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Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease

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Abstract Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection in infants and children worldwide. In addition, RSV causes serious disease in elderly and immune compromised individuals. RSV infection of children previously immunized with a formalin-inactivated (FI)-RSV vaccine is associated with enhanced disease and pulmonary eosinophilia that is believed to be due to an exaggerated memory Th2 response. As a consequence, there is currently no licensed RSV vaccine and detailed studies directed towards prevention of vaccine-associated disease are a critical first step in the development of a safe and effective vaccine. The BALB/c mouse model of RSV infection faithfully mimics the human respiratory disease. Mice previously immunized with either FI-RSV or a recombinant vaccinia virus (vv) that expresses the attachment (G) glycoprotein exhibit extensive lung inflammation and injury, pulmonary eosinophilia, and enhanced disease following challenge RSV infection. CD4 T cells secreting Th2 cytokines are necessary for this response because their depletion eliminates eosinophilia. Intriguing recent studies have demonstrated that RSV-specific CD8 T cells can inhibit Th2-mediated pulmonary eosinophilia in vvG-primed mice by as yet unknown mechanisms. Information gained from the animal models will provide important information and novel approaches for the rational design of a safe and efficacious RSV vaccine.

Keywords RSV · T cell · Th2 cell · Eosinophils · Vaccine · Immunopathology

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Introduction

RSV, a ubiquitous human pathogen first characterized in 1957 [1], is the leading cause of lower respiratory disease in infants and young children worldwide, with virtually all children having become infected within their first two years of life. It is believed that RSV infection accounts for approximately 120,000 hospitalizations and 1,500 deaths each year in the United States alone [2–4]. Primary RSV infection of infants and young children often results in acute bronchiolitis leading to inflammation-induced airway obstruction [5]. Furthermore, since disease severity has been correlated with elevated levels of IgE [6] and eosinophil cationic protein in bronchiolar secretions [7, 8], the available evidence suggests the pathology is immune-mediated. Clinically, RSV presents with a bronchiolitis that is similar to that of asthma and several epidemiological studies suggest that severe RSV infection during childhood is associated with an increased risk for the development of asthma and allergies in adulthood [9]. Interestingly, natural RSV infection does not confer lifetime immunity [10] and therefore individuals are repeatedly infected throughout life. Healthy adults develop cold- or flu-like symptoms whereas the elderly [11–14] and immunocompromised individuals [15, 16] have increased risk of severe respiratory illness. Moreover, a recent study has indicated that RSV disease burden in the elderly is similar to that of nonpandemic influenza A [17]. Because of its significant impact on human health, the development of an efficacious RSV vaccine remains a high priority.

Respiratory syncytial virus vaccine-enhanced disease

In a series of vaccine trials conducted in the 1960's, a FI-RSV vaccine was administered to children. Surprisingly, $\sim$80% of the vaccinated children experienced serious disease and were hospitalized after acquiring a natural RSV infection, as compared to only $\sim$5% of a control vaccine group [18–21]. Furthermore, the severity of the disease was found to be age dependent. The older the children at the time of vaccination, the less likely subsequent RSV infection would result in hospitalization [19, 21]. Two of the vaccinees died after contracting an RSV infection [21]. Histological analysis of the lungs of the 2 children who tragically died revealed extensive mononuclear cell infiltration including pulmonary eosinophilia [21]. Moreover, eosinophils were also found in the peripheral blood of many of the hospitalized children [22]. In the 40+ years since the FI-RSV vaccine failure, much effort has been placed into gaining a better understanding of the immunological mechanisms that led to the enhanced disease experienced by the vaccinated children. Herein we will review data obtained using small animal models that mimic RSV vaccine-enhanced disease and discuss the importance of these findings for the future design of a safe and effective RSV vaccine.

Mouse models of RSV vaccine-enhanced disease

Prior to the design of a safe and effective RSV vaccine, the underlying mechanism resulting in the failure of the FI-RSV vaccine must be better understood. RSV infection of FI-RSV-vaccinated BALB/c mice results in pulmonary eosinophilia that mimics the lung pathology observed in the FI-RSV vaccinated children [23–26]. The high incidence of exacerbated disease exhibited by the FI-RSV-vaccinated children suggests that the vaccine-enhanced disease occurred regardless of genetic differences. This is further supported

by the mouse model as the development of pulmonary eosinophilia upon FI-RSV vaccination and RSV challenge occurs in multiple strains of mice including C57BL/6 and BALB/c [23]. Due to the similarities between FI-RSV vaccinated mice and the children of the 1960's FI-RSV vaccine trials, the mouse model provides a good system to investigate the failure of the FI-RSV vaccine.

