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Prolonged Production of TNF-α Exacerbates Illness during Respiratory Syncytial Virus Infection

John A. Rutigliano*† and Barney S. Graham2*

CD8+ CTL are the main effector cells responsible for resolving viral infections. However, the CTL response to respiratory syncytial virus (RSV) infection in mice facilitates viral clearance at the expense of significant immunopathology. Previous reports have shown a strong correlation between the mechanism of CTL activity and the severity of RSV-induced illness. Furthermore, experiments in perforin knockout mice revealed that antiviral cytokine production temporally correlated with RSV-induced illness. In the current study, we show that TNF-α is the dominant mediator of RSV-associated illness, and it is also important for clearance of virus-infected cells during the early stages of infection. We also demonstrate that IFN-γ plays a protective role in conjunction with perforin/granzyme-mediated killing. Preliminary experiments in gld mice that express nonfunctional Fas ligand (FasL) revealed that RSV-induced illness is significantly reduced in the absence of FasL-mediated killing. Antiviral cytokine production was not elevated in the absence of FasL, suggesting a possible link between FasL and antiviral cytokine activity. This work shows that multiple phenotypic subsets of CD8+ CTLs respond to RSV infection, each with varying capacities for clearance of virus-infected cells and the induction of illness. In addition, the revelation that TNF-α is the principal mediator of RSV-induced illness means that administration of TNF receptor antagonists, in combination with antiviral therapy, may be an effective method to treat RSV infections. The Journal of Immunology, 2004, 173: 3408–3417.

The CD8+ CTL assumes significant responsibility for clearing most viral infections (1–3). Virus-specific CTLs use two separate mechanisms to engage in direct cell lysis. The first of these mechanisms is mediated by perforin and granzymes, which act in concert to induce apoptosis in the target cell (4–8). Granzyme B has been shown to be the most important granzyme in cytolysis (9). However, other granzymes can compensate for the absence of granzyme B, but with reduced efficiency (10, 11). Granzyme B can induce apoptosis either through disruption of the mitochondria (12–15) or by caspase activation, although there is debate over whether this process is direct or indirect (16, 17). A recent study has shown that granzyme B and perforin are complexed with the proteoglycan serglycin in cytotoxic granules, and that serglycin assists in the delivery of granzyme B to the cytoplasm (18). Perforin has been shown to be indispensable to the cytolytic process in vivo (19), but its exact role remains elusive (4–8).

The second mechanism for target cell lysis is mediated by Fas ligand (FasL),2 which is expressed by activated T cells (20) and binds to Fas on target cells. Like other members of the TNF superfamily, FasL-mediated apoptosis is initiated through the cytoplasmic protein, Fas-associated death domain protein (21). Reverse signaling is another feature common to members of the TNF receptor superfamily (22–24). Reverse signaling by FasL has been shown to serve a costimulatory function for CD8+ T cell proliferation (25–27). Because FasL has an extended t1/2 of several hours on the surface, potential hazards exist (28). Even after stimulation through the TCR that has stopped, the continued expression of FasL leads to a situation of potential nonspecific bystander lysis of uninfected cells (29–31).

Respiratory syncytial virus (RSV) is a pneumovirus that is responsible for the majority of respiratory illness and death seen in young children (32). Although RSV infections typically result in nothing more than a mild upper respiratory infection, ~5% of infected children fall prey to more severe lower respiratory tract infections and bronchiolitis. Severe lower respiratory disease results in as many as 130,000 pediatric hospitalizations annually in the U.S. (33). Similarly, RSV has been linked to high mortality in the institutionalized elderly (34) and the immunocompromised, particularly bone marrow transplant recipients (35). These strains on public health make the development of an effective RSV vaccine a high priority (36). Unfortunately, the realization of this goal has been thwarted by the legacy of failed vaccine trials in the 1960s with a formalin-inactivated, alum-precipitated RSV vaccine (FI-RSV). The FI-RSV vaccine caused more severe illness, increased rates of hospitalization, and some mortality (37–40).

