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Overexpression of Interleukin-4 Delays Virus Clearance in Mice Infected with Respiratory Syncytial Virus

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Although interleukin-4 (IL-4) expression has been implicated in vaccine-enhanced respiratory syncytial virus (RSV) disease, its role in mediating the immune response to primary RSV infection remains unclear. To assess the effect of IL-4 production on typical RSV infection, transgenic mice which either overexpress or fail to express IL-4 were challenged intranasally with RSV and their responses were compared to those of the parent strains. IL-4-deficient mice eliminated virus from the lung as quickly as did C57BL/6 controls. In contrast, mice which constitutively overexpress IL-4 showed delayed virus clearance compared with mice of the FVB/N control strain, although peak viral titers did not differ. IL-4 overexpression increased the magnitude of the subsequent antibody response. Lung lymphocytes harvested from IL-4-overexpressing mice post-RSV challenge showed diminished RSV-specific cytolytic activity compared with controls. Both IL-4-deficient and IL-4-overexpressing strains resisted rechallenge. These data imply that constitutive IL-4 expression delays or suppresses the development of a virus-specific cytotoxic lymphocyte population important in clearing primary RSV infection.

A growing body of evidence suggests that $CD4^+$ T-helper lymphocytes marshal pathological events in infectious diseases through regulated cytokine secretion. Murine T-helper (Th) cells can be classified as members of two distinct subsets based on their production of several key cytokines: type 1 Th lymphocytes characteristically secrete gamma interferon, interleukin-2 (IL-2), and tumor necrosis factor β, while type 2 lymphocytes are distinguished by the synthesis of IL-4, IL-5, IL-6, and IL-10 (20). Dominant type 1 cytokine production promotes cell-mediated immune responses and production of antibodies of the immunoglobulin G2a (IgG2a) isotype, while dominant type 2 cytokine synthesis promotes humoral responses characterized by secretion of IgG1, IgA, and IgE (19). Both patterns of cytokine secretion tend to be self-propagating and inhibit activation of Th cells of the opposite subset. In animal models of leishmaniasis, toxoplasmosis, schistosomiasis, and candidiasis, among others, a type 1-based immune response correlates with disease resolution, while dominance of the type 2 response results in disease progression (7, 12, 14, 22). Polarized type 2 cytokine responses have also been implicated in the progressive pathogenesis of human diseases such as AIDS, leprosy, and atopic disease (5, 21, 31).

Respiratory syncytial virus (RSV), a ubiquitous human pathogen, causes significant illness in infants and children during its annual epidemics. Symptoms of RSV infection range from mild coryza to severe lower respiratory tract disease. The basis for varying disease severity among infected individuals remains undefined, although severe RSV illness in children has been correlated with elevated RSV-specific IgE levels (30), a hallmark of type 2 cytokine dominance.

A formalin-inactivated, alum-precipitated RSV vaccine preparation tested in the 1960s not only failed to protect children against natural RSV infection but exacerbated both morbidity and mortality on subsequent RSV exposure (15, 16). Evidence from the BALB/c mouse model of RSV disease suggests that intramuscular immunization with inactivated viral antigens promotes increased expression of IL-4 by lung lymphocytes upon subsequent challenge with live RSV, while primary RSV challenge or rechallenge induces a cytokine profile dominated by gamma interferon production (8). Associated with these anomalously high IL-4 levels are exacerbated illness and an increase in the ratio of RSV-specific antibodies of the IgG1 isotype. Administration of an anti-IL-4 neutralizing antibody at the time of vaccination ameliorates the enhanced illness and augments $CD8^+$ cytotoxic T-lymphocyte (CTL) activation following live-RSV challenge (26).

Although these data clearly implicate aberrantly high levels of IL-4 expression in vaccine-enhanced RSV disease, the impact of elevated IL-4 levels on primary RSV infection remains undefined. Experiments with mice have demonstrated that passively transferred RSV-specific $CD4^+$ Th2 lymphocyte clones induce enhanced RSV illness upon challenge, while transferred Th1 clones ameliorate disease (1). Evidence from in vitro studies of $CD4^+$ T-cell development suggests that the presence of IL-4 at the time of initial antigen exposure may induce activation of a primarily Th2 population (23). Human conditions such as atopic disease or parasitic infection, which nonspecifically elevate IL-4 levels, might create a cytokine milieu predisposed toward a type 2 immune response to RSV, perhaps contributing to the variation in disease severity. A type 2 immune response might increase mast cell activation due to the generation of RSV-specific IgE, leading to increased release of histamine and other promoters of airway inflammation (6). In addition, elevation of IL-4 levels concurrent with viral infection appears to suppress or delay activation of virus-specific $CD8^+$ CTL populations (18, 24). The activation of virus-specific $CD8^+$ CTLs has been demonstrated to be important in eliminating RSV from the lungs of infected mice (2, 4).

