The Journal *of* Immunology

IL-13 Is Sufficient for Respiratory Syncytial Virus G Glycoprotein-Induced Eosinophilia After Respiratory Syncytial Virus Challenge

Teresa R. Johnson, Robert A. Parker, Joyce E. Johnson and Barney S. Graham

References This article **cites 75 articles**, 42 of which you can access for free at:
http://www.jimmunol.org/content/170/4/2037.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

IL-13 Is Sufficient for Respiratory Syncytial Virus G Glycoprotein-Induced Eosinophilia After Respiratory Syncytial Virus Challenge[1]

Teresa R. Johnson,[*][†] **Robert A. Parker,**[§] **Joyce E. Johnson,**[‡] **and Barney S. Graham**[2][*][†]

Although well studied in settings of helminth infection and allergen sensitization, the combined contributions of IL-4 and IL-13 and their signaling pathways in models of viral pathogenesis have not been reported. Using a murine model of respiratory syncytial virus (RSV) infection, we evaluated the contribution of IL-13, alone and in conjunction with IL-4, during immunization with recombinant vaccinia virus expressing RSV G glycoprotein (vvGs) or with formalin-inactivated RSV (FI-RSV). We showed that both IL-4 and IL-13 activity must be inhibited to modulate G-specific responses resulting in severe RSV-induced disease. Inhibition of IL-4 or IL-13 activity alone had minimal impact on disease in vvGs-immunized mice. However, treatment of IL-4-deficient mice with IL-13Ra during vvGs immunization reduced IL-5, IL-13, and eotaxin production and pulmonary eosinophilia after RSV challenge. In contrast, FI-RSV-induced immune responses were diminished when either IL-4 or IL-13 activity was blocked. After RSV challenge, these type 2 T cell responses were also diminished in vvGs-primed IL-4Rα-deficient mice. Our data suggest that secreted vvGs uses mechanisms requiring signaling through the IL-4Rα-chain by either IL-4 or IL-13 for induction of eosinophilia and is the first description of the relative contributions of IL-4, IL-13, and their receptors in viral pathogenesis. *The Journal of Immunology,* 2003, 170: 2037–2045.

Interleukin-13 is a cytokine produced by type 2 T cells with functions paralleling those of IL-4 (1–4). These shared functions of IL-4 and IL-13 are due in part to common usage of the IL-4R α-chain in both the IL-4 and IL-13 receptors (2, 5). Although IL-4 and IL-13 exhibit similar functions in B cell development and isotype switching, a preferential role for IL-13 in some inflammatory processes has been observed (6–9). Both IL-4 and IL-13 play important roles in resistance to parasitic infection in a complex regulatory network as reviewed (10). Yet, when the phenotypes of IL-4- and IL-13-deficient helminth-infected mice are compared, striking differences are observed, suggesting parallel yet distinct roles for IL-4 and IL-13 (8).

Type 2-associated cytokines have been implicated in the pathogenesis of asthma and atopy. IL-13 has been shown to be a very potent inducer of eotaxin production, suggesting a major role for this cytokine in asthma and pathogenic conditions associated with activation of type 2 T cells (6). Recent reports demonstrate that IL-13 rather than IL-4 may be the key immune mediator in asthma (7, 11). When IL-13 activity was blocked in animal models of asthma, airway hyperresponsiveness (AHR),[3] eosinophil recruit-

ment, and mucus production were inhibited, despite intact IL-4 production and function. In complementary studies, induction of IL-13 expression in transgenic mice resulted in increased AHR, infiltration of inflammatory cells (including eosinophils), mucus production, airway obstruction, and eotaxin production (9).

Respiratory syncytial virus (RSV) is the primary viral cause of respiratory infections in infants each year (12, 13) but also produces significant disease in elderly and immunocompromised patients (14–16). Although RSV infection normally results in mild to moderate disease, some infants experience more severe disease, causing 51,000–82,000 and 63,000–100,000 hospitalizations in the U.S. each year in children younger than 1 year and 5 years of age, respectively (17).

There are two disease syndromes in humans associated with RSV infection that suggest a role for type 2 cytokines in RSV pathogenesis. Elevated levels of IgE in the serum (18) and airway secretions (19) and the presence of eosinophil cationic protein in nasal secretions (20) suggest the production of type 2 cytokines during primary RSV infection and may be a correlate of severe disease. The incidence of childhood asthma is increased in those infants who experience severe RSV disease (21, 22), and the overall rate of severe RSV-induced bronchiolitis is increasing coincident with the rising incidence of asthma (17).

The second syndrome involves immunization with a formalin-inactivated RSV vaccine (FI-RSV) in the early 1960s that resulted in no protection from natural infection and caused a vaccine-enhanced illness (23, 24). Studies in mice have shown that FI-RSV immunization primes for immune responses resulting in the production of IL-4, IL-5, and pulmonary eosinophilia and severe RSV disease (25, 26). Interestingly, immunization with the RSV attachment G glycoprotein alone can induce CD4+ T cells that produce type 2 cytokines and results in eosinophil recruitment and illness on RSV challenge (27, 28), with the secreted form of RSV G (Gs) predisposing for more severe disease than the membrane-anchored form (29, 30). An epitope has been identified in the G glycoprotein that is associated with this effect (31–33). Eliminating a region of

RSV attachment protein allows induction of protective immunity without vaccine-enhanced lung eosinophilia (31), and in BALB/c mice the response to this epitope is dominated by $V\beta14^+CD4^+$ T cells (34). The G-specific response includes production of both the type 2 cytokines IL-4 and IL-13 and the type 1 cytokine IFN-γ (29, 31, 32, 34, 35).

An important question for vaccine development is whether the antigenic determinants of the RSV G were an essential factor in the FI-RSV vaccine-enhanced illness. The similar effects on induction of Th2 responses after subsequent RSV infection of G-immunized mice suggest that RSV G Ags may have played an important role in the FI-RSV vaccine-enhanced illness. However, RSV G is expressed on wild-type virus that, during a typical primary infection, leads to a Th1-type response, and primary infection with RSV causes severe disease in a minority of infants. In addition, the G-induced response in mice is dependent on the genetic background (36), is epitope dependent (31–33), and is restricted to selected TCRs (34). In contrast, FI-RSV immunization led to enhanced disease in virtually all individuals (23, 24), suggesting that it was not dependent on the genetic background or restricted to a single epitope or TCR. Finally, the pathology, illness, and immune responses elicited by FI-RSV can also be elicited by purified RSV F glycoprotein (25), especially when formulated in alum (37), indicating that this type of response is not necessarily dependent on G-specific epitopes.

Other differences have been observed in the pattern of type 2 cytokine expression in G-immunized mice compared with mice immunized with FI-RSV. For example, when IL-4 activity was inhibited during FI-RSV immunization, disease and IL-4 production following challenge was decreased, whereas production of IFN-γ was increased (38). However, when neutralizing anti-IL-4 Ab is administered during vaccinia virus (vv) Gs immunization or when IL-4-deficient mice were immunized, pulmonary eosinophilia and type 2 cytokine production were not reduced (35). With the dominant role for IL-13 in asthma (7, 11), we hypothesized IL-13 may be the primary mediator of RSV G-induced eosinophilia and enhanced lung pathology after subsequent RSV infection. In these studies, we sought to define the requirements for IL-4 and IL-13, alone or in combination, for the induction of those immune responses that predispose for severe disease following RSV challenge of vvGs-immunized mice. The contribution of each cytokine to immunization-induced immune responses was evaluated in IL-4R$\alpha^{-/-}$ mice and in IL-4$^{+/+}$ or IL-4$^{-/-}$ mice treated with IL-13 receptor antagonist (IL-13Ra).

Materials and Methods

Cells, viruses, and IL-13Ra

HEp-2 cells were purchased from the American Type Culture Collection (Manassas, VA) and maintained in EMEM supplemented with 10% FCS, glutamine, and antibiotics (10% EMEM). A laboratory stock of RSV A2 strain was prepared as previously described (39). FI-RSV was prepared as previously described (25). Stocks of vv expressing either secreted RSV G (a gift from G. W. Wertz, University of Alabama, Birmingham, AL; designated vvGs) or β-galactosidase (a gift from B. Moss, National Institutes of Health, Bethesda, MD; designated vac-lac) were generated as described (29). All virus stocks were determined to be free of *Mycoplasma* contamination by PCR analysis. Soluble IL-13Ra was constructed by in-frame fusion of the extracellular domains of the murine IL-13R α-chain with domains 2 and 3 from the constant region of the human IgG1 heavy chain (40). IL-13Ra was shown to specifically bind and neutralize IL-13 with no effect on IL-4 activity. The IL-13Ra reagent and the appropriate isotype control were provided by S. Goldman (Genetics Institute, Cambridge, MA).