Both local and systemic increases in eosinophils are characteristic of a Th2-mediated immune response [27]. This suggests that the immunized children were primed for a Th2 immune response by the vaccine. FI-RSV-vaccinated mice challenged with RSV exhibit increased levels of the Th2-associated cytokines IL-5, IL-4, IL-13, and the chemokine eotaxin [26, 28]. Furthermore, a decrease in the Th1-associated cytokine IL-12 was also observed [26]. Although the production of Th2 cytokines is only correlative with disease, many studies have shown that Th2 cytokines are necessary for the development of pulmonary eosinophilia upon RSV challenge of FI-RSV-vaccinated mice. For example, IL-4-deficient FI-RSV-immunized BALB/c mice do not develop pulmonary eosinophilia upon RSV challenge demonstrating that IL-4 plays a role in enhanced disease [23, 29]. Furthermore, mice that are depleted of IL-4, IL-10, or IL-13 also exhibit significant decreases in the level of pulmonary eosinophilia observed after challenge with RSV [29, 30]. Thus, it is apparent that Th2 cytokines are essential for the development of pulmonary eosinophilia in the FI-RSV vaccine model. In addition, loss of either IL-4 or IL-13 was sufficient to cause a decrease in virus load after RSV challenge [29]. Therefore, Th2 cytokines may also contribute to enhanced viral replication in the lung, although this mechanism is currently not well understood.

In addition to the T cell response, other factors contribute to FI-RSV vaccine-enhanced disease. Anti-RSV antibodies induced following FI-RSV vaccination have been shown to form immune complexes that may promote disease [31]. Furthermore, the antibody response induced by the FI-RSV vaccine was also found to be suboptimal in humans as natural RSV infection resulted in higher antibody titers [20, 21, 32, 33]. Another contributing factor to the enhanced disease could be the generation of carbonyl groups during the preparation of FI-RSV as reduction of these groups led to decreased pulmonary eosinophilia [34]. In support of this, pulmonary eosinophilia is also induced with RSV inactivated by glycoaldehyde, a chemical known to induce carbonyl groups [34–36]. A formalin-inactivated measles virus (FI-MV) vaccine also resulted in enhanced disease characterized by pulmonary infiltrate [37, 38] and eosinophilia [39] upon natural measles virus infection. However, the formalin-inactivated parainfluenza viruses used as controls in the FI-RSV studies did not result in enhanced disease [19, 21, 22] suggesting that the use of formalin as a fixative for these killed vaccines does not solely explain the enhanced disease observed with FI-RSV and FI-MV. Additionally, formalin-inactivated vaccines against hepatitis A virus and poliovirus have been administered without any reports of vaccine-enhanced disease [40–42]. However, given the results of the FI-RSV vaccine trial, it is likely that a successful future RSV vaccine will be a subunit, DNA, attenuated, or recombinant vaccine.

Vaccination with a recombinant vaccinia virus expressing the attachment protein of RSV

In addition to mice vaccinated with the FI-RSV vaccine, pulmonary eosinophilia is observed upon RSV challenge of BALB/c mice scarified with a recombinant vaccinia virus

expressing the G protein of RSV (vvG) [43–47]. Antibody titers do not increase above those seen with a primary RSV infection in this model [29], similar to the results obtained with FI-RSV. In addition, increases in the levels of IL-4, IL-5, IL-13, and eotaxin can be detected in vvG-primed mice challenged with RSV [23, 29, 48–53]. Although vaccination with vvG results in similar pathology as seen with FI-RSV-vaccination, several observations suggest that these two models reach the same endpoint through different means.

