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Overcoming T cell-mediated immunopathology to achieve safe RSV vaccination

Elaine M. Castilow¹ and Steven M. Varga^{1,2}

¹*Interdisciplinary Graduate Program in Immunology, 51 Newton Road, 3-532 Bowen Science Building, University of Iowa, Iowa City, IA 52242*

²*Department of Microbiology, 51 Newton Road, 3-532 Bowen Science Building, University of Iowa, Iowa City, IA 52242*

Summary

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract disease in young children. Premature infants, immunocompromised individuals, and the elderly exhibit an increased risk for the development of severe disease after RSV infection. Currently, there is not a safe and effective RSV vaccine available, in part due to our incomplete understanding of how severe immunopathology was induced following RSV infection of children previously immunized with a formalin-inactivated RSV vaccine. Much of our current understanding of RSV vaccine-enhanced disease can be attributed to the establishment of multiple mouse models of RSV vaccination. Studies analyzing the RSV-specific immune response in mice have clearly demonstrated that both CD4 and CD8 memory T cells contribute to RSV-induced immunopathology. In this review we will focus our discussion on data generated from the mouse models of RSV immunization that have advanced our understanding of how virus-specific T cells mediate immunopathology and RSV vaccine-enhanced disease.

Introduction

Respiratory syncytial virus (RSV) is a negative sense, single-stranded RNA virus that belongs to the *paramyxoviridae* family and *pneumovirinae* sub-family [1]. RSV is the leading cause of lower respiratory tract disease in young children, and it is estimated that by three years of age, virtually all children have been infected with RSV at least once [2]. Re-infection occurs frequently as sterilizing immunity is never firmly established [3–5]. RSV infections typically cause mild illness, however severe disease can occur and is often associated with symptoms such as bronchiolitis and wheezing [6]. Populations at greatest risk to develop severe disease after RSV infection include premature infants [7], persons with congenital heart disease [8–10], immunocompromised individuals [11,12], and the elderly [11,13,14]. Clinical studies have indicated that children that are hospitalized due to RSV-induced disease exhibit an increased incidence of asthma later in life as compared to children that experience a mild or asymptomatic RSV infection (recently reviewed in [15]). The potential to significantly reduce the morbidity and mortality associated with RSV infection has fueled the desire to create an effective RSV vaccine for a long time.

phone: (319) 335-7784, fax: (319) 335-9006, e-mail: steven-varga@uiowa.edu.

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In the 1960's, a formalin-inactivated RSV (FI-RSV) vaccine was tested in children. Upon subsequent natural RSV infection, 80% of the FI-RSV-immunized children required hospitalization as compared to 5% of the vaccinees that received a control formalin-inactivated parainfluenza virus vaccine [16–19]. In two cases, RSV infection after FI-RSV-immunization was fatal [17]. Histology performed on the lungs of the deceased revealed an extensive mononuclear cell infiltrate and the presence of numerous eosinophils [17]. In addition, one study found that a significantly increased number of vaccinated children had eosinophils present in the blood as compared to control children [16]. The concerns over inducing pathology due to vaccination, such as was seen with the FI-RSV vaccine trials, and the incomplete immunity formed after natural RSV infection have made the development of a successful vaccine a slow and difficult process. Here, we will discuss our current understanding of RSV vaccine-enhanced disease based on extensive studies performed in the mouse model. We will also highlight the potential obstacles presented by priming for a robust RSV-specific memory T cell response that must be overcome in order to achieve safe and effective RSV vaccination.