Mouse immunization and challenge

BALB/c IL-4$^{-/-}$ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IL-4R$\alpha^{-/-}$ mice on a BALB/c genetic background (41) were a gift from N. Noben-Troth (National Institutes of Health). Control (wild-type) BALB/c mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Mice were immunized with vac-lac, vvGs, or FI-RSV and challenged with RSV 6 weeks later as previously described (29). IL-13Ra was administered at priming or at challenge as specified in Table I. The mice were injected i.p. with 200 μg of control IgG or IL-13Ra daily at the indicated time points. Mice were anesthetized and challenged with 10^7 PFU RSV (in 0.1 ml) administered intranasally, then weighed, and scored for illness daily as described (29).

RSV titers

Mice were euthanized at the indicated days postchallenge, and lungs were removed and quick-frozen. PFU of virus were measured by plaque assay on subconfluent HEp-2 monolayers as previously described (29). Data are expressed as $\log_{10}$ (PFU per gram).

Cytokine levels

Cytokine protein levels in lung supernatants from day 4 plaque assays were quantitated by ELISA using cytokine-specific kits (R&D Systems, Minneapolis, MN).

Bronchoalveolar lavage (BAL) eosinophils

Seven days after challenge, BAL was performed as previously described (35). BAL cell pellets were differentially stained with Diff-Quick (Fisher Scientific, Pittsburgh, PA), and eosinophil numbers were counted as previously described (35).

Lung histopathology

Seven days after RSV challenge, mice were euthanized, and the left lung was removed and placed in phosphate-buffered formalin (10% formalin). Thin sections were cut from paraffin-embedded lungs and stained with H&E or with Wright's Giemsa. The stained lung sections were analyzed and scored for degree and composition of lung inflammation as previously described (29). Briefly, inflammation was assessed according to the following scale: 0, no infiltrate; 1, mixed generalized increase in interstitial mononuclear cells without widening of alveolar septa; 2, dense septal mononuclear infiltrates with septal thickening and occasional foci of intraalveolar cells; 3, significant alveolar space consolidation (intra-alveolar edema, inflammation, or hemorrhage) in addition to interstitial inflammation.

Ab assays: Ag-specific ELISAs and RSV neutralization

Prechallenge sera were collected from immunized mice by retro-orbital bleeding the day before RSV challenge. Sera were stored at $-20°$C until Ab assays were performed. Ab ELISAs were performed as previously described (29), coating the plates with purified RSV Gs isolated from RSV subtype A virus and detecting bound serum Ab with HRP-conjugated Abs to mouse IgG1 or IgG2a (Southern Biotechnology, Birmingham, AL). Data are represented as $\log_2$ values of the serum dilution producing 0.100 OD$_{450}$ unit and twice the Ag-negative well.

Table I. *Mouse immunization and treatment*[a]

Priming	Strain	Treatment	Designation
vac-lac	IL-4$^{+/+}$	IgG control	IL-4$^+$13$^+$
	IL-4$^{-/-}$	IgG control	IL-4$^-$13$^+$
	IL-4$^{+/+}$	IL-13Ra	IL-4$^+$13$^-$
	IL-4$^{-/-}$	IL-13Ra	IL-4$^-$13$^-$
vvGs	IL-4$^{+/+}$	IgG control	IL-4$^+$13$^+$
	IL-4$^{-/-}$	IgG control	IL-4$^-$13$^+$
	IL-4$^{+/+}$	IL-13Ra	IL-4$^+$13$^-$
	IL-4$^{-/-}$	IL-13Ra	IL-4$^-$13$^-$
FI-RSV	IL-4$^{+/+}$	IgG control	IL-4$^+$13$^+$
	IL-4$^{-/-}$	IgG control	IL-4$^-$13$^+$
	IL-4$^{+/+}$	IL-13Ra	IL-4$^+$13$^-$
	IL-4$^{-/-}$	IL-13Ra	IL-4$^-$13$^-$

[a] IL-4$^{+/+}$ or IL-4$^{-/-}$ BALB/c mice were immunized with vac-lac, vvGs, or FI-RSV. During immunization, mice were treated with IL-13Ra or IgG isotype control on days -1, 0, and $+1$. To denote cytokine status during priming, mice were designated IL-4$^+$13$^+$, IL-4$^-$13$^+$, IL-4$^+$13$^-$, or IL-4$^-$13$^-$ as shown. From 5 to 6 wk postimmunization, mice were challenged intranasally with 10^7 PFU live RSV.

Titers of RSV neutralizing Abs were determined by plaque reduction assay on HEp2 cell monolayers as described (42). The data are expressed as the $\log_2$ of the serum dilution resulting in 40% of the PBS control titers (i.e., 60% neutralization).

Statistical analysis

Data from multiple experiments were combined and summarized as mean $\pm$ SD. Comparisons between groups use a generalized linear model approach and adjusted for experiments. Post hoc testing was done only when there was a significant overall group effect in the analysis (e.g., between the different cytokine treatment groups or between the different priming agents). Post hoc testing used Dunnett's procedure that adjusts for multiple comparisons against a specific predefined group, either the IL-4^+13^+ control group or the vac-lac immunization group as appropriate. These post hoc analyses also adjusted for experiment when results from more than one experiment were combined. All analyses were done in SAS version 6.12 (SAS Institute, Cary, NC). p values < 0.05 were considered statistically significant.

Results

Interference with IL-13 alone does not alter disease in vvGs-primed mice

Eosinophil lung infiltration is induced in vvGs-primed mice (29) and is not inhibited by neutralization of IL-4 during vvGs immunization (35). We therefore examined the requirements of IL-13 for induction of eosinophilia in vvGs-immunized mice by blocking IL-13 function with the administration of IL-13Ra during vvGs priming or RSV challenge. Eosinophilia in IL-13Ra-treated vvGs-primed mice was examined 7 days after RSV challenge by differential staining of BAL cells. Significant recruitment of eosinophils occurred in mice immunized with vvGs, and eosinophilia was not inhibited by administration of IL-13Ra at the time of either vvGs priming or RSV challenge (Table II). Additionally, IL-13Ra administration at any time did not decrease the severity of illness or the production of type 2 associated cytokines in vvGs-immunized RSV-challenged mice (data not shown). Thus, those immune responses predisposing for severe illness and eosinophilia in vvGs-primed mice are induced in the absence of functional IL-13.

Th2 cytokine production and eosinophilia are abolished in vvGs-primed IL-4Rα-deficient mice

We have demonstrated that eosinophilia and type 2 cytokine production in vvGs-immunized RSV-challenged mice is not dependent on the individual activities of IL-4 (35) or IL-13 (Table II). Receptors for both IL-4 and IL-13 contain the IL-4Rα-chain (2, 5). Therefore, to examine the effects of the constitutive loss of both IL-4 and IL-13 signaling on severe RSV disease, IL-4Rα-deficient mice were immunized with vvGs and then challenged with RSV. Illness and viral titers did not significantly differ between IL-4Rα$^{+/+}$ and IL-4Rα$^{-/-}$ mice within the vac-lac- or the vvGs-immunized groups (data not shown). After RSV challenge, eosin-

ophilia was decreased in vvGs-primed IL-4Rα-deficient mice (Fig. 1, $p < 0.005$ relative to vvGs-primed IL-4Rα$^{+/+}$ mice). These data demonstrate that IL-4Rα-mediated signals contribute to the induction of immune responses during vvGs immunization that predispose for eosinophilia on RSV challenge.

IL-5 and IL-13 levels were significantly reduced in vvGs-immunized IL-4 Rα-deficient mice (Fig. 2; $p < 0.05$ relative to vvGs-primed IL-4Rα$^{+/+}$ mice). In addition, significant decreases were also seen in eotaxin production ($p < 0.05$), suggesting regulation of IL-5 and eotaxin by IL-4Rα-mediated signals. IFN-γ levels, however, were not altered in IL-4Rα-deficient mice (relative to wild-type controls). These data suggest that signals mediated by IL-4Rα engagement, presumably by the ligands IL-4 or IL-13, are required for the induction of type 2 cytokines in vvGs-immunized mice after RSV challenge.

Induction of eosinophilia in vvGs-immunized mice requires either IL-4 or IL-13, whereas both IL-4 and IL-13 are required for eosinophilia in FI-RSV-immunized mice

Inhibition of IL-4 (35) or IL-13 (above) alone does not reduce eosinophil recruitment in vvGs-primed mice in contrast to the effects observed in FI-RSV-immunized mice (35, 38), whereas the loss of both IL-4 and IL-13 signaling in vvGs-primed IL-4Rα-deficient mice results in reduced pulmonary eosinophilia and type 2 cytokine production (above). To evaluate the relative contributions of IL-4 and IL-13 in the generation of RSV-induced pulmonary eosinophilia and type 2 cytokine production, the functions of either IL-4 or IL-13 or both were inhibited during vvGs or FI-RSV immunization (Table I), and eosinophilia after subsequent RSV challenge was measured. In IL-4$^+13^+$ mice immunized with vvGs or with FI-RSV, significant eosinophilia was observed in BAL 7 days after RSV challenge (Fig. 3; $p < 0.0001$ relative to vac-lac-primed mice). In FI-RSV-primed mice, eosinophilia was significantly reduced when the function of IL-4 or IL-13—either alone or in combination—was interrupted (Fig. 3; $p < 0.001$ comparing FI-RSV-primed IL-4$^+13^+$ mice with each of the other FI-RSV-immunized groups). Conversely, in IL-4$^+13^-$ and IL-4$^-13^+$ mice primed with vvGs, eosinophil numbers were similar to that of IL-4$^+13^+$ mice ($p > 0.05$). However, in IL-4$^-13^-$ vvGs-immunized RSV-challenged mice, eosinophilia was significantly reduced (compared with IL-4$^+13^+$, IL-4$^+13^-$, and IL-4$^-13^+$ vvGs-primed mice, $p < 0.001$). Importantly, inhibition of eosinophil-recruiting immune responses during vvGs immunization required blockade of both IL-4 and IL-13 activity, whereas inhibition of IL-4 or IL-13 alone reduced eosinophilia in FI-RSV-primed mice. Thus, the cytokine requirements for eosinophil recruitment are different in vvGs-and FI-RSV-immunized mice.