In the mouse model of RSV infection, a large volume of work has established that CTLs contribute significantly to the severe illness that accompanies the infection (41, 42). Investigations aimed at determining the role of the CTL response during the failed FI-RSV vaccinations revealed that FI-RSV immunizations led to an IL-4-dominant immune response after live RSV challenge (43). Further efforts determined that neutralization of IL-4 during FI-RSV immunization shifted the response toward Th1 dominance, marked by increased IFN-γ production, increased cytolytic activity, and reduced illness after challenge (44). RSV infection in IL-4-overexpressing mice produced similar results (45).
More recent investigations have revealed that IL-4 diminishes perforin-mediated killing and increases FasL-mediated cytotoxicity in vivo during RSV infection (46). Subsequent studies in perforin knockout mice (PKO) showed that RSV clearance was delayed at the expense of prolonged illness, and antiviral cytokine production was temporally correlated with illness (47). The current study reveals that TNF-α contributes to clearance of virus-infected cells during the early stages of infection, but continued production of TNF-α exacerbates illness during the late stages of infection. In addition, killing mediated by perforin and granzymes, in conjunction with IFN-γ secretion, protects mice from RSV infection. These data clearly indicate that there are multiple phenotypic subsets of CD8⁺ CTLs that respond to RSV infection. These phenotypic subsets possess varying capacities to clear virus-infected cells and induce severe immunopathology. From these observations, it now becomes clear that administration of TNF receptor antagonists, in combination with antiviral therapy, may be an effective treatment to alleviate RSV illness.

Materials and Methods

Mice

Pathogen-free BALB/c mice, 8–10 wk of age, were purchased from Charles River Laboratories (Wilmington, MA). Breeding pairs of C57BL/6J-Tnfsf6gld (gld) mice and IFN-γ knockout (GKO) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The gld mice possess a spontaneous mutation in the gene encoding FasL, rendering it nonfunctional. Perforin/IFN-γ double-knockout mice (PGKO) were a gift from S. Stohlman (University of Southern California, Los Angeles, CA) (48). BALB/c PKO mice were a gift from J. Harty (University of Iowa, Iowa City, IA). All transgenic and knockout mice were H-2d, allowing for matched groups. Clinical illness was graded daily by a blinded observer. Clinical illness was determined to be free of endotoxin before use in mice (Harlan Bioproducts for Science, Indianapolis, IN).

Virus infection

The RSV challenge stock was derived from the A2 strain of RSV by sonication of HEp-2 monolayers, as previously described (49). Mice were anesthetized i.m. with ketamine (40 μg/g body weight) and xylazine (6 μg/g body weight) before intranasal inoculation with 10⁷ PFU of live RSV in 100 μl of 10% EMEM. Mice were weighed daily after infection, and percentage of weight lost was used to determine the severity of illness. Clinical illness was graded daily by a blinded observer. Clinical illness was scored, as follows: 0, no apparent illness; 1, slightly ruffled fur; 2, ruffled, but active; 3, ruffled, but inactive; 4, ruffled, inactive, hunched posture, and gaunt; 5, dead.

Plaque assays

Mice were sacrificed, and lung tissue was removed and quick frozen in liquid nitrogen. Thawed tissues were kept chilled while individually ground. Dilutions of clarified supernatant were inoculated on 80% confluent HEp-2 cell monolayers in triplicate and overlaid with 0.75% methyl cellulose in 10% EMEM. After incubation for 4 days at 37°C, the monolayers were fixed with 10% buffered formalin and stained with H&E. Plaques were counted and expressed as log₁₀ PFU/g tissue. The limit of detection is 1.8 log₁₀ PFU/g tissue in this assay.
CD49d. One hour into the incubation, 1/2 H9262 commercially available ELISA kit (R&D Systems, Minneapolis, MN). Total antiviral cytokine production in the lungs was measured using a Cytokine ELISAs protocol. Lymphocytes were isolated by centrifugation and lungs were harvested at days 4, 6, 8, and 10 postinfection. Lymphocytes were isolated manually by grinding lung tissue and were developed by addition of tetramethylbenzidine substrate. Briefly, 50 μl of supernatant from ground lungs of RSV-infected mice was thawed and added to precoated 96-well microtiter plates. Peroxidase-labeled anti-cytokine Ab was added to detect bound cytokine, and the plates were developed by addition of tetramethylbenzidine substrate.