The contribution of memory CD4 T cells to RSV vaccine-enhanced disease

Pulmonary Eosinophilia

BALB/c mice previously immunized with FI-RSV develop pulmonary eosinophilia upon RSV challenge thus mirroring the response observed in the FI-RSV vaccinated children [20–23]. RSV challenge of FI-RSV-immunized mice elicits a robust memory CD4 T cell response in the absence of a detectable memory CD8 T cell response [24,25]. The memory CD4 T cell response is comprised of both Th1 and Th2 cells as demonstrated by the detection of the Th1-associated cytokine interferon- γ (IFN- γ) and the Th2-associated cytokines interleukin-4 (IL-4), IL-5, and IL-13 in the lung after RSV challenge of FI-RSV-immunized mice [21,22,26]. IL-4-deficient mice immunized with FI-RSV fail to develop pulmonary eosinophilia after RSV challenge establishing a critical role for IL-4 in this process [23,27]. In addition, depletion of IL-13 prior to and after immunization of mice with FI-RSV also inhibits the development of pulmonary eosinophilia [27]. These data indicate that the Th2-associated cytokines IL-4 and IL-13 both promote the development of pulmonary eosinophilia upon RSV challenge of FI-RSV-immunized mice and suggest that the children vaccinated with FI-RSV underwent a memory Th2 response after contracting a natural RSV infection. Recent work has suggested that carbonyl groups formed as a result of formalin-inactivation are responsible for the induction of an RSV-specific Th2 response following RSV challenge of mice previously immunized with FI-RSV [28]. However, formalin-inactivation alone cannot account for the observed pathology as formalin-inactivation of parainfluenza, polio, and hepatitis A viruses did not result in enhanced disease or aberrant Th2 responses [16,17,19,29–31]. These studies suggest that the RSV-specific memory immune response induced by RSV infection of individuals previously immunized with FI-RSV significantly contributes to vaccine-enhanced disease.

Pulmonary eosinophilia also occurs after RSV challenge of BALB/c mice previously immunized with a recombinant vaccinia virus (vacv) expressing the attachment (G) protein of RSV (vacvG) [32–36]. Similar to the memory T cell response induced following FI-RSV immunization, the memory T cell response to the G protein consists of CD4 but not CD8 T cells [37–39]. In contrast to the memory CD4 T cell response elicited by FI-RSV vaccination where the specificity of the responding memory T cells has yet to be determined, immunization of mice with vacvG induces a memory CD4 T cell response directed against a well-defined epitope (RSV G_{183–195}) [21,40,41].

The G_{183–195}-specific memory CD4 T cell response is comprised of predominately Th1 (IFN- γ and/or tumor necrosis factor- α (TNF- α)-producing) cells with a small proportion of Th2

(IL-4-, IL-5-, and/or IL-13-producing) cells [21,23,27,34,37,40–42]. Interestingly, the diversity of the CD4 T cells capable of recognizing the G protein of RSV appears limited because the majority of RSV G_{183–195}-specific memory CD4 T cells express the V β 14 T cell receptor on their surface [41]. Depletion of the RSV G_{183–195}-specific memory CD4 T cells using an antibody directed against V β 14 reduces the development of pulmonary eosinophilia upon RSV challenge of vacvG-immunized mice [41]. In addition, the depletion of Th2 cells using an antibody specific for T1/ST2, a molecule expressed on the cell surface of Th2 cells, significantly reduces the development of pulmonary eosinophilia after RSV challenge of mice previously immunized with vacvG [43]. These results establish the necessity of an RSV-specific memory Th2 response in order to induce pulmonary eosinophilia in this model.

The role of the individual Th2-associated cytokines in mediating the development of pulmonary eosinophilia after RSV challenge of vacvG-immunized mice has been investigated. In contrast to FI-RSV immunized mice, IL-13, but not IL-4, is necessary for the development of pulmonary eosinophilia in vacvG-immunized mice [27,44]. In addition, it has also been shown that treatment of vacvG-immunized mice with recombinant IL-12, a cytokine important in the differentiation of Th1 cells, reduces pulmonary eosinophilia [45,46]. Unfortunately, the impact of IL-12 administration on IL-13 production was not examined in these studies [45, 46]. Taken together, these results demonstrate the importance of a memory Th2 response and the production of IL-13 for the development of pulmonary eosinophilia after RSV challenge of mice previously immunized with vacvG.