Table II. *BAL eosinophilia in IL-13Ra-treated vvGs-immunized mice*[a]

Priming	Treatment at Priming	Treatment at Challenge	% Eosinophils	No. of Eosinophils ($\times 10^3$)
vac-lac	IgG control	IgG control	2.8 ± 0.7	8.1 ± 8.0
	IgG control	IL-13Ra	2.7 ± 0.5	7.5 ± 7.6
	IL-13Ra	IgG control	3.4 ± 0.7	5.4 ± 1.8
vvGs	IgG control	IgG control	18.1 ± 4.0^b	69.4 ± 29.8^b
	IgG control	IL-13Ra	12.7 ± 5.9^b	90.8 ± 25.2^b
	IL-13Ra	IgG control	14.6 ± 3.9^b	31.4 ± 8.6^b

[a] BAL was performed 7 days after RSV challenge, and the BAL cells were differentially stained and counted. Data represent the mean $\pm$ SD for $n = 5–6$ mice from a single experiment.
[b] Eosinophilia is significantly greater than in vac-lac-primed mice of any treatment group ($p < 0.005$).
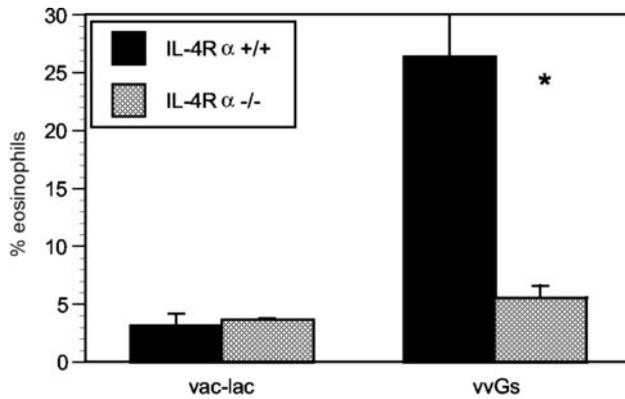
FIGURE 1. Gs-induced BAL eosinophilia is reduced in IL-4Rα-deficient mice. IL-4Rα^{+/+} or IL-4Rα^{−/−} mice were immunized with either vac-lac or vvGs and then challenged with RSV. BAL was performed 7 days after challenge, and cell pellets were differentially stained. Data represent the mean ± SD of five mice from a single experiment.

*Inhibition of IL-4 and IL-13 alters the composition and the degree of lung inflammation*

Immunization of IL-4^{+}13[+] mice with vvGs resulted in significant eosinophil recruitment to the bronchovascular bundles after RSV challenge. However, as with eosinophil infiltration to the BAL compartment, eosinophilia in the lung tissue of vvGs-immunized mice was not reduced in IL-4^{+}13[−] or IL-4^{−}13[+] vvGs-immunized mice but was significantly reduced in IL-4^{−}13[−] mice primed with vvGs and challenged with RSV compared with IL-4^{+}13[+] mice (Table III). In FI-RSV-immunized IL-4^{+}13[+] mice significant eosinophilia was observed in the tissue with eosinophil numbers reduced in IL-4^{+}13[−], IL-4^{−}13[+], and IL-4^{−}13[−] FI-RSV-primed mice ($p < 0.05$ relative to FI-RSV-immunized IL-4^{+}13[+] mice).

The severity of inflammation in the distinct histologic compartments of the lung (i.e., bronchovascular, perivenous, and interstitium) was graded using an established scoring system that has been described (29). As has been previously described (29, 30, 43, 44), vvGs-immunized RSV-challenged IL-4^{+}13[+] mice had significantly more severe lung inflammation than did vac-lac-primed IL-4^{+}13[+] mice (Table III). Generally, the severity of inflammation in
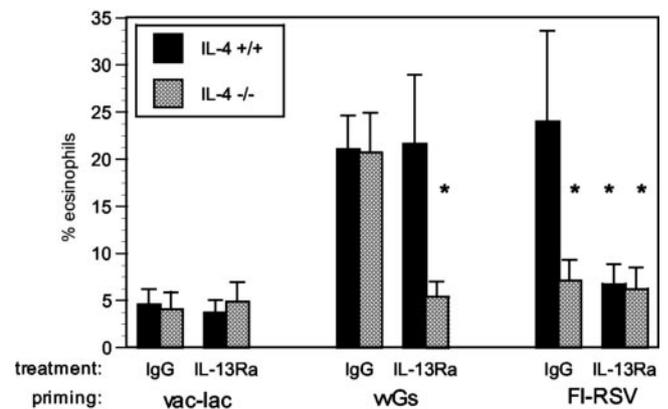
FIGURE 3. Eosinophilia is reduced by inhibition of both IL-4 and IL-13 during vvGs immunization. IL-4^{+/+} or IL-4^{−/−} mice were treated with IL-13Ra or control IgG on days −1, 0, and 1 around immunization with vac-lac, vvGs, or FI-RSV and then challenged with RSV as described in Table I. BAL was performed 7 days after challenge, and cell pellets were differentially stained. Data represent the mean ± SD for $n = 10$ from 2 combined experiments for most groups. For vac-lac- and FI-RSV-primed IL-4^{−}13[−] groups, $n = 9$, and for the vvGs-primed IL-4^{+}13[+] group, $n = 6$.

any histologic region of the lung was not significantly reduced in IL-4^{+}13[−], IL-4^{−}13[+], or IL-4^{−}13[−] vvGs-primed RSV-challenged mice (Table III). The only significant decrease in inflammation (compared with IL-4^{+}13[+] vvGs-immunized mice) was observed in the interstitial region of the IL-4^{−}13[−] mice ($p < 0.05$). In contrast, interference with IL-4 function decreased the degree of inflammation in FI-RSV-immunized mice. In the bronchovascular region, significantly less leukocyte infiltration was observed in the IL-4^{−} deficient mice regardless of the IL-13 status. Furthermore, the FI-RSV-primed IL-4^{−}13[−] mice had significantly less inflammation in the interstitial region. These data suggest that in the setting of FI-RSV immunization IL-4 has a more dominant role than IL-13 in predisposing for severe pathology after RSV challenge. Thus, the requirement for each cytokine is dependent on the nature of vaccine Ag with the disease-enhancing properties of FI-RSV immunization being more sensitive to the influences of either cytokine, particularly IL-4.
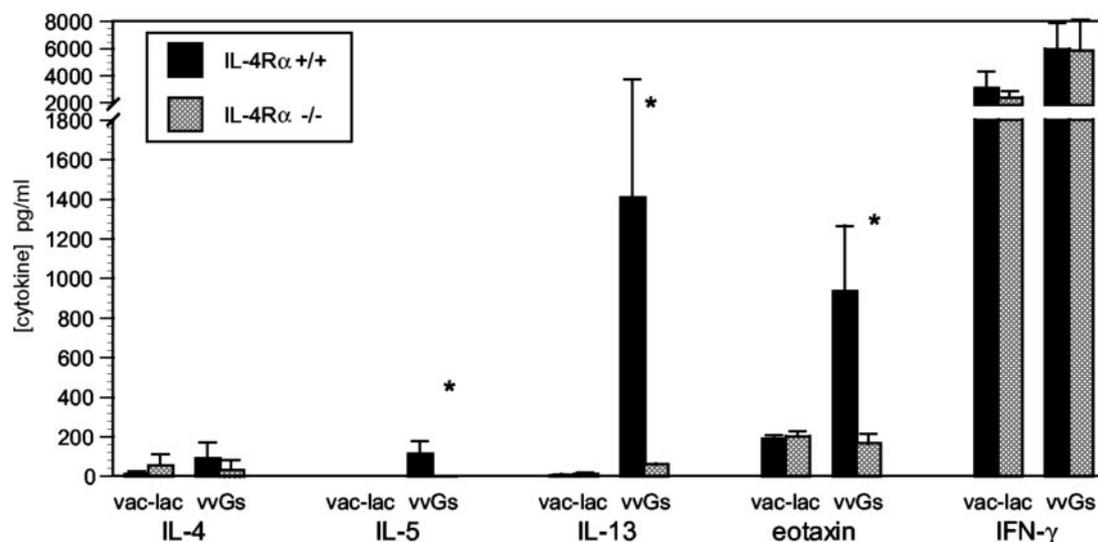
FIGURE 2. Production of type 2-associated cytokines is reduced in vvGs-primed IL-4Rα^{−/−} mice. Mice were primed and challenged as described in Fig. 1. Lung supernatants at 4 days postchallenge were examined in cytokine-specific ELISAs. Data represent the mean ± SD of five mice from a single experiment.