IL-4 and IL-13 share multiple signaling molecules, including the IL-4 receptor α chain (IL-4Rα) [54]. Using IL-4Rα-deficient mice, it has been shown that IL-4 and/or IL-13 are necessary for the development of pulmonary eosinophilia in vvG-primed mice [29]. Further work has been done to distinguish the individual roles of these two cytokines. In contrast to the requirement for IL-4 in the FI-RSV model, depletion of IL-4 from BALB/c mice during priming with vvG [23] and the use of IL-4-deficient mice [23, 29] has shown that in the absence of IL-4, pulmonary eosinophilia still develops in this model. Moreover, depletion of IL-13 either at immunization or challenge in mice vaccinated with vvG did not prevent the development of pulmonary eosinophilia [29]. However, depletion of IL-13 in conjunction with the absence of IL-4 was found to be effective in decreasing pulmonary eosinophilia, in contrast to FI-RSV-immunized mice where depletion of either IL-4 or IL-13 was sufficient for decreased pulmonary eosinophilia [29]. Using IL-13-deficient BALB/c mice, our recent data indicates a crucial role for this cytokine because vvG-primed mice challenged with RSV do not develop pulmonary eosinophilia in the complete absence of IL-13 (Table 1). The difference between our data and that previously published could result from incomplete depletion of IL-13 or the need to have IL-13 deficiency at both immunization and challenge in order to prevent the development of a Th2 environment. Overall, these data demonstrate that IL-13, but not IL-4, is important for the development of pulmonary eosinophilia in vvG-primed mice.

Several additional observations underscore the importance of a Th2 response for the development of vvG vaccine-enhanced disease. T1/ST2 is a receptor predominately expressed on the cell surface of Th2 cells [55]. Depletion of Th2 cells using an anti-T1/ST2 antibody resulted in decreased eosinophilia, decreased CD4 T cells, and decreased CD8 T cells upon RSV challenge [56]. T1/ST2 antibody treatment also led to reduced levels of TNF-α, IFN-γ, and IL-5 [56]. Separate studies have shown that in vivo neutralization of the chemokine eotaxin mirrors T1/ST2 depletion in that pulmonary eosinophilia is decreased [49]. These studies also showed a reduction in IL-5 levels and the number of CD4 T cells after RSV challenge [49]. It is apparent from these data that Th2 cells and eotaxin are both necessary for the development of pulmonary eosinophilia in vvG-primed mice. Parallel studies have not been performed in the FI-RSV vaccine model.

Table 1 Pulmonary eosinophilia is decreased in IL-13-deficient mice

Strain	Treatment	Percent Eosinophils (± SD)	Total Number Eosinophils (x 10^3 ± SD)
BALB/c	vvβ-gal + RSV[a]	0.9 ± 0.7	5.9 ± 4.7
BALB/c	vvG + RSV	15.2 ± 7.7	132.6 ± 38
IL-13 KO	vvβ-gal + RSV	0.1 ± 0.2	1.2 ± 2
IL-13 KO	vvG + RSV	1.5 ± 0.6[b]	48.6 ± 2.8[b]

[a] Mice were scarified with 3×10^6 PFU of indicated recombinant vaccinia and challenged with 2×10^6 PFU RSV 3 weeks later. Eosinophils were determined in the BAL on day 7 post-challenge

[b] Significantly ($P < 0.05$) decreased compared to BALB/c vvG + RSV

In BALB/c mice, the G protein of RSV is known to contain an I-E^d-restricted CD4 T cell epitope ($G_{183–195}$) [50, 51, 53]. Interestingly, there is no evidence to date that the G protein elicits an H-2^d-restricted CD8 T cell response [57, 58]. Adoptive transfer of RSV G-specific T cells into RSV infected mice was sufficient for the development of pulmonary eosinophilia [59]. The RSV $G_{183–195}$ epitope elicits a mixed Th1 and Th2 response as determined by production of IFN-γ, IL-4, IL-5, and IL-13 [50, 53]. RSV $G_{183–195}$-specific CD4 T cells have been shown to predominately express the Vβ14 chain as part of their T cell receptor [53]. Depletion of the V$\beta14^+$ T cells in vvG-immunized mice led to decreased pulmonary eosinophilia [25, 52] and decreased IL-4, IL-5, IL-13, eotaxin, and IFN-γ levels [25, 52]. Therefore, RSV G-specific V$\beta14^+$ T cells are required for the enhanced disease observed in vvG-primed mice.

In FI-RSV vaccinated mice, there is no measurable response to the RSV $G_{183–195}$ epitope [28]. Furthermore, there is no predominance of V$\beta14^+$ T cells as is seen in vvG-immunized mice, and depletion of V$\beta14^+$ T cells does not affect the development of pulmonary eosinophilia [25]. These data cumulatively suggest that V$\beta14^+$ T cells contribute to the pathology of vvG-mediated enhanced disease but do not significantly contribute to FI-RSV vaccine-enhanced disease.