To assess the impact of IL-4 expression on the immune

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response to RSV, transgenic mice which either fail to express IL-4 through targeted gene disruption or which constitutively express IL-4 from the immunoglobulin (Ig) promoter were challenged with RSV. Comparison of the immune responses of these transgenic mice with those of their control parent strains indicates that a nonspecific systemic elevation of IL-4 levels delays viral clearance and alters antibody isotype selection.

MATERIALS AND METHODS

Mice. FVB/N strain-based TC.UG mice which express IL-4 as a transgene fused to the Ig promoter-enhancer as described by Tepper et al. (27) were kindly provided by Phillip Leder (Harvard University, Boston, Mass.). These mice show constitutive IL-4 expression by B cells with minimal thymic and peripheral T-cell transgene expression, normal thymus and splenic T-cell profiles and concanavalin A responsiveness, and elevated IgE and IgG1 responsiveness to antigen stimulation (3, 27). IL-4-deficient mice on a C57BL/6 strain background (IL-4 −/−) generated by targeted disruption of the IL-4 gene as described by Kopf et al. (17) were generously provided by O. Kanagawa (Washington University, St. Louis, Mo.). These IL-4 −/− mice fail to mount type 2 cytokine responses to antigen challenge (17). Eight-week-old female pathogen-free mice of the control strains FVB/N and C57BL/6 were purchased from Harlan Laboratories (Indianapolis, Ind.). All mice were housed and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* as previously described (10).

Virus challenge. The RSV challenge stock was derived from HEp2 cells infected with A2 strain RSV as previously described (11). Mice were anesthetized intramuscularly with xylazine-ketamine prior to intranasal challenge with 10^7 PFU of RSV as previously described (10).

Plaque assays. Lung tissue was removed, weighed, and immediately quick-frozen in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (10% EMEM). Tissues were maintained at 4°C while individually ground. Clarified lung supernatants were diluted and inoculated onto subconfluent HEp2 cell monolayers in Costar 12-well plates (Costar, Cambridge, Mass.). After 1 h, plates were covered with 0.75% methylcellulose in 10% EMEM and incubated for 4 days at 37°C. Monolayers were then fixed with 10% buffered formalin and stained with hematoxylin-eosin. Plaques were counted with the aid of a dissecting microscope. Lungs from six IL-4 −/−, five C57BL/6, or seven IL-4 OE or FVB/N mice were assayed per time point.

RSV-specific Ig isotype enzyme-linked immunosorbent assay. Soluble RSV fusion (F) protein was bound to the solid phase on Immunolon II 96-well plates (Nunc, Roskilde, Denmark) at 20 ng/well. Plates were blocked with phosphate-buffered saline (PBS) containing 10% fetal bovine serum and washed with PBS–0.5% Tween. Twofold serial dilutions (100-μl) of mouse serum, collected 6 weeks after RSV challenge, were added to the wells, starting at a dilution of 1:640 in PBS, and incubated for 1 h. After washing, 100 μl of rabbit antimurine IgG1, IgG2a, or IgM conjugated to horseradish peroxidase (Zymed Laboratories, South San Francisco, Calif.) at a dilution of 1:5,000 was added to each well and incubated for 1 h. For IgE, sheep antimurine IgE (Serotec, Oxford, England) at a dilution of 1:1,000, biotinylated goat anti-sheep Ig at 1:1,000, and then streptavidin-horseradish peroxidase (Zymed Laboratories) at 1:5,000 were added to each well. Plates were washed and incubated with TMB substrate (Sigma Chemical Co., St. Louis, Mo.) for 10 min. Substrate development was stopped with 2.5 M H_2SO_4, and the optical density at 450 nm was determined. A serum dilution was considered positive if the optical density at 450 nm was greater than 0.2 after subtraction of the background. Data are represented as averages of $\log_2$ geometric means from six mice/strain ± the standard deviation (SD).