Table III. *Lung inflammation scores[a]*

Priming	Group	Eosinophilia[b]	Bronchovascular	Perivenous	Interstitial
vac-lac	IL-4$^+$13$^+$	0.0 ± 0.0	0.9 ± 0.5	0.8 ± 0.6	1.2 ± 0.7
	IL-4$^-$13$^+$	0.0 ± 0.0	1.1 ± 0.3	1.0 ± 0.0	1.4 ± 0.5
	IL-4$^+$13$^-$	0.0 ± 0.0	1.2 ± 0.5	1.0 ± 0.5	1.4 ± 0.7
	IL-4$^-$13$^-$	0.0 ± 0.0	1.0 ± 0.5	0.9 ± 0.3	1.6 ± 0.6
vvGs	IL-4$^+$13$^+$	50.0 ± 11.3	1.9 ± 0.5	1.3 ± 0.5	2.1 ± 0.4
	IL-4$^-$13$^+$	30.0 ± 25.2	2.0 ± 0.5	1.5 ± 0.4	2.0 ± 0.4
	IL-4$^+$13$^-$	44.5 ± 28.2	1.7 ± 0.6	1.4 ± 0.5	2.0 ± 0.7
	IL-4$^-$13$^-$	6.5 ± 20.6^c	1.5 ± 0.4	1.3 ± 0.3	1.4 ± 0.7^c
FI-RSV	IL-4$^+$13$^+$	50.0 ± 0.0	2.4 ± 0.5	1.5 ± 0.8	2.0 ± 0.7
	IL-4$^-$13$^+$	0.0 ± 0.0^c	1.4 ± 0.6^c	1.2 ± 0.6	1.3 ± 0.6
	IL-4$^+$13$^-$	17.6 ± 16.3^c	2.2 ± 0.3	1.4 ± 0.6	1.4 ± 0.7
	IL-4$^-$13$^-$	0.0 ± 0.0^c	1.5 ± 0.6^c	1.4 ± 0.5	1.0 ± 0.8^c

[a] H&E-stained thin sections of lung tissue from 7 days postchallenge were scored for severity of inflammation in a blinded manner. Data represent the pooled mean ± SD for 10 mice from 2 separate experiments except for eosinophilia in FI-RSV-primed mice, in which $n = 5$ mice from a single experiment.
[b] Percentage of eosinophils in the infiltrate surrounding the bronchovascular bundles.
[c] These groups have significantly lower scores than those for the IL-4$^+$13$^+$ mice of the same immunization group. $p < 0.05$.

Cytokine production after challenge of FI-RSV- and vvGs-primed mice is altered by inhibition of IL-4 and IL-13 function

IL-4, IL-5, IL-13, IFN-γ, and eotaxin levels in lung supernatants at day 4 postchallenge were measured by ELISA (Table IV). Cytokine production in vac-lac-immunized mice was not significantly altered by any intervention. Similarly, IL-10 and IFN-γ levels were not significantly altered in vvGs-immunized mice by any intervention when compared with IL-4$^+$13$^+$ mice, and only in the FI-RSV-primed IL-4−13- mice were IL-10 levels significantly reduced ($p < 0.01$) with no significant changes in IFN-γ production. IL-4$^+$13$^+$ mice immunized with vvGs or FI-RSV produced significantly more IL-4, IL-5, IL-10, IL-13, IFN-γ, and eotaxin than did vac-lac-primed mice ($p < 0.05$). In vvGs-immunized mice, IL-4 and IL-13 levels were significantly reduced in IL-4$^-$13$^+$ and IL-4$^-$13$^-$ mice ($p < 0.05$ relative to IL-4$^+$13$^+$ mice), but not in IL-4$^+$13$^-$ mice, whereas IL-5 production in vvGs-immunized mice was significantly altered only in vvGs-immunized IL-4$^-$13$^-$ mice ($p < 0.05$). The fact that IL-13Ra treatment of IL-4$^{+/+}$ vvGs-immunized mice did not reduce IL-13 levels is not unexpected because the IL-13Ra reagent blocks the function of secreted IL-13, not production of the cytokine. It is also possible that IL-13Ra administration increased IL-13 levels by altering its clearance. Furthermore, the levels of IL-4, IL-13, and eotaxin showed a statistically significant interaction between the IL-4 groups (IL-4$^{+/+}$ vs IL-4$^{-/-}$) and the IL-13 groups (IgG- vs IL-13Ra-treated) with cytokine levels for the IL-4$^-$13$^-$ group significantly different from the levels expected from the combination of the IL-4 group effect and the IL-13 group effect. These data suggest that the immune responses induced by vvGs immunization require either IL-4 or IL-13 and that modulation of these responses at the time of immunization requires interference with the activity of both cytokines.

When cytokine production in FI-RSV-immunized RSV-challenged mice was examined, different patterns were observed. In IL-4$^+$13$^-$ mice, IL-5, IL-13, and eotaxin production was significantly altered ($p < 0.05$ relative to IL-4$^+$13$^+$ mice). However, in the absence of IL-4 (and independent of the presence of IL-13), levels of IL-4, IL-5, IL-13, and eotaxin were significantly reduced ($p < 0.05$, comparing IL-4$^+$13$^+$ mice with either IL-4$^-$13$^+$ or IL-4$^-$13$^-$ mice). In contrast to vvGs-immunized mice, IL-13Ra treatment had minimal impact beyond the inhibition of IL-4, with an interaction observed only in IL-5 production. Thus, IL-4 is a critical regulatory component in the generation of memory T cell responses during FI-RSV immunization.

Table IV. *Cytokine production in immunized mice after RSV challenge[a]*

Priming	Group	IL-4	IL-5	IL-10	IL-13	IFN-γ	Eotaxin
vac-lac	IL-4$^+$13$^+$	13.0 ± 11.9	2.1 ± 3.1	149.1 ± 138.8	9.2 ± 12.5	269 ± 227	48.3 ± 47.8
	IL-4$^-$13$^+$	2.0 ± 1.6	52.9 ± 91.0	264.1 ± 270.8	17.7 ± 28.2	246 ± 294	61.4 ± 26.7
	IL-4$^+$13$^-$	6.9 ± 7.0	1.8 ± 1.5	236.8 ± 173.9	4.5 ± 5.2	247 ± 263	42.5 ± 32.5
	IL-4$^-$13$^-$	5.0 ± 1.6	7.9 ± 16.8	204.2 ± 155.8	1.4 ± 1.0	77 ± 82	57.5 ± 40.4
vvGs	IL-4$^+$13$^+$	119.7 ± 39.7	109.6 ± 108.7	274.9 ± 264.4	352.4 ± 150.9	1323 ± 827	296.5 ± 127.6
	IL-4$^-$13$^+$	7.5 ± 3.7^b	46.6 ± 59.1	260.6 ± 314.3	134.8 ± 135.0^b	2339 ± 1088	210.9 ± 141.9
	IL-4$^+$13$^-$	226.4 ± 109.1^b	168.8 ± 93.0	299.3 ± 299.4	514.9 ± 208.6	2139 ± 1967	426.2 ± 183.8
	IL-4$^-$13$^-$	6.3 ± 5.6^b	21.9 ± 33.7^b	250.5 ± 239.1	88.1 ± 78.5^b	1715 ± 1080	151.8 ± 86.7
FI-RSV	IL-4$^+$13$^+$	166.3 ± 93.2	157.0 ± 106.2	456.2 ± 363.0	545.2 ± 196.9	1377 ± 656	772.8 ± 264.1
	IL-4$^-$13$^+$	3.8 ± 2.3^b	13.0 ± 16.2^b	334.8 ± 314.3	112.2 ± 85.6^b	954 ± 720	373.0 ± 220.4^b
	IL-4$^+$13$^-$	114.2 ± 67.4	74.2 ± 41.7^b	398.6 ± 331.9	367.7 ± 150.8^b	2415 ± 1785	377.1 ± 233.2^b
	IL-4$^-$13$^-$	3.7 ± 4.8^b	55.2 ± 82.1^b	214.3 ± 161.8^b	51.4 ± 27.7^b	2183 ± 2225	192.3 ± 82.0^b

[a] Cytokine levels in day 4 lung supernatants were measured by specific ELISA. Data are represented as mean ± SD for 8–10 mice from 2 combined experiments. Limit of detection is 2 pg/ml for IL-4, IL-5, IL-10, and IL-13 kits and 10 pg/ml for IFN-γ and eotaxin kits.
[b] Statistically significantly difference relative to IL-4$^+$13$^+$ mice of same priming group.