Contrary to what is seen with FI-RSV vaccination, vaccine-enhanced disease after vvG vaccination is dependent upon genetic background (Table 2). H-2^d (BALB/c, DBA/2n, and B10.D2) and some H-2^b (BALB.B and 129) mice vaccinated with vvG develop pulmonary eosinophilia upon RSV challenge [44, 50]. Interestingly, other H-2^b mice (C57BL/6 and C57BL/10), in addition to H-2^k mice (CBA/Ca, CBA/J, C3H, BALB.K, and B10.BR), do not develop pulmonary eosinophilia [44, 50]. Introducing H-2^b background genes by crossing C57BL/6 mice with either BALB/c or B10.D2 mice ablated pulmonary eosinophilia whereas H-$2^{d/k}$ mice developed eosinophilia after RSV challenge [44]. These data suggest that non-MHC-linked genes contribute to the lack of pulmonary eosinophilia in H-2^b mice. Whereas, the observed difference in pulmonary eosinophilia was independent of changes in viral titer and T cell responsiveness [44], mice exhibiting pulmonary eosinophilia also experienced a decrease in the ratio of CD8 to CD4 T cells in the BAL after RSV challenge [44]. Depletion of either CD8 T cells or IFN-γ in vvG-immunized C57BL/6 mice allowed for the development of pulmonary eosinophilia whereas neither treatment was effective in BALB.K mice [60]. Therefore it would seem that each strain of resistant mice potentially have distinct mechanisms of inhibiting pulmonary eosinophilia, and that in C57BL/6 mice, CD8 T cells and the production of IFN-γ are essential for protection (Table 2, discussed below).

In separate studies, G protein sensitization of BALB/c, B6.C-H2^d, BALB.B, C57BL/6, SJL, and C3H/HeJ mice was sufficient for the development of pulmonary eosinophilia upon RSV challenge [63]. However, when primed with a G peptide encompassing the 185–193 epitope, BALB.B, C57BL/6, SJL, and C3H mice did not develop pulmonary eosinophilia whereas BALB/c and B6.C-H2^d mice did [63], supporting the results above. One explanation for the difference in the Hancock et al. studies and the studies performed by Hussell et al. discussed above is a difference in the route of priming. Hussell et al. scarified mice with vvG on the posterior whereas Hancock et al. gave G protein preparations intramuscularly [44, 63]. Overall, these studies show that the development of pulmonary eosinophilia is dependent both on the route of priming as well as on the background of the vaccinated mice.

Table 2 The influence of mouse strain and T cell responses on RSV vaccine-enhanced disease

Strain	Immunization	T Cell Response (CD4 or CD8)	Manipulation	Eosinophilia	Reference
H-2^d					
BALB/c	vvβ-gal	–	–	NO	[46, 47]
BALB/c	vvG	CD4	–	YES	[46, 59]
BALB/c	vvG	CD4	αVβ14	NO	[25, 52]
BALB/c	vvG	CD4	αT1/ST2	NO	[56]
BALB/c	vvF	CD4/CD8	–	NO	[60, 61]
BALB/c	vvF	CD4/CD8	αCD8 or β_2M KO	YES	[60, 61]
BALB/c	vvG + vvF	CD4/CD8	–	YES	M.R. Olson and S.M. Varga, unpublished observation
BALB/c	vvG/M2	CD4/CD8	–	NO	[61]
BALB/c	vvG/M2	CD4/CD8	IFN-γ KO	NO*	M.R. Olson and S.M. Varga, unpublished observation
BALB/c	vvG/M2	CD4/CD8	IFN-γ/Perforin KO	NO*	M.R. Olson and S.M. Varga, unpublished observation
BALB/c	FI-RSV	CD4	–	YES	[26, 62]
BALB/c	FI-RSV	CD4	αVβ14	YES	[25]
BALB/c	FI-RSV + vvM2	CD4/CD8	–	NO	M.R. Olson and S.M. Varga, unpublished observation
DBA/2n	vvG	NA	–	YES	[44]
B10.D2	vvG	NA	–	YES	[44]
H-2^b					
C57BL/6	vvG	CD4/CD8	–	NO	[44]
C57BL/6	vvG	CD4/CD8	αCD8	YES	[44]
C57BL/6	vvG	CD4/CD8	αIFN-γ	YES	[44]
C57BL/6	FI-RSV	CD4	–	YES	
C57BL/10	vvG	NA	–	NO	[44]
BALB.B	vvG	NA	–	YES	[44]
129	vvG	NA	–	YES	[44]
H-2^k					
All Tested[a]	vvG	NA	–	NO	[44]
H-2$^{d/b}$					
All Tested[b]	vvG	NA	–	NO	[44]
H-2$^{d/k}$					
All Tested[c]	vvG	NA	–	YES	[44]