The presence of a memory Th2 response is necessary, but not sufficient for the development of pulmonary eosinophilia after RSV challenge of mice previously immunized with vacvG. Data examining the number of memory Th2 cells present in the lungs after RSV challenge of mice previously co-immunized with vacvG and a recombinant vacv expressing the M2 protein of RSV (vacvM2, discussed in detail below) demonstrates that these mice mount a reduced yet detectable Th2 response without the subsequent development of pulmonary eosinophilia [24]. This suggests that there is a minimum number of memory Th2 cells required to induce the development of pulmonary eosinophilia.

Chemokines produced by infected pulmonary epithelial cells following RSV exposure mediate the influx of numerous inflammatory cells into the lung. Th2 cells express the chemokine receptors CCR3 and CCR4 and are recruited into the lung by the production of the chemokines CCL11, CCL17, and CCL22 [47]. Eosinophils express CCR3 and CCR5 and are recruited into the lungs in part by CCL11 production after RSV challenge of vacvG-immunized mice [27, 48,49]. In addition, RSV challenge of mice previously immunized with vacvG also results in a significant increase in the amount of CCL17 mRNA [50] and CCL22 protein [44] in the lung. These data suggest that chemokine production in the lung contributes to the development of pulmonary eosinophilia through the recruitment of both Th2 cells and eosinophils.

IL-13-deficient vacvG-immunized mice, which exhibit decreased pulmonary eosinophilia after RSV challenge as compared to wild-type controls, have significantly decreased CCL11 and CCL22 production in the lung [44]. Anti-CCL11 treatment reduces, but does not ablate pulmonary eosinophilia after RSV challenge of vacvG-immunized mice suggesting that additional chemokines are involved in the recruitment of eosinophils into the lung in this model [49]. CCL24 is one potential chemokine that could contribute to the recruitment of eosinophils after RSV challenge of mice previously immunized with vacvG. Although CCL24 has not yet been examined in RSV models, data from infections with pneumonia virus of mice, a virus belonging to the same sub-family as RSV, shows a positive correlation between the production of CCL24 mRNA and the development of pulmonary eosinophilia [51]. These results suggest that the development of pulmonary eosinophilia upon RSV challenge of mice previously immunized with vacvG requires the initial recruitment of Th2 cells into the lung by chemokines

such as CCL11, CCL17 and/or CCL22 followed by the recruitment of eosinophils from the blood into the lung by CCL11 and other undefined chemokines such as CCL24.

RSV challenge of BALB/c mice immunized with a vacv expressing the fusion (F) protein of RSV (vacvF) also elicits a memory CD4 T cell response to a defined epitope (RSV F₅₁₋₆₆) [52–54]. In contrast to mice previously immunized with vacvG or FI-RSV, there is a readily detectable F-specific memory CD8 T cell response elicited after RSV challenge of vacvF-immunized mice [25,38]. Interestingly, vacvF-immunized mice do not develop pulmonary eosinophilia upon RSV challenge [32,33,52,54]. RSV challenge of mice previously immunized with vacvF exhibit a memory Th1 response in the absence of a detectable memory Th2 response [52,54]. This supports data from FI-RSV- and vacvG-immunized mice demonstrating the necessity of an RSV-specific memory Th2 response for the development of pulmonary eosinophilia after RSV challenge.

In the absence of IFN- γ , F-specific memory CD4 T cells produce IL-5 and mice develop pulmonary eosinophilia after RSV challenge demonstrating a protective role for IFN- γ in vacvF-immunized mice [52]. The phenotypic change from a memory Th1 response to a memory Th2 response in the absence of IFN- γ occurs even though the number of memory Th2 cells formed after vacvF-immunization of wild-type or IFN- γ -deficient mice is not significantly different [52]. These data suggest that IFN- γ inhibits the expansion of the memory Th2 cells and thus prevents the development of pulmonary eosinophilia upon RSV challenge of vacvF-immunized wild-type mice. In addition, depletion of IFN- γ has been shown to result in the development of pulmonary eosinophilia in vacvG-immunized C57BL/6 mice, which do not normally develop pulmonary eosinophilia upon RSV challenge [35].