Synthetic peptides
RSV 82–90 (SYIGSINNI) is derived from the M2 protein of the RSV A2 strain, and influenza virus nucleoprotein 147–155 (TYQRTRALV) is derived from the influenza virus A/Puerto Rico/8/34 nucleoprotein. Peptides were synthesized by Anaspec (San Jose, CA), and confirmed to be >95% pure by the National Institute of Allergy and Infectious Diseases peptide core facility (Bethesda, MD). Both peptides are H-2Kd restricted.

Intracellular cytokine staining (ICS)
Mice were sacrificed and lungs were harvested at days 4, 6, 8, and 10 postinfection. Lymphocytes were isolated manually by grinding lung tissue between the frosted ends of two sterile glass microscope slides in RPMI 1640 containing 10% FBS. Lymphocytes were isolated by centrifugation postinfection. Lymphocytes were isolated manually by grinding lung tissue between the frosted ends of two sterile glass microscope slides in RPMI 1640 containing 10% FBS. Lymphocytes were isolated by centrifugation postinfection. Lymphocytes were isolated manually by grinding lung tissue between the frosted ends of two sterile glass microscope slides in RPMI 1640 containing 10% FBS.

Cytokine ELISAs
Total antiviral cytokine production in the lungs was measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

Results
Clearance of RSV-infected cells occurs with delayed kinetics in gld mice
The supernatants from ground lungs were used to examine viral clearance on days 4, 6, 8, and 10 postinfection (Fig. 1). Wild-type (WT) mice demonstrated a typical pattern of clearance, with the virus titer falling below the level of detection on day 8. However, gld mice harbored significantly higher levels of virus at day 6 postinfection, and low levels of virus were still detected on day 8. These results demonstrate that although RSV is cleared in WT mice, FasL makes a significant contribution to viral clearance.

Statistical analysis
Data from individual mouse experiments were maintained in a Paradox database. Statistical analysis was performed by transferring data from the database into SAS Institute statistical software (Chapel Hill, NC) to perform ANOVA using Kruskal-Wallis and Wilcoxon rank sum tests. Comparisons were made between individual experiments by statistical modeling and trend analysis calculated by the General Linear Model method in the SAS program. Values of p <0.05 as determined by ANOVA were considered statistically significant.

FIGURE 3. CD8+ T cell activity is not elevated in the lungs of RSV-infected gld mice. RSV-infected mice were sacrificed at various days postinfection, and IFN-γ production by CD8+ T cells was determined by ICS. The graph shows the absolute number of CD8+CD3+ lymphocytes that stained positive for IFN-γ. The data are a single representative mouse from each group that was sacrificed on day 3 postinfection. The data are a single representative of four experiments, with averages and SDs calculated from results with four mice per group.

FIGURE 4. NK cell activity is not altered in the lungs of RSV-infected gld mice. RSV-infected mice were sacrificed at days 3–7 postinfection, and IFN-γ production by NK cells was determined by ICS. The graph shows the absolute number of DX5+ NK cells that stained positive for IFN-γ. The data are a single representative of four experiments, with averages and SDs calculated from results for four mice per group. Value of p >0.05 at all days.