[a] BALB.K, C3H, B10.BR, CBA/J, and CBA/Ca mice

[b] F1 generations of BALB/c × C57BL/6 and B10.D2 × C57BL/6 mice

[c] F1 generations of DBA/2 × C3H, BALB/c × BALB.K, BALB/c × B10.BR, B10.D2 × BALB.K, and B10.D2 × B10.BR mice

[d] NA information not available

* Similar levels of eosinophilia compared to vvβ-gal-immunized control mice after RSV challenge

The contribution of G to FI-RSV vaccine-enhanced disease

Due to the similarities found between FI-RSV vaccinated mice and vvG-vaccinated mice, the role of the G protein in FI-RSV preparations has been examined. FI-RSV preparations that are deficient in the G protein of RSV (FI-RSVΔG) or in the immunodominant epitope RSV $G_{183–195}$ (FI-RSVΔGpep) do not differ in their ability to induce pulmonary eosinophilia when compared to unaltered FI-RSV [24]. However, the levels of eotaxin (FI-RSVΔG, FI-RSVΔGpep) and IL-5 (FI-RSVΔGpep) were decreased in mice vaccinated with FI-RSV lacking either the G protein or the CD4 peptide as compared to mice vaccinated with wild-type FI-RSV [24]. This may mean that these molecules are not necessary for the development of pulmonary eosinophilia in FI-RSV vaccinated mice, or that other factors in addition to the G protein contribute to FI-RSV vaccine-enhanced disease compensate for the loss of the protein or epitope (discussed above). Interestingly, FI-RSVΔG and FI-RSVΔGpep did allow for increased viral titers after RSV challenge suggesting that the presence of the G protein within FI-RSV provides some protection [24].

FI-RSV and vvG vaccinations both result in enhanced disease upon RSV challenge characterized by Th2 responses and pulmonary eosinophilia. Despite these similarities, it appears that independent mechanisms control disease development following vaccination with FI-RSV vs. vvG. This idea is supported by the differing cytokine requirements, dependence on background genes, cellular responses and epitope responsiveness between the two models. Future studies exploring the individual mechanisms that result in the pathology seen in these two models will deepen our understanding of the failures of RSV vaccines and assist in the development of safe and effective vaccines.

CD8 T cell regulation of CD4 T cell-induced immunopathology

CD8 T cells play a critical role in the adaptive host response against intracellular bacterial and viral pathogens. These cells are capable of not only recognizing and destroying infected cells, but are also capable of producing effector cytokines that promote the inflammatory state (e.g. IFN-γ and TNF-α). Although these are the most common roles of CD8 T cells during the resolution of infection, these cells also contribute important regulatory functions that limit CD4 T cell-driven immunopathology in several model systems.

Mice lacking β_2-microglobulin (β_2M), and thus a CD8 T cell response, suffer from a profound wasting disease after intracranial infection with lymphocytic choriomeningitis virus (LCMV) [64]. This disease is characterized by an exacerbated CD4 T cell response that is responsible for the development of clinical wasting symptoms, such as enhanced weight loss and systemic disease [65]. Furthermore, CD4 T cells isolated from LCMV-infected β_2M-deficient hosts can transfer wasting disease into naïve β_2M-deficient, but not β_2M-sufficient hosts [65]. These data suggest that CD8 T cells are critical for the regulation of the LCMV-specific CD4 T cell response. Additionally CD8 T cells have been shown to play a regulatory role in OVA-induced asthma/allergy models. In these models, CD8 T cells inhibit IgE antibody titers, the proliferation of Th2 cells and the secretion of Th2 cytokines [66–70]. In these same models, CD8 T cells have also been described to reduce the secretion of the Th2-associated chemokine CCL11 [71] and increase the presence of Th1-attracting chemokines (e.g. CXCL10) [72]. These systems highlight the important immunoregulatory role of CD8 T cells in suppressing CD4 T cell-mediated immunopathology.

RSV-specific CD8 T cell responses

T cells play a key role in the clearance of RSV. Children with T cell deficiencies have difficulty clearing the virus and are more susceptible to subsequent infection with RSV [15, 73]. Additionally, mice deficient in T cells become chronically infected whereas wild-type mice readily clear the virus [74]. Depletion of CD8 T cells alone does not cause chronic infection, but results in delayed virus clearance. These data suggest that although CD8 T cells play a role in eliminating RSV, CD4 T cells can also contribute to virus clearance [74]. Several CD8 effector molecules appear to play a role in the clearance of virus. For example, CD8 T cells from IFN-γ-deficient animals exhibit a decreased ability to mediate virus clearance when adoptively transferred into infected hosts [75]. Additionally, mice deficient in functional FasL expression and also depleted of TNF-α demonstrate delayed virus clearance [76].