In vacvF-immunized IFN- γ -deficient mice challenged with RSV, the production of CCL11, CCL17 and CCL22 is significantly increased as compared to vacvF-immunized wild-type mice [52]. In addition, studies performed on vacvG-immunized mice undergoing a challenge RSV infection suggest that the development of pulmonary eosinophilia is dependent upon CCL11 and CCL22 [44,48,49]. Taken together, these data suggest that the CCL17- and CCL22-dependent recruitment of Th2 cells and the CCL11-dependent recruitment of eosinophils contribute to pulmonary eosinophilia after RSV challenge of mice previously immunized with either vacvG or vacvF.

Systemic Disease

Systemic disease including weight loss occurs after RSV challenge of BALB/c mice previously immunized with either vacvG or vacvF [34,41,43,49,52,55]. Studies have shown that depletion of TNF- α from either vacvG- or vacvF-immunized mice significantly reduces weight loss after RSV challenge [56]. In both vacvG- and vacvF-immunized mice, TNF- α depletion decreases IFN- γ production by CD4 T cells [56] suggesting that IFN- γ production by memory CD4 T cells may also contribute to weight loss. Further support of the contribution of Th1 cells to weight loss comes from experiments where vacvG-immunized mice treated with recombinant IL-12 prior to and after RSV challenge exhibit both enhanced memory CD4 T cell IFN- γ responses as well as enhanced weight loss [45,46]. Finally, IFN- γ -deficient vacvF-immunized mice challenged with RSV do not lose weight demonstrating a role for IFN- γ in mediating weight loss [52]. These data suggest that a robust memory Th1 response contributes to weight loss in multiple models of RSV vaccination.

The contribution of memory CD8 T cells to RSV vaccine-enhanced disease

Pulmonary Eosinophilia

In contrast to immunization with FI-RSV, vacvG, or vacvF, RSV challenge of BALB/c mice immunized with vacvM2 elicits a memory CD8 T cell response in the absence of a detectable

memory CD4 T cell response [25,57,58]. Mice co-immunized with vacvG and vacvM2 do not develop pulmonary eosinophilia upon RSV challenge demonstrating a protective role for M2-specific memory CD8 T cells [24,59]. Importantly, mice co-immunized with FI-RSV and vacvM2 after RSV challenge do not develop pulmonary eosinophilia [24]. These results suggest an important failure of the FI-RSV vaccine was its inability to prime for an RSV-specific memory CD8 T cell response. Moreover, these data demonstrate that M2-specific memory CD8 T cells can inhibit the development of pulmonary eosinophilia after RSV challenge of previously immunized mice.

The presence of an F-specific memory CD8 T cell response in vacvF-immunized mice is necessary to prevent the development of pulmonary eosinophilia after RSV challenge. RSV challenge of vacvF-immunized mice deficient in β_2 -microglobulin, a component of the major histocompatibility complex molecule responsible for peptide epitope presentation to CD8 T cells, results in pulmonary eosinophilia [59]. In addition, depletion of CD8 T cells from vacvF-immunized mice leads to the production of detectable levels of Th2-associated cytokines upon RSV challenge [54]. These data indicate that F-specific memory CD8 T cells inhibit the development of pulmonary eosinophilia upon RSV challenge of vacvF-immunized mice.

The mechanism of inhibition of pulmonary eosinophilia by M2- and F-specific memory CD8 T cells has been examined. IFN- γ -deficient mice immunized with vacvF exhibit an RSV-specific Th2 response and develop pulmonary eosinophilia after challenge with RSV [52]. Therefore, these data suggest that wild-type mice previously immunized with vacvF do not develop pulmonary eosinophilia after RSV challenge due to the inhibition of the RSV-specific memory Th2 response by IFN- γ produced by F-specific memory CD8 T cells. M2-specific memory CD8 T cells can also produce IFN- γ [24,57,59]. However, in contrast to vacvF-immunized mice, M2-specific memory CD8 T cell inhibition of pulmonary eosinophilia does not require IFN- γ [24]. The precise mechanism of M2-specific memory CD8 T cell inhibition of pulmonary eosinophilia is currently under investigation in our laboratory.