Pathology. Lungs were inflated via the left main bronchus and fixed in 10% buffered formalin phosphate. Sections stained with hematoxylin and eosin were examined in a blinded fashion. Inflammatory infiltrates were assessed morphologically for location, thickness, and cell composition. Inflammatory aggregates around airways and vessels were assessed at the point of minimal diameter of the structure. The entire lung section, including alveolar spaces, bronchovascular bundles at all levels, and interstitium, was reviewed, and inflammation was semiquantitatively evaluated as follows: for interstitial-alveolar cellularity, 0 = no infiltrate, 1 = mild, generalized increase in cellularity of the alveolar septa without thickening of the septa or significant airspace consolidation, 2 = dense septal mononuclear infiltrates with thickening of septa, 3 = significant alveolar consolidation in addition to interstitial inflammation, including alveolar hemorrhage and edema; for peribronchovascular infiltrates, 0 = no infiltrate, 1 = infiltrate up to four cells thick in most vessels, 2 = infiltrate five to seven cells thick in most vessels, 3 = infiltrate greater than seven cells thick in most vessels. Data are expressed as means ± standard errors (five IL-4 −/− and C57BL/6, seven IL-4 OE, and six FVB/N mice).

Direct cytotoxicity assay. Lungs of 10 IL-4 OE and 10 FVB/N mice were harvested 8 days after RSV challenge. Lymphocytes were isolated from the lungs by manual separation from the lung capsule, followed by density centrifugation on Ficoll-Hypaque as described previously (26). Target cells were generated by infecting *H-2^q^* MLE 12 mouse lung epithelial cells (American Type Culture Collection, Rockville, Md.) with A2 strain RSV at a multiplicity of infection of 1 for 16 h. Control (uninfected) target cells were prepared from the same MLE 12 cell passage. During the preparation of effector cells, the target cells were incubated with ^{51}Cr (100 μCi/10^7 cells) for 60 min at 37°C, washed three times in RPMI with 10% fetal bovine serum, and distributed in V-bottom 96-well plates (Costar Corp., Cambridge, Mass.) at 2 × 10^4 cells in 100 μl per well. Twofold dilutions of effector lymphocytes in RPMI–10% fetal bovine serum starting at 10^6 cells in 100 μl per well, were added to triplicate wells with RSV-infected or uninfected target cells, resulting in a top effector-to-target cell ratio of 50:1. Plates were centrifuged for 30 s at 150 × *g* prior to 4 h of incubation at 37°C in 5% CO_2. Cells were then gently pelleted, and 100 μl of supernatant per well was transferred for determination of released ^{51}Cr counts per minute (cpm) in a Cobra Autogamma gamma counter (Packard Instruments, Meriden, Conn.). Spontaneous and total ^{51}Cr release was determined by treating labeled target cells with RPMI only or 5% Triton X-100 detergent (Sigma Chemical Co.), respectively. The percentage of specific ^{51}Cr release from target cells is defined as 100 × (sample cpm − background cpm)/(total cpm − background cpm) (2). Each assay was performed twice with lymphocytes pooled from five mice per strain; data are represented as means ± SD.

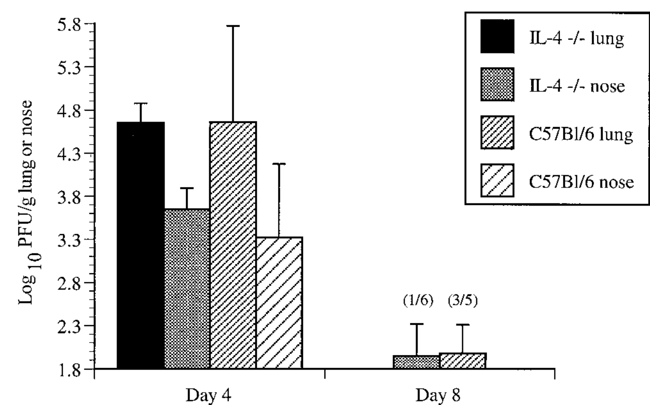

FIG. 1. Viral replication in lungs and noses of IL-4 −/− and C57BL/6 control mice 4 and 8 days post-RSV challenge. Viral titers on day 4 postinfection, the peak of viral replication in the mouse model, did not differ significantly between the strains. By day 8, IL-4 −/− mice show no detectable virus (<1.8 $\log_{10}$ PFU/g) in the lungs while three of five control C57BL/6 mice display reduced but still detectable viral titers. One of six IL-4 −/− mice had detectable virus in the nose at day 8 post-RSV challenge (six IL-4 −/− mice and five controls per time point).