CD8 T cells recognize three major RSV antigenic determinants in H-2^d BALB/c mice (Table 3). The RSV M2 protein contains a H-2K^d-restricted CD8 T cell epitope between amino acids 82 and 90. CD8 T cells responding to $M2_{82–90}$ comprise 30–50% of the pulmonary CD8 T cells at the peak of the response, approximately 8 days after an acute RSV infection [77]. A subdominant CD8 T cell epitope has been identified within the RSV M2 protein ($M2_{127–135}$) and is also restricted by H-2K^d [79]. The fusion (F) protein of RSV contains a subdominant H-2K^d-restricted CD8 T cell epitope between amino acids 85 and 93 and comprises approximately 5% of the acute CD8 T cell response in BALB/c mice [77]. Both the subdominant $M2_{127–135}$- and $F_{85–93}$-specific CD8 T cell responses appear to follow similar kinetics as the dominant $M2_{82–90}$-specific CD8 T cell response.

Recently several CD8 T cell epitopes have been described in H-2^b C57BL/6 mice (Table 3). An H-2D^b-restricted CD8 T cell epitope has been identified in the RSV matrix (M) protein (a.a. 187–195) that has similar kinetics and magnitude to that of the BALB/c $M2_{82–90}$ CD8 T cell response [81]. As discussed above, the RSV G protein contains no H-2^d-restricted CD8 T cell epitopes. However, this same protein elicits the second largest identified RSV-specific CD8 T cell epitope in C57BL/6 mice, accounting for

Table 3 The RSV-specific CD8 T cell response

Background	Protein	Amino acid #	Optimal stimulating peptide	Restriction	% of pulmonary CD8 T cells (acute infection)	References
BALB/c (H-2^b)	M2	82–90	SYIGSINNI	H-2K^d	~30–50%	[77, 78]
BALB/c	M2	127–135	VYNTVISYI	H-2K^d	NA	[79]
BALB/c	F	85–93	KYKNAVTEL	H-2K^d	~1–5%	[80]
C57BL/6 (H-2^d)	M	187–195	NAITNAKII	H-2D^b	~13%	[81, 82]
C57BL/6	G	177–188	SNNPTCWAICKR	*H-2D^b*	~8%	[82]
C57BL/6	NP	57–64	ANHKFTGL	*H-2D^b*	~7%	[82]
C57BL/6	F	433–442	KTFSNGCDYV	*H-2D^b*	~7%	[82]
C57BL/6	NP	360–368	NGVINYSVL	*H-2D^b*	~2.5%	[82]
C57BL/6	F	250–258	YMLTNSELL	*H-2D^b*	~2.5%	[82]

Optimal stimulating peptides were determined by their ability to induce IFN-γ detected by intracellular cytokine staining (ICS). Restriction elements in italics are the predicted restriction of each peptide determined by SYFPEITHI (http://www.syfpeithi.com) and BIMAS (http://www.bimas.dcrt.nih.gov/molbio/hla_bind) prediction databases. NA = data not available

approximately 8% of the total CD8 T cells in the lung after acute RSV infection. This epitope is also H-2D^b-restricted and lies between amino acids 177 and 188 [82]. Several additional RSV CD8 T cell epitopes were described in C57BL/6 mice each accounting for less than 8% of the pulmonary CD8 T cell response including; $NP_{57–64}$, $F_{433–442}$, $NP_{360–368}$, and $F_{250–258}$ [82]. The MHC class I restriction of these subdominant epitopes is predicted to be H-2D^b, but has not been directly determined ([82], Table 3).

RSV-specific CD8 T cells inhibit vvG-induced RSV vaccine-enhanced disease

As described above, RSV vaccine-enhanced disease results from sensitization of BALB/c mice with vvG followed by intranasal RSV infection (Table 2). This enhanced disease is largely characterized by a robust pulmonary CD4 T cell response, with both Th1 and Th2 components, and pulmonary eosinophilia [46, 59]. In contrast, mice immunized with a recombinant vaccinia virus expressing the RSV F protein (vvF) generated both CD4 and CD8 T cell responses after subsequent RSV infection and did not develop pulmonary eosinophilia [59]. However, vvF-immunized mice lacking a CD8 T cell response (either by antibody depletion or by use of β_2M-deficient mice) develop pulmonary eosinophilia [60, 61]. Taken together, these data suggest that RSV-specific CD8 T cells are capable of inhibiting RSV vaccine-enhanced disease.