Systemic Disease

Significant weight loss occurs after RSV challenge of BALB/c mice previously immunized with vacvM2 [60]. Since there is no detectable memory CD4 T cell response to the M2 protein, this indicates a direct role for memory CD8 T cells in mediating weight loss [25,57,58]. Thus, memory CD8 T cell responses, although capable of preventing the development of pulmonary eosinophilia, can induce systemic immunopathology during secondary RSV infections.

Whereas weight loss in vacvF-immunized mice was found to be dependent upon IFN- γ [52], data from our laboratory suggests that weight loss in vacvM2 and vacvG co-immunized mice occurs independently of IFN- γ (Olson and Varga, unpublished data). A role for TNF- α in mediating weight loss in both vacvG- and vacvF-immunized mice has been established [56] suggesting that M2-specific memory CD8 T cells may contribute to weight loss through the production of TNF- α . As with findings regarding the development of pulmonary eosinophilia in RSV immunization models, the mechanism of memory CD8 T cell-mediated weight loss may differ depending on the model system. Further investigation of each model will provide us with a better understanding of the cytokines that contribute to memory CD8 T cell-induced immunopathology.

The relationship between pulmonary eosinophilia and systemic disease

To this point, we have discussed the development of pulmonary eosinophilia and the development of systemic disease as measured by weight loss and clinical illness separately. Because so much RSV literature focuses on the development of pulmonary eosinophilia, the relationship between eosinophilia and systemic disease is of significant interest. Studies

depleting Th2 cells during RSV challenge of vacvG-immunized mice demonstrate that weight loss can occur in the absence of pulmonary eosinophilia [43]. Recent work examining the development of pulmonary eosinophilia and systemic disease in vacvF-immunized mice has also shown that systemic disease can develop in the absence of pulmonary eosinophilia and a detectable Th2 response [52]. In these studies, IFN- γ was found to protect against pulmonary eosinophilia while promoting the development of systemic disease. Together, these data suggest that RSV-specific memory Th2 responses lead to the development of pulmonary eosinophilia, and that RSV-specific memory Th1 responses lead to systemic disease. This implies that future vaccines should elicit a balanced immune response as skewing away from a memory Th2 response toward a memory Th1 response may result in enhanced immunopathology.

In order to determine precisely how harmful the induction of an RSV-specific memory Th2 response is, the role of eosinophils during RSV challenge of previously immunized mice needs to be determined. In mice undergoing a primary RSV infection, it has been found that eosinophils contribute to viral clearance [61]. This has not been directly tested in mice undergoing a memory response to RSV, however, work from vacvF-immunized mice and IL-13-deficient vacvG-immunized mice, neither of which develop pulmonary eosinophilia, demonstrate that viral clearance is not significantly altered in the absence of eosinophils [44, 52]. More research is necessary in order to more precisely determine the specific contributions of Th2 cells and eosinophils to the development of RSV vaccine-enhanced disease.

Mechanisms of RSV vaccine-enhanced disease

It has been shown that the cytokines required for the development of pulmonary eosinophilia and weight loss in BALB/c mice undergoing a secondary RSV infection are dependent upon the initial immunization strategy [23,44,52,56]. In addition, the mechanisms of inhibition of pulmonary eosinophilia differ depending on the model system in question [24,52]. Based on studies performed in the RSV mouse model, we have developed models of how memory CD4 and CD8 T cells act in concert to induce RSV vaccine-enhanced disease following different RSV immunization strategies (Figure 1).

Figure 1A shows our proposed mechanism of RSV vaccine-enhanced disease in vacvG-immunized mice. RSV G₁₈₃₋₁₉₅-specific memory Th2 cells are recruited to the lung by the production of CCL11, CCL17, and/or CCL22 and produce IL-4, IL-5, and/or IL-13. IL-5 induces the production of eosinophils from precursors in the bone marrow [62] and is necessary for the recruitment of eosinophils into the lung [63, 64]. The production of IL-13 by G₁₈₃₋₁₉₅-specific memory Th2 cells and other inflammatory cells is required for the development of pulmonary eosinophilia. Eosinophils can be recruited into the lung by CCL11 and CCL24. Despite the abundant production of IFN- γ produced in the lung by RSV G₁₈₃₋₁₉₅-specific memory Th1 cells, pulmonary eosinophilia still develops which is dependent upon the presence of an undefined minimum number of RSV G₁₈₃₋₁₉₅-specific memory Th2 cells that express the V β 14 T cell receptor [24, 40, 41]. RSV G₁₈₃₋₁₉₅-specific memory Th1 cells contribute to weight loss through the production of TNF- α and/or IFN- γ .

