription factor important for the differentiation of Th2 CD4 T cells, are dispensable for the increased clinical illness, weight loss, and enhanced pause (Penh) associated with VACV-G immunization [68]. While it remains clear that memory CD4 T cells mediate the enhanced pathology associated with VACV-G immunization, it is not clear that a distinct subset of CD4 T cells is necessary to mediate disease.

Immunization with either FI-RSV or VACV-G fails to prime a memory CD8 T cell response and induces a weak neutralizing antibody response that permits a CD4 T cell dominated memory response [59, 69–71]. The induction of either CD8 T cell memory or neutralizing antibody responses may limit the excessive pathology associated with either FI-RSV or VACV-G immunization by limiting the secondary expansion of memory CD4 T cells and promoting viral clearance following RSV infection. In agreement, induction of CD8 T cell memory responses has been shown to inhibit the pulmonary eosinophilia associated with either FI-RSV or VACV-G immunization through reduction in the magnitude of the CD4 T cell response following RSV infection [70]. However, induction in CD8 T cell memory is also associated with increased weight loss [70]. In addition, improvement of the neutralizing antibody response induced by FI-RSV immunization following TLR3 agonist supplementation leads to significantly reduced histopathology following RSV challenge [69]. Together these data suggest that inhibition of CD4 T cell memory responses may limit the severity of RSV vaccine-enhanced disease symptoms.

Conclusion

Effective clearance of RSV infection with limited disease requires a proper balance between the effector and regulatory functions of the immune response. CD4 T cells play a critical role in maintaining this balance. However, environments that do not induce a proper CD4 T cell response, such as in infants and following either FI-RSV or VACV-G immunization, can result in CD4 T cell-mediated disease. Improper CD4 T cell responses such as induction of Th2-, Th9-, and Th17-related cytokines have been observed in RSV-infected infants and have been shown to induce disease in the mouse model of primary RSV infection and in various vaccination settings. Regulation of the immune response by Tregs and CD4 T cell production of IL-10 works to limit immune-mediated disease, but these responses may not be sufficient to fully prevent disease following either vaccination or primary RSV infection. Thus, understanding the requirements for induction of a proper effector and regulatory CD4 T cell response in infants will greatly aid the development of an effective RSV vaccine.

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