This regulation is not unique to BALB/c mice as CD8 T cells also play an important role in the inhibition of pulmonary eosinophilia in other strains of mice. In H-2^b C57BL/6 mice, vvG-immunization does not lead to the development of pulmonary eosinophilia after subsequent RSV challenge [44]. This is likely because similar to vvF-immunized BALB/c mice; the RSV G protein contains both a CD4 and a CD8 T cell epitope in the H-2^b haplotype [50, 82]. Furthermore, C57BL/6 mice immunized with vvG that have been depleted of CD8 T cells develop pulmonary eosinophilia after subsequent RSV challenge [44]. Taken together these data suggest that RSV-specific CD8 T cells play a role in the inhibition of RSV vaccine-enhanced disease in multiple mouse strains.

Until recently, the epitopes recognized by F-specific CD4 and CD8 T cells were uncharacterized making identification and quantification of these cells difficult in the in vivo BALB/c mouse model for RSV vaccine-enhanced disease. However, a CD4 T cell epitope in the RSV G protein ($G_{183–195}$) and a CD8 T cell epitope in the RSV M2 protein ($M2_{82–90}$) have now been identified. A recombinant vaccinia virus, created by Srikiatkhachorn and Braciale [61], that expresses the RSV G protein engineered to also contain the $M2_{82–90}$ CD8 T cell epitope (vvG/M2) has further aided in determining the role of CD8 T cells in inhibiting RSV vaccine-enhanced pulmonary eosinophilia. Mice immunized with vvG/M2 exhibited significantly reduced levels of pulmonary eosinophilia compared to vvG-immunized animals. Additionally, the concurrent M2-specific CD8 T cell response inhibited the levels of Th2 cytokines (i.e. IL-4 and IL-5) produced after in vitro stimulation of lung mononuclear cells harvested from RSV challenged, vvG/M2-immunized mice [61]. However, increased levels of pulmonary IFN-γ were detected in vvG/M2-immunized mice compared to vvG-immunized mice after RSV challenge, suggesting that IFN-γ production by CD8 T cells may play a role in the inhibition of pulmonary eosinophilia [61].

As mentioned above and shown in Table 2, RSV M2-specific CD8 T cells are able to effectively abrogate vvG-induced pulmonary eosinophilia after RSV challenge. However, it is unclear if other RSV-specific CD8 T cells can achieve this same inhibitory effect. Recent data from our laboratory demonstrate that mice immunized with a 1:1 ratio of vvG

and vvM2 have significantly reduced levels of pulmonary eosinophilia compared to vvG-immunized mice after RSV challenge. Chang et al. [80] have identified a subdominant CD8 T cell epitope within the F-protein of RSV (Table 3). In contrast, mice immunized with a 1:1 ratio of vvG and vvF have similar levels of eosinophilia compared to vvG-immunized mice (M.R. Olson and S.M. Varga unpublished observation, Table 2). Furthermore, there are only 2-fold more M2-specific CD8 T cells in the lungs of vvG + vvM2-immunized mice compared to F-specific CD8 T cells in vvG + vvF-immunized mice. Taken together, these data suggest that F-specific CD8 T cells are unable to inhibit vvG-induced pulmonary eosinophilia. These results are intriguing because as described above and in Table 2, F-specific CD8 T cells inhibit vvF-induced eosinophilia after RSV challenge [60, 61]. Furthermore, F-specific CD8 T cells appear to require IFN-γ to inhibit vvF-induced pulmonary eosinophilia [60] whereas M2-specific CD8 T cells do not require IFN-γ for inhibition of vvG-induced eosinophilia in BALB/c mice (M.R. Olson and S.M. Varga, unpublished observations). These data highlight important differences between the pathology-inducing Th2 responses invoked by vvG- and vvF-immunization and potential differences in regulatory abilities of RSV-specific CD8 T cells.