The model presented in Figure 1B represents the mechanism for the development of RSV vaccine-enhanced disease in vacvG and vacvM2 co-immunized mice. Pulmonary eosinophilia is inhibited by the introduction of an M2-specific memory CD8 T cell response. This likely occurs due to the reduction of the G₁₈₃₋₁₉₅-specific memory Th2 response by inhibition of the production of the Th2 recruiting chemokines, CCL17 and CCL22. It is currently unclear how M2-specific memory CD8 T cells inhibit the production of CCL17 and CCL22. One possibility is that the M2-specific memory CD8 T cells may kill RSV infected epithelial cells that serve as a source of CCL17 and CCL22. However, enhanced viral clearance has not been

demonstrated upon the introduction of an M2-specific memory CD8 T cell response during a G_{183–195}-specific memory CD4 T cell response suggesting this may not represent the primary mechanism of inhibition of pulmonary eosinophilia [24]. M2-specific memory CD8 T cells and G_{183–195}-specific memory Th1 cells can contribute to weight loss, presumably through the production of TNF- α and/or IFN- γ .

Finally, Figure 1C outlines a potential model for the development of RSV vaccine-enhanced disease in vacvF-immunized mice. Eosinophilia does not normally develop upon RSV challenge of vacvF-immunized mice due to inhibition of the F_{51–66}-specific memory Th2 response by IFN- γ . In contrast to the limited T cell receptor repertoire utilized by G_{183–195}-specific memory CD4 T cells [40, 41], F_{51–66}-specific memory CD4 T cells express a diverse T cell receptor repertoire [52]. Thus, the more conventional F_{51–66}-specific memory CD4 T cell response may be much less polarized and more susceptible to regulation by IFN- γ . The source of inhibitory IFN- γ in vacvF-immunized mice has not yet been identified, however F-specific memory CD8 T cells are likely candidates. The individual characteristics of M2- and F-specific memory CD8 T cells that result in different mechanisms of inhibition of pulmonary eosinophilia remain undefined. IFN- γ and TNF- α production from the F-specific memory CD8 T cells and/or the F-specific memory Th1 cells may mediate weight loss.

Concluding Remarks and Future Perspective

Many current RSV vaccine efforts seek to induce an RSV-specific memory T cell response. Therefore, in order to achieve safe and effective RSV vaccination, we first need to thoroughly understand the mechanisms by which immunopathology can result from a RSV-specific memory T cell response. The studies briefly summarized here suggest that the induction of a memory CD8 T cell response is of great importance to inhibit the generation of a memory Th2 response that is responsible for the development of pulmonary eosinophilia. Unfortunately, the production of Th1-associated cytokines by memory CD8 T cells and/or memory Th1 cells can also lead to enhanced disease. Induction of a carefully balanced and controlled immune response will be critical for a vaccine that seeks to cause minimal harm upon natural RSV infection.

Young calves infected with bovine RSV develop severe disease with similar pathology to what is observed in young children infected with human RSV [65]. In addition, RSV challenge of calves previously immunized with FI-RSV results in enhanced pulmonary disease in the presence of a Th2 response [66,67]. The use of recombinant vaccinia virus vaccines and DNA vaccines has been effective at reducing the development of disease in this natural host model [68,69]. These results provide hope for the future development of a safe and effective vaccine for use in humans. Over the coming years, we believe that additional work using the various RSV immunization models will help elucidate the underlying mechanisms of how RSV-specific memory T cells induce immunopathology and the various manifestations of RSV vaccine-enhanced disease. Additionally, continued work examining specimens from children hospitalized due to RSV infection will further our knowledge of the factors that predispose and contribute to severe disease in young children upon initial RSV infection. Overall, we believe the results from the murine studies will provide important insight as to what steps can be taken to prevent RSV vaccine-induced immunopathology in humans.