RESULTS

Overexpression of IL-4 delays RSV clearance. To determine if IL-4 production at the time of challenge might be required for virus clearance, viral titers in the lungs and noses of IL-4 −/− mice were compared to those in the C57BL/6 parental control mice. Viral titers in either the lung or nose did not differ significantly between the two strains at day 4, previously established as the peak of RSV replication in the mouse (11). Six of six IL-4 −/− mice reduced viral titers to undetectable levels by day 8 post-RSV infection, while low RSV titers could still be detected in lungs from three of five C57BL/6 control mice at this time point (Fig. 1). RSV replication in the nose was undetectable in all IL-4 −/− mice but one at the day 8 time point, suggesting that elimination of virus at the mucosal epithelium of the upper and lower respiratory tracts does not depend on IL-4-induced responses.

FVB/N mice showed a pattern typical of the previously established BALB/c mouse model of RSV, maintaining about 6.2 $\log_{10}$ PFU/g of lung at the day 4 peak of RSV replication and reducing the virus to undetectable levels by day 8 postchallenge (Fig. 2). In contrast, mice which constitutively overexpress IL-4 (IL-4 OE) fail to clear RSV from the lungs in the same time period, with no significant reduction in viral titers seen through 11 days postchallenge. By day 21 postchallenge, no virus could be detected in the lungs, indicating that viral clearance was delayed rather than abrogated in the IL-4 OE strain. Both IL-4

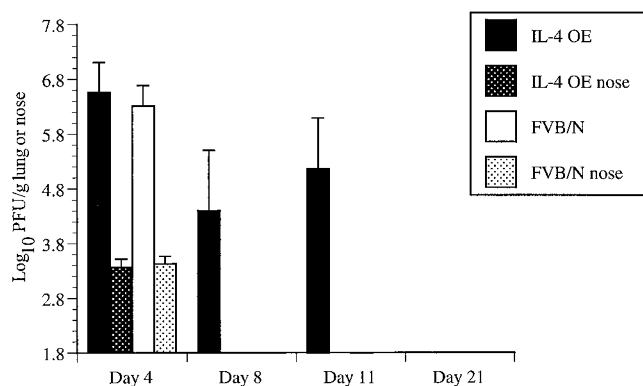

FIG. 2. Viral replication in lungs and noses of IL-4-overexpressing and FVB/N control mice. RSV titers in the lungs 4 days post-RSV challenge reached equivalent levels in both IL-4 OE and parental control strains. The FVB/N controls reduced viral titers to undetectable levels by day 8 postinfection, while the IL-4 OE mice failed to reduce viral titers below peak levels through day 11 postchallenge. Both transgenic and control mice cleared detectable RSV from the nose by day 8 and from the lungs by day 21 postchallenge (seven mice per time point).

OE and FVB/N controls eliminated all detectable virus from the nose by day 8 post-RSV challenge.

IL-4 overexpression alters lymphocyte migration into the lung in response to RSV challenge. Lungs from the transgenic, knockout, and control mouse strains were harvested 8 days post-RSV challenge, previously shown in the BALB/c mouse model to be the time point of maximal pathological lesions (11). Hematoxylin-eosin-stained lung sections show that alterations in the density and location of the lymphocytic infiltrate correlate with the presence of IL-4 (Fig. 3). C57BL/6 control mice (Fig. 3b) demonstrate a relatively dense lymphocytic infiltrate around arteries and airways, with histiocytes apparent in the interstitial spaces. In contrast, the IL-4 −/− mice display a much sparser lymphocytic infiltrate in all compartments, with no evidence of alveolar pneumonitis and minimal thickening of the alveolar septa apparent in the C57BL/6 controls (Fig. 3a). Semiquantitative assessment of the cellular infiltrate demonstrates little difference in cellular density in bronchovascular regions but a significant decrease in interstitial-alveolar cellularity in IL-4 −/− mice compared to controls (Table 1). Small lymphocytes constituted the major infiltrating cell population in both groups.