CD8 T cell inhibition of FI-RSV-induced RSV vaccine-enhanced disease

As mentioned above, mice immunized with FI-RSV develop a robust Th2-driven CD4 T cell response and pulmonary eosinophilia after subsequent RSV challenge [23, 26, 62]. Our laboratory as well as others have previously described the ability of RSV M2-specific CD8 T cells to inhibit vvG-induced eosinophilia [61]. Previous work has demonstrated that whereas both vvG- and FI-RSV-immunization lead to development of pulmonary eosinophilia after RSV challenge, the mechanisms underlying each are different (see above discussion). Our laboratory has recently demonstrated that M2-specific CD8 T cells inhibit both vvG- and FI-RSV-induced pulmonary eosinophilia after RSV challenge (Table 2).

Mechanisms of CD8 T cell inhibition of RSV vaccine-enhanced disease

Data from OVA-induced allergy models and correlative data from the BALB/c mouse model of RSV vaccine-enhanced disease suggests a role of IFN-γ in the ability of CD8 T cells to regulate pulmonary eosinophilia [61, 69, 70, 83]. However, unpublished data from our laboratory and data from Srikiatkhachorn et al. [61] suggest that IFN-γ secretion by RSV-specific CD8 T cells is not required to inhibit RSV vaccine-enhanced pulmonary eosinophilia in BALB/c mice (Table 2). It is currently unclear what CD8 T cell effector molecules are required for this inhibition. Myers et al. [84] describes a regulatory antigen-specific CD8 T cell population generated after OVA, poly(I:C), and anti-41BB immunization that regulates proliferation of CD4 T cells in an IFN-γ- and TGF-β-dependent manner [84]. Both CD4 T regulatory cells (Treg) and subsets of CD8 T suppressor T cells (Ts) secrete TGF-β [85], which has wide anti-inflammatory properties and affects a range of adaptive and innate immune responses [86]. More specifically, TGF-β potently inhibits CD4 T cell proliferation in the absence of co-stimulation and the production of IL-2 in activated T cells [84]. It is possible that CD8 Ts cells are generated after RSV challenge of vvG/M2-primed mice and that these Ts cells regulate the Th2 response that leads to enhanced pulmonary eosinophilia and lung pathology. However, the role of TGF-β in this system has not yet been examined. The anti-inflammatory cytokine IL-10 has also been

implicated as an effector molecule of CD8 Ts cells and also plays a role in the inhibition of T cell responses [87, 88]. Data from our laboratory has demonstrated no increase in the frequency of M2-specific CD8 T cells expressing T regulatory cell-associated markers such as Foxp3, GITR or IL-10 (M.R. Olson and S.M. Varga, unpublished observations).

Perforin and TNF-α are important CD8 T cell effector molecules that play a role in viral clearance and contribute to the overall inflammatory environment of infected hosts. Although neither perforin nor TNF-α is required to clear RSV infection, a deficiency in either molecule results in a delay in virus clearance [76, 89]. These data suggest that perforin and TNF-α play a role in virus clearance, however it is currently unclear if these effector molecules contribute to the ability of CD8 T cells to inhibit vvG-induced pulmonary eosinophilia after RSV challenge. Previous studies suggest that delayed clearance of human metapneumovirus (a close relative to RSV) infection exacerbates the Th2-type response in the lung, thereby linking kinetics of viral clearance with the severity of the Th2 response [90]. It is possible that the concurrent CD8 T cell response in vvG/M2-immunized mice clears RSV infection more rapidly than vvG-immunized mice in a perforin- or TNF-α-dependent mechanism, thus reducing the Th2 response that drives pulmonary eosinophilia. Studies are currently underway in our laboratory to resolve these lingering questions.

Conclusions

RSV is the leading cause of hospitalization and lower respiratory tract infection in children under 5 years of age. The tragedy that occurred during the FI-RSV vaccine trial underscores the importance of carefully analyzing immune responses to vaccines in order to avoid unanticipated side affects. This tragic incident has impeded the development of a RSV vaccine for over forty years in great part because the underlying mechanisms of enhanced disease were never clarified. In addition, because natural infection does not induce long-term immunity, it is unclear which, if any, facets of the immune system will provide the most beneficial host response without priming for a memory response that causes enhanced immunopathology similar to that exhibited by the FI-RSV vaccine recipients. Although much effort has been expended in analyzing the underlying mechanisms, specifically the role of Th2 cytokines in mediating RSV vaccine-enhanced disease, only recently has a potential regulatory role for CD8 T cells been proposed. These data strongly suggest that novel RSV vaccines should aim at inducing balanced CD4 and CD8 T cell responses to enhance effectiveness and minimize CD4 T cell driven immunopathology.

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