In addition to our primitive understanding of the processes leading to pulmonary eosinophilia and weight loss, the mechanisms directing goblet cell hyperplasia, mucus production, and pulmonary function remain largely uninvestigated in models of memory immune responses to RSV and should be a focus of future work. Therefore, continued work investigating the memory immune response to RSV is important for a thorough understanding of how to prevent

immunopathology induced by RSV-specific memory T cells. Only then can we rationally design a T cell-based RSV vaccine.

Executive Summary

Introduction

- Respiratory syncytial virus (RSV) is an important human pathogen that can cause serious respiratory tract disease in multiple populations.
- A formalin-inactivated RSV (FI-RSV) vaccine resulted in enhanced morbidity and mortality in young children upon natural RSV infection. Autopsy of the deceased revealed an extensive pulmonary mononuclear cell infiltrate and the presence of numerous eosinophils.

The contribution of memory CD4 T cells to RSV vaccine-enhanced disease

- Immunization of BALB/c mice with either FI-RSV or a recombinant vaccinia virus (vacv) expressing the attachment (G) protein of RSV (vacvG) results in pulmonary eosinophilia and weight loss upon challenge RSV infection.
- The development of pulmonary eosinophilia in vacvG-immunized is dependent upon the presence of a memory Th2 response and the production of IL-13, as well as CCL11, CCL17, and CCL22 production.
- In mice immunized with a vacv expressing the fusion (F) protein of RSV (vacvF), a memory Th2 response, increased CCL11, CCL17, and CCL22 production, and the development of pulmonary eosinophilia occurs only in the absence of IFN- γ .
- Memory CD4 T cells have been reported to contribute to weight loss through the production of TNF- α , and/or IFN- γ , depending on the immunization strategy.

The contribution of memory CD8 T cells to RSV vaccine-enhanced disease

- Co-immunization of mice with vacvG and a vacv expressing the M2 protein of RSV (vacvF) decreases Th2-associated cytokine production and prevents the development of pulmonary eosinophilia.
- Mice co-immunized with FI-RSV and vacvM2 also do not develop pulmonary eosinophilia.
- Mice immunized with vacvF are no longer protected against pulmonary eosinophilia in the absence of CD8 T cells.
- CD8 T cells contribute to weight loss through yet unidentified mechanisms.

The relationship between pulmonary eosinophilia and systemic disease

- Murine studies suggest that pulmonary eosinophilia and systemic disease are independent aspects of RSV vaccine-enhanced disease.

Model of mechanisms of RSV vaccine-enhanced disease

- We present proposed models of RSV vaccine-enhanced disease specific for each of the following immunization strategies: A) vacvG, B) vacvG and vacvM2, and C) vacvF.

Concluding Remarks and Future Perspective

- We believe that continued work with various models of RSV immunization in mice will provide additional insights regarding not only the mechanisms of RSV

vaccine-enhanced disease in humans, but also which factors predispose individuals for severe disease upon RSV infection.

- The data reviewed here indicates that both Th1 and Th2 CD4 T cell responses, as well as CD8 T cell responses can contribute to RSV vaccine-enhanced disease. Therefore, a RSV vaccine that induces a balanced and controlled immune response will likely be most efficacious.

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Figure 1.

Models of the mechanisms of respiratory syncytial virus (RSV) vaccine-enhanced disease. Proposed models for the cells and cytokines required for the development of pulmonary eosinophilia and weight loss in mice immunized with (A) a recombinant vaccinia virus (vacv) expressing the attachment (G) protein of RSV (vacvG), (B) co-immunization with vacvG and a vacv expressing the M2 protein of RSV (vacvM2), and (C) a recombinant vacv expressing the fusion (F) protein of RSV (vacvF).