Table V. *Viral titers in the lung after RSV challenge[a]*

Priming	Group	Day 4	Day 7
vac-lac	IL-4$^+$13$^+$	6.98 ± 0.44	3.43 ± 1.47
	IL-4$^-$13$^+$	6.99 ± 0.44	3.46 ± 0.78
	IL-4$^+$13$^-$	7.06 ± 0.88	3.73 ± 0.45
	IL-4$^-$13$^-$	7.07 ± 0.28	3.68 ± 1.00
vvGs	IL-4$^+$13$^+$	5.98 ± 0.63	1.50 ± 0.00
	IL-4$^-$13$^+$	5.91 ± 0.71	1.54 ± 0.11
	IL-4$^+$13$^-$	6.23 ± 0.41	1.69 ± 0.26
	IL-4$^-$13$^-$	6.37 ± 0.56	1.59 ± 0.19
FI-RSV	IL-4$^+$13$^+$	5.66 ± 0.60	1.59 ± 0.18
	IL-4$^-$13$^+$	3.80 ± 1.16^b	2.01 ± 0.55
	IL-4$^+$13$^-$	3.18 ± 0.98^b	1.50 ± 0.00
	IL-4$^-$13$^-$	2.21 ± 0.77^b	1.51 ± 0.01

[a] Lungs were removed 4 and 7 days after RSV challenge, and viral titers were subsequently measured by plaque assay on subconfluent HEp2 monolayers. Data represent the pooled mean log$_{10}$ (PFU/gram lung) ± SD for 9–10 mice from 2 separate experiments. The limit of detection is 1.8 log$_{10}$ (PFU/gram lung).

[b] These groups have significantly lower scores than those for the IL-4$^+$13$^+$ mice of the same immunization group. $p < 0.05$.

IL-4 and IL-13 inhibit viral clearance in FI-RSV-immunized mice but not in vvGs-primed mice

Peak RSV replication occurs 3–5 days after infection of mice, and virus is cleared around 8 days postinfection (39). Lack of IL-4 or IL-13 did not alter peak viral titers in vac-lac- or vvGs-primed mice (Table V). In FI-RSV-immunized mice, neutralization of IL-4 during priming has been shown to increase cytotoxic T lymphocyte killing and reduce peak viral titers (38). Therefore, the decrease in day 4 viral titers of FI-RSV-primed IL-4$^-$13$^+$ and IL-4$^-$13$^-$ would be predicted. However, the decreased titers in IL-4$^-$13$^-$ FI-RSV-immunized mice are unexpected and may suggest a role for IL-13 in CTL activity. A recent report demonstrates the ability of IL-13 to synergize with GM-CSF and CD40 ligand to increase CTL activity (45). These data suggest that, whereas both IL-4 and IL-13 affect the induction of those immune responses that result in viral clearance in FI-RSV-primed mice, neither cytokine is critical to the induction of antiviral responses during vvGs immunization.

IL-4 and IL-13 alter Ag-specific Ab levels but not neutralizing Ab activity

The reduction in viral titers reported in Table V above may be due to increased titers of RSV-specific Abs. Therefore, RSV G-specific Ab and RSV-neutralizing Ab responses were measured (Table VI). As reported previously (29), vvGs immunization induced very low levels of neutralizing Ab. FI-RSV immunization resulted in higher levels of neutralizing activity that were significantly higher than vac-lac- or vvGs-primed mice ($p < 0.001$). Additionally, interference with IL-4 or IL-13 function did not alter neutralizing activity in most treatment groups. Only in FI-RSV-immunized IL-4$^-$13$^-$ mice was a significant increase in neutralizing Ab titers observed ($p < 0.05$ relative to IL-4$^+$13$^+$ FI-RSV-primed mice). Therefore, the increased antiviral activity that reduced viral titers in FI-RSV-immunized IL-4$^-$13$^+$ and IL-4$^+$13$^-$ mice was not due to increased neutralizing Ab activity.

Abs to RSV Gs were not detected in any vac-lac-primed mouse, whereas significant Ab responses were present in both vvGs- and FI-RSV-immunized mice (Table VI). Although unable to make IgE, the capacity of IL-4-deficient mice to produce IgG is intact (46). However, it has been reported that responses are biased to Abs with an IgG2a isotype with some decrease in IgG1 isotype. This pattern is reflected in our data in which titers of G-specific IgG2a Abs are significantly greater in IL-4$^-$13$^+$ and IL-4$^-$13$^-$ mice relative to IL-4$^+$13$^+$ mice primed with either vvGs or FI-RSV. However, only in FI-RSV-primed IL-4$^-$13$^+$ and IL-4$^-$13$^-$ mice is a concomitant decrease in the IgG1 isotype observed. Furthermore, in vvGs-primed IL-4$^+$13$^-$ mice, inhibition of IL-13 activity alone significantly increases both IgG1 and IgG2a Ab titers, underscoring a more prominent role for IL-13 in G-specific responses. Although significant differences were observed in G-specific Ab responses, these alterations did not reflect changes observed in viral titers (Table V) or lung histopathology (Table III; particularly in vvGs-immunized mice), suggesting that these parameters are correlates of altered T cell responses.

Discussion

Immunization with vvGs does not require IL-4 to induce pulmonary eosinophilia following RSV challenge (35). In contrast, FI-RSV-induced eosinophilia is IL-4 dependent (35, 38). However,

Table VI. *Ab responses in immunized mice[a]*

Priming	Group	G-Specific ELISA Ab Production		
		IgG1 titers	IgG2a titers	Neutralizing Ab Titers
vac-lac	IL-4$^+$13$^+$	NDT	NDT	3.49 ± 0.99
	IL-4$^-$13$^+$	NDT	NDT	3.11 ± 0.91
	IL-4$^+$13$^-$	NDT	NDT	3.67 ± 1.14
	IL-4$^-$13$^-$	NDT	NDT	3.36 ± 1.19
vvGs	IL-4$^+$13$^+$	10.69 ± 2.07	10.64 ± 2.62	3.84 ± 1.10
	IL-4$^-$13$^+$	9.82 ± 2.14	13.62 ± 1.02^b	3.45 ± 0.80
	IL-4$^+$13$^-$	12.15 ± 0.88^b	11.71 ± 2.53^b	3.85 ± 1.02
	IL-4$^-$13$^-$	10.84 ± 1.14	13.01 ± 0.81^b	4.45 ± 1.48
FI-RSV	IL-4$^+$13$^+$	12.27 ± 1.50	9.72 ± 2.60	4.48 ± 1.77
	IL-4$^-$13$^+$	8.86 ± 1.37^b	10.77 ± 2.47^b	4.59 ± 1.51
	IL-4$^+$13$^-$	11.44 ± 1.27	8.98 ± 2.07	5.19 ± 1.29
	IL-4$^-$13$^-$	9.41 ± 1.31^b	12.45 ± 0.36^b	5.40 ± 1.73^b

[a] Sera were collected from immunized mice before RSV infection. G-specific Ab titers were determined by ELISA, and data are expressed as the log$_2$ of the serum dilution producing 0.100 OD$_{450}$ U. Neutralizing Ab titers were measured in an RSV plaque reduction assay. Data are expressed as the log$_2$ of the serum dilution resulting in 40% plaque formation of the PBS control wells. Data represent the mean ± SD of 10 serum samples from 2 separate experiments. The limits of detection are 6.0 and 3.25 for the ELISA and neutralization assays, respectively. NDT, Not detected.

interference with IL-4 activity during FI-RSV immunization results in only a partial reversal of the type 2-associated disease profiles (35, 38). Additionally, in FI-RSV-immunized RSV-challenged mice, IL-4 is the dominant cytokine found in the lung, whereas IL-13 is expressed at higher levels in vvGs-primed mice (35). Therefore, we examined the role of IL-13 in RSV disease, alone and in combination with IL-4, in vvGs- and FI-RSV-immunized mice. The studies presented here describe the first detailed analysis of the combined contribution of these cytokines to the induction of virus-specific type 2 T cell responses and demonstrate distinct requirements for IL-4 and IL-13 in the induction of RSV-specific immune responses during immunization with vvGs and with FI-RSV.