Lungs from the FVB/N parental control strain show a predominantly intra-alveolar infiltrate with periarterial and perivenous components (Fig. 3c). The IL-4 OE mice demonstrate increased lymphocyte density in the interstitial and intraalveolar regions (Fig. 3d), with an increase in the overall number of infiltrating lymphocytes and focal areas of intraalveolar edema not seen in controls. This increased interstitial-alveolar cellularity in IL-4 OE mice persists through day 11 postchallenge (Table 1; photo not shown). Despite alterations in the location and density of inflammatory cells, the cellular infiltrate in both IL-4 OE and FVB/N mice also consisted nearly exclusively of small lymphocytes.

Overexpression of IL-4 diminishes RSV-specific cytotoxicity. To assess the effects of IL-4 overexpression on CTL activity, lymphocytes were harvested from the lungs of IL-4 OE and FVB/N mice 8 days after RSV challenge and assayed directly for cytolytic activity in a standard ^{51}Cr release assay (Fig. 4). Lymphocytes from the FVB/N parental control strain specifically lyse approximately 20% of RSV-infected target cells at a 50:1 effector-to-target cell ratio without in vitro stimulation, demonstrating no detectable lysis of uninfected cells. In contrast, lymphocytes from IL-4 OE mice demonstrated no cytotoxicity against infected or uninfected target cells.

IL-4 overexpression increases the magnitude of the antibody response. Serum from mice of all four strains was collected 6 weeks after RSV challenge and assayed for the presence of RSV-specific antibodies (Fig. 5). IL-4 −/− mice demonstrate generally reduced antibody production compared to the control C57BL/6 mice. IL-4 −/− mice did produce some antibody, mostly of the IgG2a isotype, presumably induced by the presence of IFN-γ at the time of challenge.

Antibody responses in the FVB/N parental control strain did not appear robust, with peak titers similar to those of IL-4 −/− mice. The IL-4 OE mice, however, mounted an antibody response much greater in magnitude than that of the parental controls. IL-4 OE mice produced approximately equivalent amounts of IgG1 and IgG2a, reflecting the presence of both IL-4 and IFN-γ at the time of challenge. Three of six IL-4 OE mice but no FVB/N controls produced detectable RSV-specific IgE titers, indicating that the overexpression of IL-4 exerted some effect on anti-RSV antibody isotype selection (Fig. 5).

IL-4 overexpression or deficiency does not increase susceptibility to rechallenge. Mice of all four strains proved resistant to reinfection with the same dose and stock of RSV 6 weeks after the initial challenge. RSV titers were undetectable in both lungs and noses after rechallenge, and lymphocytic infiltrates in the lungs followed the same patterns as the initial response (data not shown). Weight loss did not differ significantly between IL-4 −/− or IL-4 OE mice and the respective parental control strain after primary challenge or rechallenge (data not shown).

DISCUSSION

In these studies, we investigated the effects of overexpression of IL-4 or deficiency in IL-4 production on the immune response to primary RSV challenge. Constitutive overexpression of IL-4 prior to RSV infection delays viral clearance, increases the density of the lymphocytic infiltrate in the lungs, and diminishes induction of primary CTL responses, whereas mice which express no IL-4 clear RSV readily after primary infection with minimal pathology. In addition, IL-4 overexpression leads to the synthesis of RSV-specific IgE. This altered response to primary RSV challenge does not impair the ability to eliminate virus upon rechallenge in the mouse.

Studies with other infection systems, including influenza virus challenge with coadministered exogenous IL-4 and recombinant IL-4-expressing vaccinia virus, have demonstrated delayed viral clearance in the presence of IL-4 (18, 24). The studies with transgenic mice described here suggest that constitutive expression of IL-4 also promotes an immune response that clears the virus inefficiently, extending the prior observations. One explanation for this failure to clear the virus lies in the suppression of an active CTL population. Evidence obtained with the influenza virus and recombinant vaccinia virus models demonstrated reduced CTL activation following both primary and secondary stimulation in the setting of transient IL-4 exposure (18, 24). Similarly, the studies described here show that lung lymphocytes from IL-4-overexpressing mice harvested 8 days after RSV challenge demonstrated no detectable RSV-specific cytotoxicity. In comparison, lung lymphocytes from the FVB/N parental control strain specifically lysed RSV-infected target cells without in vitro stimulation. Since RSV-specific CD8$^+$ CTLs have demonstrated importance in viral clearance (2, 4), suppression of CD8$^+$ CTL activation could contribute to the prolonged viral replication seen in the