FIGURE 1. Clearance of RSV-infected cells occurs with delayed kinetics in gld mice. The graphs show the absolute number of CD8+ T cells and NK cells that stained positive for IFN-γ in lung tissues of wild-type (WT) and gld mice. RSV-infected mice were sacrificed at various days postinfection, and IFN-γ production by NK cells was determined by ICS. The data are a single representative of four experiments, with averages and SDs calculated from results for four mice per group. Value of p >0.05 at all days.
Clinical illness is significantly diminished in gld mice

Mice were weighed and scored for illness daily after infection for 12 days. WT mice experienced peak weight loss in excess of 20% on days 7–8 postinfection (Fig. 2A). In contrast, peak weight loss in gld mice was less than 7% at day 7 postinfection. In addition to reduced weight loss, gld mice exhibited no visible signs of illness at any time during the course of infection, including ruffled fur, inactivity, or gaunt posture (Fig. 2B). Combined with the minimal weight loss seen in gld mice, it is apparent that illness is significantly abrogated in RSV-infected gld mice. These data clearly show that perforin-mediated cytotoxicity contributes very little to RSV-associated illness, but it is sufficient to efficiently eliminate the virus without the assistance of FasL-mediated killing.

Cytolytic lymphocytes from RSV-infected gld mice are not functionally compromised

We next asked whether cytolytic lymphocytes from gld mice were functionally impaired in their ability to respond to RSV. To this end, we performed ICS for IFN-γ in CD8+ CTLs isolated directly from the lungs of gld mice. As shown in Fig. 3, gld mice were not inhibited in their ability to produce IFN-γ in response to stimulation by the well-characterized H-2Kd peptide from the M2 protein of RSV (50). The pattern of IFN-γ production and the peak response were similar in WT and gld mice. IFN-γ levels were slightly reduced in gld mice, but these differences were not biologically significant. A negative control using a peptide from the flu nucleoprotein led to undetectable IFN-γ production. The PMA/ionomycin-stimulated positive controls induced IFN-γ production in nearly 50% of CD8+ T cells (data not shown). These data confirm that the effects seen in gld mice are not the result of an inability of gld CTLs to respond to RSV.

CTLs are not the only lymphocytes capable of responding to infections. NK cells also respond to infections during the earliest stages of the immune response, and they use the same cytolytic machinery as CTLs. The NK cell response to RSV infection peaks at ~day 4 postinfection (51). To address the possibility that a deficiency in NK cell function was responsible for the aberrant viral clearance and/or the diminished illness in gld mice, we used ICS to measure IFN-γ production by NK cells isolated directly from the lungs of gld mice at days 3–7 postinfection. We saw no major differences in NK cell activity by this parameter (Fig. 4), although the pattern of reduced IFN-γ production was also seen in NK cells from gld lungs. However, these differences were not significant. The flu nucleoprotein served as a negative control and again produced undetectable IFN-γ production, while the PMA/ionomycin-stimulated positive controls induced IFN-γ production in >25% of NK cells (data not shown). We then looked at absolute numbers of CD8+ T cells and NK cells isolated directly from the lungs of gld mice, because a disparity in cell numbers might differentially affect immune function. As shown in Fig. 5, both CD8+ T cell and NK cell numbers were similar in WT and gld mice at all time points. The slight differences in cell numbers were not significant when multiple comparisons are taken into account. From these results, we conclude that the differences in disease outcome observed in RSV-infected gld mice are not caused by otherwise dysfunctional cytotoxic lymphocytes, and that the differences noted were due to the absence of functional FasL expression.

Total antiviral cytokine production is unaltered in the lungs of RSV-infected gld mice

CTLs counter viral infections not only by direct cell lysis, but also by the elaboration of antiviral cytokines such as IFN-γ and TNF-α. In our previous report, we observed that production of IFN-γ and...
TNF-α was temporally correlated with the illness profiles of both the WT and PKO mice (47). We therefore measured total IFN-γ and TNF-α production in gld mice by ELISA on multiple days postinfection in RSV-infected gld mice. IFN-γ production was similar in WT and gld mice at each time point (Fig. 6A). The same pattern was seen when we measured TNF-α (Fig. 6B). We also measured MIP-1α and MIP-1β (Fig. 6, C and D). Interestingly, production of IFN-γ, MIP-1α, and MIP-1β was significantly increased in gld mice on day 7 postinfection. No significant differences were seen at any other days. These data indicate that antiviral cytokine production is not augmented in the absence of FasL-mediated cytolysis during RSV infection.