Inhibition of either IL-4 or IL-13 alone reduces eosinophilia, the severity of pulmonary inflammation, and type 2 cytokine production in FI-RSV-immunized RSV-challenged mice. In contrast, both IL-4 and IL-13 activity must be blocked to diminish vvGs-induced eosinophilia and type 2 cytokine production. These data suggest that different immune mechanisms are used by vvGs and FI-RSV to initiate $CD4^+$ T cell responses that predispose for eosinophilia and type 2 cytokine production after RSV challenge. Several factors may contribute to these differential cytokine requirements by vvGs and FI-RSV. First, the effects of adjuvant during immunization must be considered, because RSV-specific immune responses are influenced quantitatively and qualitatively by adjuvant (47). Thus, the alum-precipitated FI-RSV and the live vaccinia virus vector of vvGs may activate distinct signaling pathways. Yet, it has been demonstrated that it is not merely the adjuvant that establishes eosinophil-recruiting immune responses, but rather the viral Ag also contributes to the phenotype of these responses (43). Secondly, the site of immunization may contribute to the induction of these distinct responses. Bembridge et al. (30) demonstrated that vvGs given intradermally led to pulmonary eosinophilia, but vvGs given i.p. did not. These data may suggest that the context of Ag presentation is important and that APC subpopulations influence differentiation of naive cells into Th1 or Th2 cells (48). Thirdly, antigenic load impacts the T cell differentiation process (49–52). This may play a role in the differential cytokine requirements for vvGs- and FI-RSV-induced immune response because FI-RSV, a formalin-inactivated virus, is administered at a single time point whereas immunization with vvGs, the replication of which may be detected for >2 wk postinfection (53), results in prolonged production of Ag.

The precise antigenic composition of an immunogen may influence the cytokine requirements for T cell differentiation. For example, requirements for IL-4 have been shown to vary depending on the strain of parasite used for infection, given that IL-4-deficient mice are resistant to only some strains of *Leishmania major* (54). It has also been shown that the stage of parasite life cycle influences cytokine requirements with IL-13 required for expulsion of *Nippostrongylus brasiliensis* adult worms, but not eggs (55). Hancock et al. (43) demonstrated that responses to purified RSV fusion (F) glycoprotein might be modulated by the Th1-inducing adjuvant QS-21, whereas immune responses induced by purified RSV G were not significantly altered.

Our data underscore the complexity of the responses that result in immunopathology following viral challenge. Although interference with IL-4 or IL-13 activity resulted in concomitant decreases in IL-5 production and pulmonary eosinophilia in FI-RSV-immunized mice after RSV challenge, only inhibition of both IL-4 and IL-13 resulted in significant decreases in IL-5 production and in pulmonary eosinophilia in vvGs-immunized RSV-challenged mice. There are conflicting data describing the requirements of IL-5 for eosinophil recruitment in paramyxovirus-infected mice.

Whereas Schwarze et al. (56) report a lack of eosinophilia and airway hyperresponsiveness in IL-5-deficient mice during primary infection with RSV, other groups have been unable to reproduce the findings that primary RSV infection elicits IL-5 and eosinophilia in wild-type mice. In addition, using pneumonia virus of mice (PVM) or Sendai virus (natural paramyxovirus pathogens in mice), Domachowske et al. (57) observed significant pulmonary eosinophilia in IL-5-deficient mice. Macrophage-inflammatory protein-1α (MIP-1α) is produced after RSV and PVM infection of mice (58–60). PVM infection of mice deficient in either MIP-1α or its receptor CCR1 resulted in reduced eosinophilia (59, 60). Similarly, RSV-infected MIP-1$\alpha^{-/-}$ mice had significantly less pulmonary inflammation, although no eosinophils were present in either MIP-1$\alpha^{+/+}$ or MIP-1$\alpha^{-/-}$ mice (58). Thus, the fact that interference with either IL-4 or IL-13 function alone during vvGs immunization did not significantly alter IL-5 production or eosinophil recruitment may suggest that other inflammatory mediators such as MIP-1α may be more important in RSV-specific eosinophilic responses.

It has recently been reported that the enhanced respiratory disease in FI-RSV-immunized mice, as measured by AHR is the result of immune complex deposition in the lung after RSV challenge, with AHR being reduced in C3- and IgG-deficient mice (61). Thus, the formation of immune complexes could contribute to the enhanced illness and pathology described in our studies. However, pulmonary histopathology was not reduced in FI-RSV-immunized RSV-challenged C3- or IgG-deficient mice (61). Additionally, interference with IL-4 or IL-13 function resulted in changes in pulmonary eosinophilia or illness without concomitant changes in Ab production. Therefore, although a role for Ab cannot be ruled out, these data suggest that T cells and their products are the factors that mediate the illness and more severe pathology observed in vvGs-immunized mice.

These studies have not defined which components of the FI-RSV vaccine-induced immune response were responsible for modifying the RSV titers after challenge. Although both IL-4$^-$13$^+$ and IL-4$^+$13$^-$ mice immunized with FI-RSV had lower virus titers postchallenge, there is no indication that neutralizing activity or Ab levels were different between groups. Previous work from our laboratory has demonstrated that anti-IL-4 treatment of FI-RSV-immunized mice (38) or FI-RSV immunization of IL-4-deficient mice resulted in decreased RSV titers and increased CTL activity (38). We and other groups have also shown that excessive amounts of IL-4 inhibit CTL function and can alter the mechanism of CTL activity (62–65) to a less efficient Fas-Fas ligand-dominated pathway (66).

Antiviral activity of eosinophils has been described for paramyxoviruses (67, 68) and for HIV-1 (69). Although direct antiviral activity of isolated eosinophils and of recombinant eosinophil RNases have been shown in vitro against RSV (67, 68), our data demonstrate high viral titers after RSV challenge of vvGs- and FI-RSV-immunized mice that develop abundant pulmonary eosinophilia. However, this is not necessarily a contradiction. In in vivo studies reported by Rosenberg and Domachowske (68), eosinophil infiltration peaks on day 3 postinfection when virus is still present in the lung and, therefore, when the antiviral activity of eosinophils may be detected. However, the kinetics of eosinophil recruitment after RSV challenge is different with peak eosinophilia occurring days 6 and 7 postchallenge (36). Peak viral titers occur at day 4 postchallenge, a time at which little pulmonary inflammation has yet occurred, and virus is cleared on days 7–9 postinfection (39). In immunized mice, viral clearance occurs 1–2 days sooner. Thus, in vvGs- or FI-RSV-immunized mice, viral clearance is

nearly complete before significant numbers of eosinophils are recruited to the lung, making it difficult to draw any conclusions about the antiviral activity of eosinophils against RSV in vivo. Therefore, it is predominantly the antiviral activity of T cells, not eosinophils, which mediate viral clearance of RSV in these studies.

Reports describing mice deficient in IL-4, IL-13, IL-4Rα, or Stat6 infected with a variety of parasites have shown different disease profiles, depending on the immunodeficiency (54, 55, 70–74). Collectively, these studies demonstrate that multiple and redundant mechanisms exist in vivo for generation of immune responses associated with the Th2 phenotype. The data reported here confirm these findings and extend them to virus-specific immune responses. As with *N. brasiliensis* infection of IL-4Rα-deficient mice (41), we demonstrate a complete ablation of IL-5-producing immune responses in vvGs-primed, RSV-challenged mice and significant reductions in IL-4 and IL-13 production. Chemokine production has not previously been reported in these mice; we show that in the absence of IL-4Rα signaling, production of eotaxin is also substantially reduced. However, levels of IL-13 in vvGs-immunized IL-4Rα-deficient mice are still significantly higher than in vac-lac-primed mice, demonstrating the presence of alternative mechanisms for generating type 2 cytokine production that do not require IL-4Rα-mediated signals for induction or establishment of that memory cell population. NK T cells have been suggested as one cell population that may be responsible for IL-4 production in the absence of IL-4Rα-mediated signaling (41, 74) and may serve as a source of IL-13 in the experiments reported here.

The data reported here show that, although both vvGs and FI-RSV immunization predispose for production of type 2 cytokines and pulmonary eosinophilia after challenge, the cytokine requirements for the induction of these immune responses are distinct. We have demonstrated that IL-13 is sufficient for mediating the type 2 cytokine response and eosinophilia induced by immunization with vvGs and can compensate for the absence of IL-4. This is in contrast to the type 2 response induced by FI-RSV that is strictly IL-4 dependent. In addition, vvGs immunization favors IL-13 production (29), whereas FI-RSV immunization favors IL-4 production (38). Whether these distinctions in the pattern of cytokine production and requirements are based on the strength of the response induced by secreted RSV G, different Ag delivery systems with different durations or quantities of Ag delivery, or distinct RSV-specific Ags eliciting the response has not been determined. The G-induced CD4$^+$ T cell response is targeted to an immunodominant peptide (31–33). However, recently it has been reported that the response to the immunodominant G epitope is I-E^d-restricted with a predominant Vβ14 TCR usage, suggesting the epitope-specific response may be limited to selected genotypes (75). This genetically restricted T cell response is not consistent with the FI-RSV vaccine-enhanced disease that occurred in virtually all human vaccinees (23), but could be relevant to the pathogenesis of severe disease in naturally acquired primary infection, which occurs in relatively few individuals. The collective data indicate that G-induced immune responses cannot fully account for the FI-RSV vaccine-enhanced illness and that factors other than RSV G antigenicity contribute to the vaccine-enhanced disease induced by FI-RSV immunization. These factors may include the obligate MHC class II Ag processing of the particulate and nonreplicating FI-RSV immunogen, the lack of MHC class I Ag presentation, or the adjuvant properties of alum. However, the data suggest that there are multiple pathways for inducing responses associated with type 2 cytokines. Defining these pathways and their mechanisms of regulation is important for determining the appropriate composition of a safe and effective RSV vaccine.