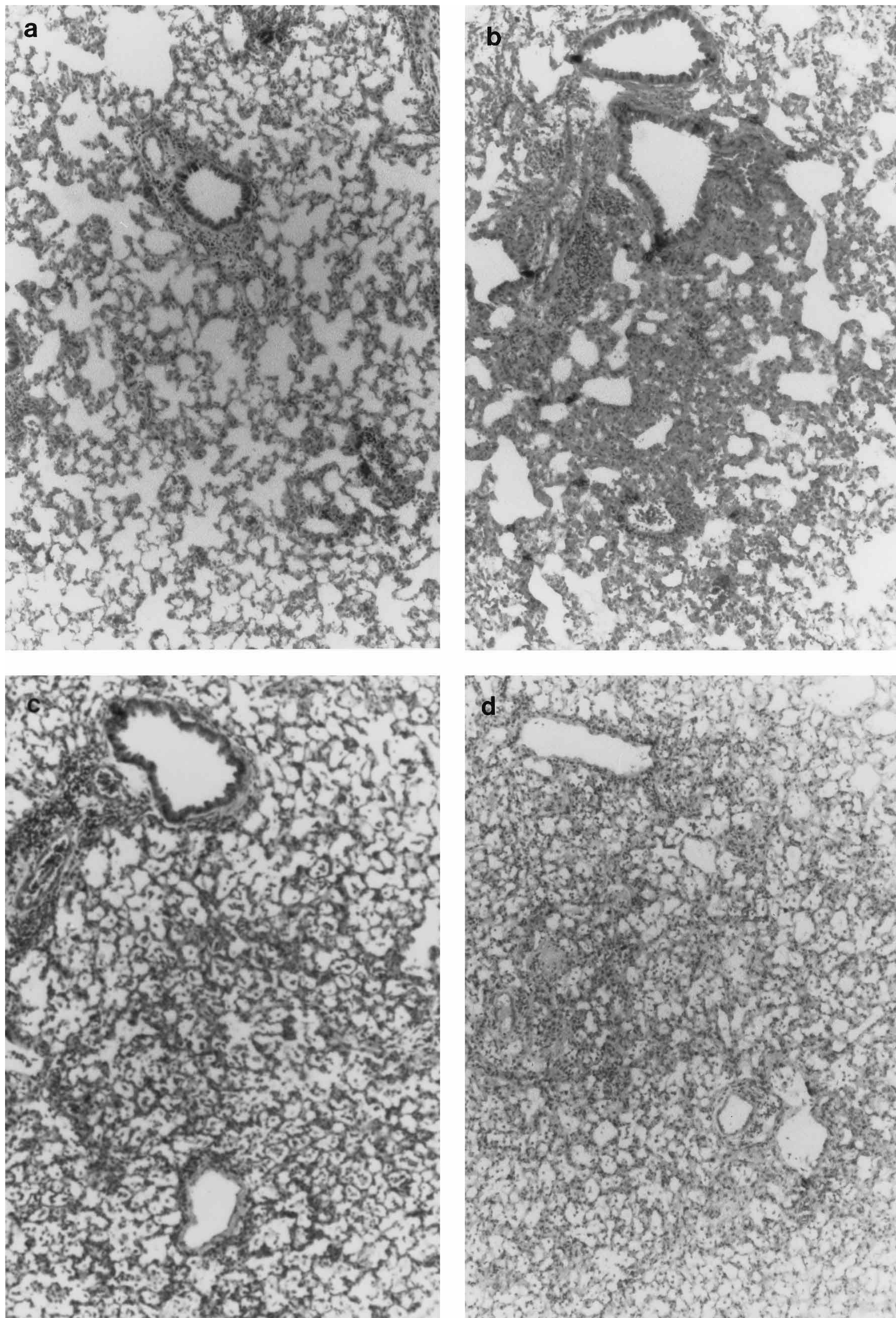

FIG. 3. Histopathological examination of lung sections 8 days post-RSV challenge. Hematoxylin-eosin staining of lung sections from IL-4 −/− mice (a) reveals no evidence of pneumonitis and a diminished lymphocytic infiltrate compared to the C57BL/6 controls (b). In addition to the periarterial and perivenous cellular infiltration in the C57BL/6 controls, histiocytes can be seen in the interstitial spaces, accompanied by thickening of alveolar septa. Lungs from mice of the FVB/N strain (c) show a predominantly intra-alveolar infiltrate, with a less marked perivenous and periarterial component. IL-4-overexpressing mice display a dense interstitial and intra-alveolar response with a notable increase in the overall number of infiltrating lymphocytes (d).