RSV-induced illness is abrogated in the absence of TNF-α

We have previously shown that antiviral cytokine production was elevated and prolonged in RSV-infected PKO mice (47). However, this exaggerated pattern of antiviral cytokine production was not seen in gld mice. Several labs have demonstrated a protective role for IFN-γ during RSV infection (52-55), and when we infected GKO mice with RSV, we observed similar results. GKO mice exhibited slightly more weight loss than wild-type mice (Fig. 7A), suggesting the possibility that production of IFN-γ does, in fact, play a protective role in response to primary RSV infection during both the inductive phase of the immune response, managed by NK cells, as well as during the effector phase, which is controlled by CD8+ CTLs. However, the differences were small, and the clearance of virus-infected cells in GKO mice was not statistically significant (Fig. 7B).

In a previous report, we showed that antiviral cytokine production temporally correlated with RSV-induced illness (47). Because our results from the current study further confirmed that IFN-γ plays a protective role in response to RSV infection, we hypothesized that TNF-α was the likely culprit that was mediating the illness that accompanies severe RSV infection. We therefore administered anti-TNF-α Ab to WT, GKO, gld, and PGKO mice to elucidate the roles of both cytokines during primary RSV infection. As expected, all mice exhibited significant reductions in illness when TNF-α was depleted, especially during the time of peak illness (Fig. 8). Isotype control-treated PGKO mice displayed the most severe illness pattern (Fig. 8D). The illness in PGKO mice was delayed, which was consistent with our previous observations in PKO mice (47). These data not only reaffirm the previous reports that have demonstrated a protective role for perforin and IFN-γ, but they clearly demonstrate that TNF-α exacerbates illness during RSV infection.

In addition to illness, we also surveyed the impact of anti-TNF-α treatment on clearance of virus-infected cells. In this experiment, we again infected WT, GKO, gld, and PGKO mice. Interestingly, we saw delayed clearance in all strains that received anti-TNF-α treatment (Fig. 9). By day 8 postinfection, virus titer

**FIGURE 6.** Total antiviral cytokine production is not elevated or prolonged in the lungs of RSV-infected gld mice. ELISAs were performed using lung supernatants to determine the concentration of total IFN-γ (A), TNF-α (B), MIP-1α (C), and MIP-1β (D) in the lungs of RSV-infected gld mice. The data are a single representative of four experiments. Averages and SDs were calculated from results with four mice per group. Value of p < 0.05 at day 7 in A, C, and D.
leads to a significant reduction in illness and a slight delay in clearance of virus-infected cells.

In a previous study, we examined the CTL response to primary infection in the absence of perforin. The results from that study revealed delayed clearance of virus-infected cells and reduced cytolytic activity (47), which was consistent with previous work in FasL-dominant killing environments (43, 45, 56). In addition, we observed delayed and prolonged illness in the PKO mice. This prolonged illness temporally correlated with elevated and exaggerated production of the antiviral cytokines IFN-γ and TNF-α. These data led us to hypothesize that antiviral cytokine production was contributing to the exacerbated illness seen in the setting of a high IL-4/FasL-dominant immune response to RSV infection. In the current study, we saw a significant reduction in illness in gld mice (Fig. 2). Moreover, total production of IFN-γ and TNF-α in the lungs, as well as MIP-1α and MIP-1β, was neither elevated nor prolonged (Fig. 6), but was still temporally correlated with RSV-induced illness.

Several groups have established that IFN-γ plays a protective role during RSV infection (52–55). These reports are consistent with early findings from our lab in which an IFN-γ response to live virus challenge was associated with decreased illness and improved cytolytic activity (43), as well as data gathered from experiments in GKO mice that are presented in this work (Fig. 7). We have previously described severe illness in WT and PKO mice despite augmented IFN-γ production, leaving TNF-α as the most likely cause of severe RSV-induced immunopathology. In the current study, we show that anti-TNF-α administration results in significantly attenuated illness (Fig. 8), while also causing a slight delay in clearance of virus-infected cells (Fig. 9).