References

1. Lucey, D. R., M. Clerici, and G. M. Shearer. 1996. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. *Clin. Microbiol. Rev. 9:532.*
2. de Vries, J. E., and G. Zurawski. 1995. Immunoregulatory properties of IL-13: its potential role in atopic disease. *Int. Arch. Allergy Immunol. 106:175.*
3. Punnonen, J., and J. E. de Vries. 1994. IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. *J. Immunol. 152: 1094.*
4. Deckers, J. G. M., S. de Hau, F. J. van der Woude, S. W. van der Kooij, M. R. Daha, and C. van Kooten. 1998. IL-4 and IL-13 augment cytokine- and CD40-induced RANTES production by human renal tubular epithelial cells in vitro. *J. Am. Soc. Nephrol. 9:1187.*
5. Lin, J.-X., T.-S. Migone, M. Tsang, M. Friedman, J. A. Weatherbee, L. Zhou, A. Yamauchi, E. T. Bloom, J. Mietz, S. John, and W. J. Leonard. 1995. The role of shared receptor motifs and common stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL- 4, IL-7, IL-13, and IL-15. *Immunity 2:331.*
6. Li, L., Y. Xia, A. Nguyen, Y. H. Lai, L. Feng, T. R. Mosmann, and D. Lo. 1999. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J. Immunol. 162:2477.*
7. Grunig, G., M. Warnock, A. E. Wakil, R. Venkayya, F. Brombacher, D. M. Rennick, D. Sheppard, M. Mohrs, D. D. Donaldson, R. M. Locksley, and D. B. Corry. 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science 282:2261.*
8. Bancroft, A. J., A. N. J. McKenzie, and R. K. Grencis. 1998. A critical role for IL-13 in resistance to intestinal nematode infection. *J. Immunol. 160:3453.*
9. Zhu, Z., R. J. Homer, Z. Wang, Q. Chen, G. P. Geba, J. Wang, Y. Zhang, and J. A. Elias. 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest. 103:779.*
10. McKenzie, A. N. J. 2000. Regulation of T helper type 2 cell immunity by interleukin-4 and interleukin-13. *Pharmacol. Ther. 88:143.*
11. Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, and D. D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science 282:2258.*
12. La Via, W. V., M. I. Marks, and H. R. Stutman. 1992. Respiratory syncytial virus puzzle: clinical features, pathophysiology, treatment, and prevention. *J. Pediatr. 121:503.*
13. Ruuskanen, O., and P. L. Ogra. 1993. Respiratory syncytial virus. *Curr. Prob. Pediatr. 23:50.*
14. Dowell, S. F., L. J. Anderson, J. Gary, D. D. Erdman, J. F. Plouffe, J. File, B. J. Marston, and R. F. Breiman. 1996. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. *J. Infect. Dis. 174:456.*
15. Falsey, A. R., E. E. Walsh, and R. F. Betts. 1990. Serologic evidence of respiratory syncytial virus infection in nursing home patients. *J. Infect. Dis. 162:568.*
16. Hall, C. B., K. R. Powell, N. E. MacDonald, C. L. Gala, M. E. Menegus, S. C. Suffin, and H. J. Cohen. 1986. Respiratory syncytial viral infection in children with compromised immune function. *N. Engl. J. Med. 315:77.*
17. Shay, D. K., R. C. Holman, R. D. Newman, L. L. Liu, J. W. Stout, and L. J. Anderson. 1980. Bronchiolitis-associated hospitalizations among US children, 1980 –1996. *JAMA 282:1440.*
18. Bui, R. H. D., G. A. Molinaro, J. D. Kettering, D. C. Heiner, D. T. Imagawa, and J. St. Geme. 1987. Virus-specific IgE and IgG4 antibodies in serum of children infected with respiratory syncytial virus. *J. Pediatr. 110:87.*
19. Welliver, R. C., D. T. Wong, M. Sun, J. Middleton, R. S. Vaughn, and P. L. Ogra. 1981. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N. Engl. J. Med. 305: 841.*
20. Garofalo, R., J. L. L. Kimpen, R. C. Welliver, and P. L. Ogra. 1992. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. *J. Pediatr. 120:28.*
21. Martinez, F. D., A. L. Wright, L. M. Taissig, C. J. Holberg, M. Halonen, W. J. Morgan, and the Group Health Medical Associates. 1995. Asthma and wheezing in the first six years of life. *N. Engl. J. Med. 332:133.*
22. McIntosh, K. 1976. Bronchiolitis and asthma: possible common pathogenetic pathways. *J. Allergy Clin. Immunol. 57:595.*
23. Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen, and R. H. Parrott. 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am. J. Epidemiol. 89:422.*
24. Kapikian, A. Z., R. H. Mitchell, R. M. Chanock, R. A. Shvedoff, and C. E. Stewart. 1969. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am. J. Epidemiol. 89:405.*
25. Graham, B. S., G. S. Henderson, Y.-W. Tang, X. Lu, K. M. Neuzil, and D. G. Colley. 1993. Priming immunization determines T helper cytokine mRNA expression patterns in lungs of mice challenged with respiratory syncytial virus. *J. Immunol. 151:2032.*

26. Stott, E. J., G. Taylor, L. A. Ball, K. Anderson, K. K. Y. Young, A. M. Q. King, and G. W. Wertz. 1987. Immune and histopathological responses in animals vaccinated with recombinant vaccinia viruses that express individual genes of human respiratory syncytial virus. *J. Virol. 61:3855.*

27. Roberts, S. R., D. L. Lichtenstein, L. A. Ball, and G. W. Wertz. 1994. The membrane-associated and secreted forms of the respiratory syncytial virus attachment glycoprotein G are synthesized from alternative initiation codons. *J. Virol. 68:4538.*

28. Alwan, W. H., W. J. Kozlowska, and P. J. M. Openshaw. 1994. Distinct types of lung disease caused by functional subsets of antiviral T cells. *J. Exp. Med. 179:81.*

29. Johnson, T. R., J. E. Johnson, S. R. Roberts, G. W. Wertz, R. A. Parker, and B. S. Graham. 1998. Priming with secreted glycoprotein G of respiratory syncytial virus (RSV) augments interleukin-5 production and tissue eosinophilia after RSV challenge. *J. Virol. 72:2871.*

30. Bembridge, G. P., R. Garcia-Beato, J. A. Lopez, J. A. Melero, and G. Taylor. 1998. Subcellular site of expression and route of vaccination influence pulmonary eosinophilia following respiratory syncytial virus challenge in BALB/c mice sensitized to the attachment G protein. *J. Immunol. 161:2473.*

31. Sparer, T. E., S. Matthews, T. Hussell, A. J. Rae, B. Garcia-Barreno, J. A. Melero, and P. J. M. Openshaw. 1998. Eliminating a region of respiratory syncytial virus attachment protein allows induction of protective immunity without vaccine-enhanced lung eosinophilia. *J. Exp. Med. 187:1921.*

32. Srikiatkhachorn, A., W. Chang, and T. J. Braciale. 1999. Induction of Th-1 and Th-2 responses by respiratory syncytial virus attachment glycoprotein is epitope and major histocompatibility complex independent. *J. Virol. 73:6590.*

33. Tebbey, P. W., M. Hagen, and G. E. Hancock. 1998. Atypical pulmonary eosinophilia is mediated by a specific amino acid sequence of the attachment (G) protein of respiratory syncytial virus. *J. Exp. Med. 188:1967.*

34. Varga, S. M., E. L. Wissinger, and T. J. Braciale. 2000. The attachment (G) glycoprotein of respiratory syncytial virus contains a single immunodominant epitope that elicits both Th1 and Th2 CD4$^+$ T cell responses. *J. Immunol. 165: 6487.*

35. Johnson, T. R., and B. S. Graham. 1999. Secreted respiratory syncytial virus G glycoprotein induces interleukin-5 (IL-5), IL-13, and eosinophilia by an IL-4-independent mechanism. *J. Virol. 73:8485.*

36. Hussell, T., A. Georgiou, T. E. Sparer, S. Matthews, P. Pala, and P. J. M. Openshaw. 1998. Host genetic determinants of vaccine-induced eosinophilia during respiratory syncytial virus infection. *J. Immunol. 161:6215.*

37. Hancock, G. E., D. J. Hahn, D. J. Speelman, S. W. Hildreth, S. Pillai, and K. McQueen. 1994. The pulmonary immune response of BALB/c mice vaccinated with the fusion protein of respiratory syncytial virus. *Vaccine 12:267.*

38. Tang, Y.-W., and B. S. Graham. 1994. Anti-IL-4 treatment at immunization modulates cytokine expression, reduces illness, and increases cytotoxic T lymphocyte activity in mice challenged with respiratory syncytial virus. *J. Clin. Invest. 94:1953.*