TABLE 1. Lung inflammation scores after RSV challenge[a]

Strain	Interstitial inflammation		Periarterial inflammation		Perivenous inflammation	
	Day 8	Day 11	Day 8	Day 11	Day 8	Day 11
IL-4 −/−	0.7 ± 0.2	ND	0.5 ± 0.0	ND	1.2 ± 0.1	ND
C57BL/6	1.6 ± 0.4	ND	0.7 ± 0.1	ND	1.9 ± 0.2	ND
IL-4 OE	1.2 ± 0.2	0.7 ± 0.1	1.0 ± 0.0	0.7 ± 0.2	1.0 ± 0.0	1.1 ± 0.1
FVB/N	1.0 ± 0.0	0.4 ± 0.1	1.0 ± 0.0	0.4 ± 0.1	1.2 ± 0.2	0.8 ± 0.2

[a] Values (units are defined in Materials and Methods) represent means ± standard errors; ND, not determined.

lungs of IL-4 OE mice. IL-4 influences the differentiation of $CD4^+$ T-cell subpopulations, which may be reflected in altered lymphocyte migration into the lungs. A graded increase in the density of interstitial and intra-alveolar lymphocytic infiltrates correlates with increasing amounts of IL-4 in these studies. IL-4 −/− mice demonstrate sparse lymphocytic infiltration, while IL-4 OE mice show the greatest cellular density, with the lymphocytes distributed largely in interstitial spaces rather than around blood vessels and airways. This increased infiltrate in IL-4 OE mice may stem from prolonged exposure to RSV antigens due to delayed viral clearance, which might promote continued recruitment of lymphocytes. Additionally, IL-4 has been shown to alter expression of cell surface adhesion molecules such as VCAM-1 on endothelium, acting synergistically with other cytokines to selectively enhance T-cell infiltration of sites of inflammation (28). Such alterations could lead to increased recruitment of effector cells into the interstitial spaces or to selective homing of effector cell subsets which cause immunopathology out of proportion to viral clearance. Alternatively, recruitment of the optimal effector cell population after RSV challenge may depend on type 1 cytokine-mediated effects on lymphocyte migration. Since IL-4 and other type 2 cytokines suppress activation of Th1 lymphocytes, failure to secrete chemokines or cytokines necessary to attract $CD8^+$ CTLs or other effector populations to the site of viral infection could also result in inflammation in the absence of effective viral clearance.

The disparity between impaired viral clearance in the lungs and efficient clearance in the noses of IL-4 OE mice may also reflect altered trafficking patterns, in which effector cells may be preferentially routed to the mucosal epithelium in the presence of constitutively expressed IL-4. However, the ability of IL-4 −/− mice to eliminate the virus from the nasal mucosa suggests that the composition of the immune response needed for efficient viral clearance from the lungs and nose may be differentially regulated. IL-6, which is associated with production of secretory IgA and should not be impaired by IL-4 overexpression, may induce a local immune response sufficient to eliminate virus replication in the nasal epithelium. Antibody production may also be responsible for the eventual clearance of the virus seen by day 21 in IL-4 OE mice; passive transfer of RSV-specific immune serum has been shown to effect viral clearance in mice (9), and the vigorous RSV-specific antibody response which develops in the weeks after challenge may be sufficient to finally eliminate RSV replication.

Investigators have used in vitro models of T-cell development to explore the effects of cytokines on antigen-specific immune development, providing illuminating but often contradictory evidence. Although evidence exists that the genetic background determines the "default" phenotype of activated Th-cell precursors (13), equally compelling studies suggest that the composition of the cytokine milieu and the presence of IL-4 at the time of activation determine whether a Th cell commits to a Th2 phenotype (23). In human conditions such as atopic disease or parasitic infection, constitutively elevated levels of IL-4 may create an environment in which any primary