Previous work has shown a correlation between TNF-α and immunopathology associated with RSV infection (57, 58). In one of these studies, our lab reported that inhibition of TNF-α before infection led to increased cytopathology of HEp-2 cells, whereas beginning treatment after infection had a minimal impact on cytopathology of HEp-2 cells (57). Additional studies in vivo led to the conclusion that endogenous levels of TNF-α were beneficial, but did not eliminate the possibility that elevated levels of TNF-α may be injurious. The current study expands upon our initial findings. We now provide empirical evidence to show that TNF-α produced in response to RSV infection during the inductive phase of the immune response is protective. This is most likely a result of the NK cell response, which is an important component of early immunity to RSV. However, the new data presented in this manuscript also demonstrate that prolonged production of TNF-α results in significant illness during the effector phase of the immune response. So, while T cell responses are crucial for efficient elimination of RSV, an overzealous T cell response leads to lung injury and significant immunopathology through the production of TNF-α, which was not shown by our previous report (57).

A second report by Hussell et al. (58) examined the role of TNF-α in response to RSV infection in the setting of immunized, WT mice. Their report showed that WT mice immunized with a recombinant vaccinia virus expressing either the G or F protein of RSV 2 wk before RSV challenge had significantly reduced illness when treated with anti-TNF-α. The results from the current study are consistent with those of Hussell et al., but several important distinctions are evident. The previous study was focused on an immune response stemming from vaccinia-primed memory in WT mice, which is dissimilar to our studies of primary infection in several strains of knockout mice. In addition, the previous study is heavily focused on general memory CD4 T cell responses, whereas the current study is concerned with NK and CD8 T cell responses to primary RSV infection. Importantly, our study also defines the
contribution of TNF-α to viral clearance and immunopathology relative to other effector T cell functions, which has not been examined previously in such comprehensive detail. Although the previous study demonstrated that the memory CD4 T cell response to RSV infection improves in the setting of TNF-α inhibition, we show that during primary infection, TNF-α production during the inductive immune response serves an important protective role, while exaggerated production of TNF-α during the adaptive phase of the immune response is harmful and induces significant lung immunopathology. Moreover, we demonstrate that perforin-mediated killing and IFN-γ production combine to play a protective role in response to primary RSV infection.

It has been reported that TNF-α can induce lung injury (59). In this particular study, lung injury occurred in the absence of perforin and FasL expression, as long as TNF-α was present. Another study has presented evidence that up-regulation of the antiapoptotic gene IEX-1L protects cells from TNF-α-induced apoptosis during RSV infection (60). However, another group has demonstrated that IEX-1L is not expressed in vivo (61). Other reports have shown that TNF-α, like FasL, can be responsible for bystander killing of uninfected cells (62, 63). Overzealous production of TNF-α in the context of an IL-4-dominant immune response may therefore be responsible for more severe illness in this way.

Our current results present evidence that FasL may also play a role in the induction of RSV-induced illness, as evidenced by the fact that illness was significantly reduced in gld mice (Fig. 2). It has recently been reported that epithelial lung injury in humans with acute lung injury or acute respiratory distress syndrome is associated with an up-regulation of FasL-mediated apoptosis (64). In addition, the up-regulation of FasL expression in these patients was associated with increased apoptosis, as determined by TUNEL staining. A cursory examination of these data would suggest that FasL may be directly responsible for the observed pathology. However, one group has reported that CTLs from gld mice were reduced in their ability to proliferate in response to alloantigenic stimulation (25). This group went on to show that reverse signaling through FasL was necessary for maximum T cell proliferation (26, 27). This is not surprising, considering that other members of the TNF superfamily have similarly been shown to participate in reverse signaling (22–24). We therefore hypothesize that reverse signaling through FasL leads to increased production of TNF-α, which exacerbates disease.