39. Graham, B. S., M. D. Perkins, P. F. Wright, and D. T. Karzon. 1988. Primary respiratory syncytial virus infection in mice. *J. Med. Virol. 26:153.*

40. Donaldson, D. D., M. J. Whitters, L. J. Fitz, T. Y. Neben, H. Finnerty, S. L. Henderson, J. O'Hara, D. R. Beier, K. J. Turner, C. R. Wood, and M. Collins. 1998. The murine IL-13 receptor α2: molecular cloning, characterization, and comparison with murine IL-13 receptor-α1. *J. Immunol. 161:2317.*

41. Noben-Trauth, N., L. D. Shultz, F. Brombacher, J. Urban, H. Gu, and W. E. Paul. 1997. An interleukin 4 (IL-4)-independent pathway for CD4$^+$ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc. Natl. Acad. Sci. USA 94:10838.*

42. Openshaw, P. J. M., S. L. Clarke, and F. M. Record. 1992. Pulmonary eosinophilic response to respiratory syncytial virus infection in mice sensitized to the major surface glycoprotein G. *Int. Immunol. 4:493.*

43. Hancock, G. E., D. J. Speelman, K. Heers, E. Bortell, J. Smith, and C. Cosco. 1996. Generation of atypical pulmonary inflammatory responses in BALB/c mice after immunization with the native attachment (G) glycoprotein of respiratory syncytial virus. *J. Virol. 70:7783.*

44. Srikiatkhachorn, A., and T. J. Braciale. 1997. Virus-specific, CD8$^+$ T lymphocytes down regulate Th2 type cytokine secretion and pulmonary eosinophilia during experimental murine respiratory syncytial virus infection. *J. Exp. Med. 186:421.*

45. Ahlers, J. D., I. M. Belyakov, M. Terabe, D. D. Donaldson, E. K. Thomas, and J. A. Berzofsky. 2002. A push-pull approach to maximize vaccine efficacy: abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L. *Proc. Natl. Acad. Sci. USA 99:13020.*

46. Kopf, M., G. Le Gros, M. Bachmann, M. C. Lamers, H. Bluethmann, and G. Kohler. 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature 362:245.*

47. Neuzil, K. M., J. E. Johnson, Y.-W. Tang, J.-P. Prieels, M. Slaoui, N. Gar, and B. S. Graham. 1997. Adjuvants influence the quantitative and qualitative immune response in BALB/c mice immunized with respiratory syncytial virus FG subunit vaccine. *Vaccine 15:525.*

48. Rissoan, M.-C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal Malefyt, and Y.-J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science 283:1183.*

49. Constant, S., C. Pfeiffer, A. Woodard, T. Pasqualini, and K. Bottomly. 1995. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4$^+$ T cells. *J. Exp. Med. 182:1591.*

50. Hosken, N. A., K. Shibuya, A. W. Heath, K. M. Murphy, and A. O'Garra. 1995. The effect of antigen dose on CD4$^+$ T helper cell phenotype development in a T cell receptor-αβ-transgenic model. *J. Exp. Med. 182:1579.*

51. Rizzo, L. V., R. H. DeKruyff, D. T. Umetsu, and R. R. Caspi. 1995. Regulation of the interaction between Th1 and Th2 T cell clones to provide help for antibody production in vivo. *Eur. J. Immunol. 25:708.*

52. Rogers, P. R., and M. Croft. 2000. CD28, Ox-40, LFA-1, and CD4 modulation of Th1/Th2 differentiation is directly dependent on the dose of antigen. *J. Immunol. 164:2955.*

53. Johnson, T. R., J. E. Fischer, and B. S. Graham. 2001. Construction and characterization of recombinant vaccinia viruses co-expressing a respiratory syncytial virus protein and a cytokine. *J. Gen. Virol. 82:2107.*

54. Noben-Trauth, N., W. E. Paul, and D. L. Sacks. 1999. IL-4- and IL-4 receptor-deficient BALB/c mice reveal differences in susceptibility to *Leishmania major* parasite substrains. *J. Immunol. 162:6132.*

55. Urban, J. F., Jr., N. Noben-Trauth, D. D. Donaldson, K. B. Madden, S. C. Morris, M. Collins, and F. D. Finkelman. 1998. IL-13, IL-4Rα, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis. Immunity 8:255.*

56. Schwarze, J., G. Cieslewicz, E. Hamelmann, A. Joetham, L. D. Shultz, M. C. Lamers, and E. W. Gelfand. 1999. IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. *J. Immunol. 162:2997.*

57. Domachowske, J. B., C. A. Bonville, A. J. Easton, and H. F. Rosenberg. 2002. Pulmonary eosinophilia in mice devoid of interleukin-5. *J. Leukocyte Biol. 71:966.*

58. Haeberle, H. A., W. A. Kuziel, I.-J. Dieterich, A. Casola, Z. Gatalica, and R. P. Garofalo. 2001. Inducible expression of inflammatory chemokines in respiratory syncytial virus-infected mice: role of MIP-1α in lung pathology. *J. Virol. 75:878.*

59. Domachowske, J. B., C. A. Bonville, J. L. Gao, P. M. Murphy, A. J. Easton, and H. F. Rosenberg. 2000. The chemokine macrophage-inflammatory protein-1α and its receptor CCR1 control pulmonary inflammation and antiviral host defense in paramyxovirus infection. *J. Immunol. 165:2677.*

60. Domachowske, J. B., C. A. Bonville, J. L. Gao, P. M. Murphy, A. J. Easton, and H. F. Rosenberg. 2000. MIP-1α is produced but it does not control pulmonary inflammation in response to respiratory syncytial virus infection in mice. *Cell. Immunol. 206:1.*

61. Polack, F. P., M. N. Teng, P. L. Collins, G. A. Prince, M. Exner, H. Regele, D. D. Lirman, R. Rabold, S. J. Hoffman, C. L. Karp, et al. 2002. A role for immune complexes in enhanced respiratory syncytial virus disease. *J. Exp. Med. 196:859.*

62. Fischer, J. E., J. E. Johnson, R. K. Kuli-Zade, T. R. Johnson, S. Aung, R. A. Parker, and B. S. Graham. 1997. Overexpression of interleukin-4 delays virus clearance in mice infected with respiratory syncytial virus. *J. Virol. 71:8672.*

63. Aung, S., Y.-W. Tang, and B. S. Graham. 1999. Interleukin-4 diminishes CD8$^+$ T respiratory syncytial virus-specific cytotoxic T-lymphocyte activity in vivo. *J. Virol. 73:8944.*

64. Moran, T. M., H. Isobe, A. Fernandez-Sesma, and J. L. Schulman. 1996. Interleukin-4 causes delayed virus clearance in influenza virus-infected mice. *J. Virol. 70:5230.*

65. Sharma, D. P., A. J. Ramsay, D. J. Maguire, M. S. Rolph, and I. A. Ramshaw. 1996. Interleukin-4 mediates down regulation of antiviral cytokine expression and cytotoxic T-lymphocyte responses and exacerbates vaccinia virus infection in vivo. *J. Virol. 70:7103.*

66. Aung, S., and B. S. Graham. 2000. IL-4 diminishes perforin-mediated and increases Fas ligand-mediated cytotoxicity in vivo. *J. Immunol. 164:3487.*

67. Rosenberg, H. F., and J. B. Domachowske. 1999. Eosinophils, ribonucleases and host defense: solving the puzzle. *Immunol. Res. 20:261.*

68. Rosenberg, H. F., and J. B. Domachowske. 2001. Eosinophils, eosinophil ribonucleases, and their role in host defense against respiratory virus pathogens. *J. Leukocyte Biol. 70:691.*

69. Klebanoff, S. J., and R. W. Coombs. 1996. Virucidal effect of stimulated eosinophils on human immunodeficiency virus type 1. *AIDS Res. Hum. Retroviruses 12:25.*

70. Fallon, P. G., E. J. Richardson, G. J. McKenzie, and A. N. J. McKenzie. 2000. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J. Immunol. 164:2585.*

71. Mohrs, M., B. Lederman, G. Köhler, A. Dorfmuller, A. Gessner, and F. Brombacher. 1999. Differences between IL-4- and IL-4 receptor α-deficient mice in chronic leishmaniasis reveal a protective role for IL-13 receptor signaling. *J. Immunol. 162:7302.8.*

72. Chiaramonte, M. G., D. D. Donaldson, A. W. Cheever, and T. A. Wynn. 1999. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest. 104:777.*

73. McKenzie, G. J., P. G. Fallon, C. L. Emson, R. K. Grencis, and A. N. J. McKenzie. 1999. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J. Exp. Med. 189:1565.*

74. Jankovic, D., M. C. Kullberg, N. Noben-Trauth, P. Caspar, J. M. Ward, A. W. Cheever, W. E. Paul, and A. Sher. 1999. Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J. Immunol. 163:337.*

75. Kumar, H., A. Belperron, S. W. Barthold, and L. K. Bockenstedt. 2000. CD1d deficiency impairs murine host defense against the spirochete, *Borrelia burgdorferi. J. Immunol. 165:4797.*