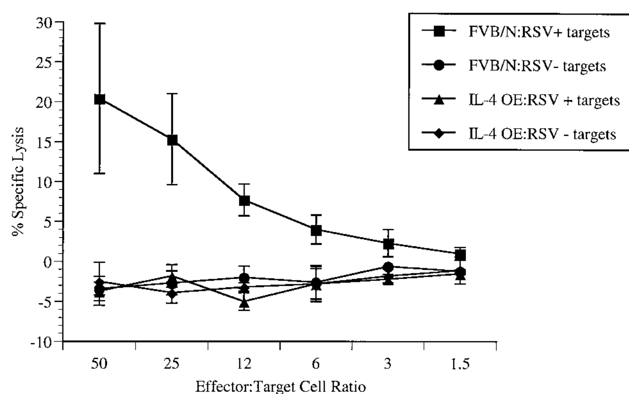

FIG. 4. Lymphocytes isolated from the lungs of FVB/N but not IL-4 OE mice lyse RSV-infected target cells in a direct cytotoxicity assay. Lymphocytes were harvested from the lungs of FVB/N and IL-4 OE mice 8 days post-RSV challenge and assayed directly for cytolytic activity against RSV-infected target cells without in vitro stimulation. Lymphocytes from FVB/N control mice demonstrate specific lysis of RSV-infected target cells, with about 20% cytotoxicity at a 50:1 effector-to-target cell ratio. Lymphocytes from the lungs of IL-4 OE mice show no detectable RSV-specific cytotoxicity at this time point. The data shown represent the range of two distinct assays utilizing effector cells pooled from five mice per strain ± SD.

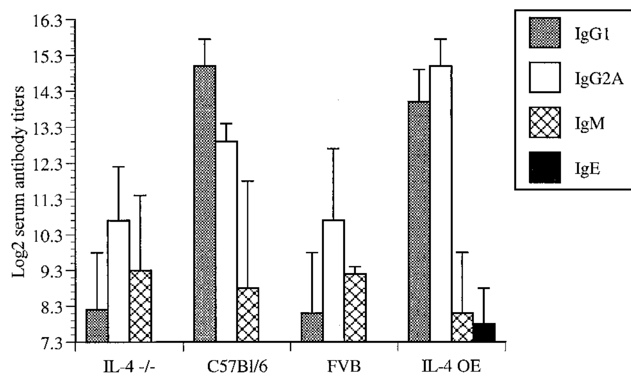

FIG. 5. RSV-specific serum antibody isotype titers 6 weeks post-RSV challenge. IL-4 −/− mice display lower overall titers of antibody to the RSV fusion protein than does the C57BL/6 control strain, and the dominance of the IgG2a isotype suggests a predominantly type 1 cytokine response at the time of challenge. C57BL/6 control mice produce significantly more IgG1 antibody in response to RSV than do IL-4 −/− mice ($P < 0.01$). IL-4 OE mice display generally elevated antibody titers compared to the control FVB/N strain, with significant increases in IgG2a ($P < 0.01$) and IgG1. Although IL-4 OE mice produced approximately equivalent levels of IgG1 and IgG2a, three of these transgenic mice displayed detectable RSV-specific IgE (six mice per group; significance determined by Student's t test).

antigenic stimulation occurs in the context of high IL-4 levels. Thus, these conditions could shift the immune response to primary RSV infection to a type 2 effector profile. The transfer studies of Alwan et al. have demonstrated that RSV-specific Th2 clones clear the virus ineffectively and increase RSV disease after challenge (1), and studies in which inactivated-RSV vaccine was given to mice show that a small increase in the number of IL-4-secreting lung lymphocytes after RSV challenge correlates with enhanced illness (25).

These studies may provide a model for understanding how inherently skewed immune environments among individuals might contribute to the range of disease severity seen in response to RSV infection. Increases in RSV-specific IgE in infants, correlated with disease severity and a tendency to wheeze for years subsequent to RSV infection (29), may reflect a type 2 cytokine response to RSV induced by atopic disease or other pre-existing underlying conditions. In light of the evidence from in vitro studies which suggests that the cytokine environment may influence not only the primary but the secondary effector response to antigenic stimulation, excessive production of IL-4 during primary RSV infection may increase susceptibility to reinfection and concomitant illness throughout life. The ability of the IL-4-deficient mouse and the failure of the IL-4-overexpressing mouse to clear the virus suggest that vaccines or therapies that minimize induction of a type 2 cytokine environment might improve viral clearance and reduce immunopathogenesis associated with disease. Further studies with this model will allow insight into the effects of IL-4 on lymphocyte trafficking, local production of chemical mediators of inflammation, and airway responsiveness and whether CTL activation is suppressed directly by IL-4 or indirectly by reduction of type 1 cytokine synthesis.

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