NK cells possess the same cytolytic machinery as CD8+ CTLs. Hussell and Openshaw (51) have reported that NK cell responses to primary RSV infection peak on day 4 postinfection. In addition, NK cells are the most ardent producers of IFN-γ during this time. When we examined NK cell function in gld mice at days 3–7 postinfection, we saw no significant differences compared with

FIGURE 8. Illness is significantly reduced in RSV-infected mice that receive anti-TNF-α treatment. WT (A), GKO (B), gld (C), and PGKO (D) mice were infected with RSV and weighed daily to measure illness. Mice received 200 µg of either isotype control or anti-TNF-α Ab every other day, starting at day −1. Averages and SDs were calculated from results with the following mouse numbers: WT, n = 36 through day 9, 33 at day 10, 32 at day 11, and 13 at day 12; GKO, n = 10 at day 9, 7 at day 10, and 3 at day 11; gld, n = 13 through day 9, and 4 through day 12; PGKO, n = 6 through day 9, and 3 through day 11. Value of p < 0.05 at days 1–12 in A, days 6 and 8–10 in B, days 6–9 and 11–12 in C, and days 2–10 in D.
WT mice (Fig. 4), which matched the results we saw in CD8^+ T cells (Fig. 3). From these results, we conclude that our data are not the result of impaired NK cell activity in the absence of functional FasL.

Because T cells are responsible for the severe immunopathology seen during RSV infection, it is logical to target CTLs for the development of efficacious therapies against RSV. In the quest to elucidate which subsets of CD8^+ CTL will efficiently clear virus-infected cells without attendant immunopathology, it is important to understand that CTL responses are dependent upon the pathogen, target organ, and route of inoculation (67). Delayed clearance of mouse hepatitis virus, CMV, and RSV has been observed in PKO mice (47, 67–69). Similarly, clearance of lymphocytic choriomeningitis virus (LCMV) was delayed in granzyme B-deficient mice (70). Virus-infected hepatocytes rely on FasL-mediated cytolysis (71), but FasL is not needed for resolution of LCMV infection (72, 73). Roles for both the granule exocytosis pathway and FasL have been demonstrated in influenza infection (74). In addition, a recent study has shown that high viral loads of LCMV lead to ablation of T cell responses and persistence of the disease (75). Although part of the disparity in T cell responses to different pathogens is indeed dependent on the milieu of the response, the particular T cell subsets involved also play an important role in the resolution of an infection. Two subtypes of CD8^+ T cells, designated Tc1 and Tc2, have been described (76–78). These designations reflect the typical cytokine profiles of each population, and are nearly identical with the Th1/Th2 model, in which Th1 CD4^+ T cells produce IFN-γ and Th2 cells produce IL-4 and IL-5. Furthermore, heterogeneity of naive and memory CD8^+ T cell populations that extends far beyond a simple Tc1/Tc2 distinction has been described and reviewed elsewhere (76–78). Some of these subsets can be defined by the preferential use of different effector functions in response to various virus infections. Similarly, some immunocompromised individuals, such as those who suffer from familial hemophagocytic lymphohistiocytosis, in which T cells express little or no perforin (79), possess abnormal T cell populations that respond to virus infections in ways that do not mirror what is seen in a normal, healthy individual. For this reason, it is important to attain a full understanding of which CD8^+ CTL subsets effectively respond to RSV infections.

With this study, we have now achieved a greater understanding of which CTL subsets are most efficient at clearing RSV-infected cells without attendant immunopathology. Presently, we hypothesize that a protective immune response to RSV is generated by perforin/granzyme-mediated cytolysis and IFN-γ production. FasL and TNF-α also contribute to clearance of virus-infected cells. However, FasL is inefficient compared with other mechanisms, while continued production of TNF-α during the CTL response is harmful and exacerbates illness despite its protective role early. Moreover, we hypothesize that reverse signaling through FasL facilitates TNF-α production. It is known that the p55 TNF-α receptor is the main conduit for TNF-α-induced cytolytic activity (80–82). For this reason, antiviral therapies that target TNF-α or the TNF receptor may hold great promise in the quest to alleviate suffering induced by RSV infection.
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