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Improved Effector Activity and Memory CD8 T Cell Development by IL-2 Expression during Experimental Respiratory Syncytial Virus Infection[1]

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Respiratory syncytial virus (RSV) is a major cause of lower respiratory infection in young children and the elderly. Studies of mice suggest that RSV suppresses the effector activity of CD8 T cells and the development of pulmonary CD8 T cell memory, in which the impaired effector activity could be recovered by in vitro IL-2 treatment. To investigate the effect of in vivo IL-2 expression on RSV immunity, mice were infected with RSV followed by administration of replication-defective adenovirus expressing IL-2. The effector activity of RSV M2-specific CD8 T cells and the development of CD8 T cell memory in the lung was significantly increased by IL-2 expression. Furthermore, the Ab responses against RSV were augmented by IL-2. Interestingly, weight loss and illness caused by RSV challenge were substantially reduced by IL-2 priming, suggesting that the pathogenesis of RSV-related disease could be prevented by IL-2-mediated enhancement of beneficial immune responses. Thus, our results show that IL-2 has potential to be used as a vaccine adjuvant against RSV infection. ***The Journal of Immunology*****, 2004, 172: 503–508.**

Respiratory syncytial virus (RSV)[3] is the most important cause of severe lower respiratory tract infection in infants and young children worldwide (1, 2). Natural infection with RSV provides limited protection from reinfection and subsequent diseases, as demonstrated by the recurrence of severe RSV infections throughout individual's lifetime (3, 4). However, no effective antiviral therapy or vaccine is currently available. Thus, the development of RSV therapeutics and vaccines has long been a priority for infants and young children (2).

RSV-specific CD8 T cell responses are involved in the clearance of the virus and recovery from infection. In BALB/c mice, previous studies have demonstrated that virus-specific CTLs are sufficient for effective virus clearance during primary infection (5, 6). In addition to the role in virus clearance, it has been suggested that CD8 T cells play an important role in the regulation of the differentiation and activation of Th2 CD4 T cells, which mediate the enhanced lung pathology by the recruitment of eosinophils into the lungs during RSV infection (7, 8). However, the immune mechanisms underlying the balance among protection, disease, and recovery from infection still remain to be determined.

Substantial numbers of Ag-specific memory T cells persist in the lung after clearance of respiratory virus infection such as influenza and sendai virus (9–11), and these cells may play an important role in the control of secondary infection (11, 12). In case of RSV, repeated infection is a frequent event in the human population, indicating that protective immunity to reinfection may be incomplete and of short duration (3, 4). We have previously shown that RSV induces immune dysfunction of lung CD8 T cell effector activity and impairs the development of peripheral CD8 T cell memory (13, 14). Thus, these reports suggest a possible mechanism to explain the limited duration of protective immunity against RSV infection and frequent reinfection events in the human population. Interestingly, it also has been shown that suppression of effector activity of RSV-specific CD8 T cells could be recovered by ex vivo IL-2 treatment (13).

IL-2 has pleotropic effects on the immune system which include stimulation of proliferation, lytic activity, and cytokine secretion of T lymphocytes (15, 16). In addition, it has been proposed that IL-2 is required for generating and maintaining memory T cells (17, 18). Thus, it is of interest to see whether in vivo IL-2 expression reverses RSV-induced immunosuppression of CD8 T cells in the respiratory tract and confers prolonged beneficial immunity against RSV reinfection.

In this study, we tested a hypothesis that supplementary IL-2 expression by recombinant adenovirus during primary RSV infection could reverse immunosuppression and improve the effector activity and development of memory CD8 T cells. Our results in this study showed that IL-2 priming increases the effector activity of CD8 T cells, development of pulmonary memory CD8 T cells, and humoral responses, leading to reduced disease severity during RSV challenge.

Materials and Methods

Mice

Six-week-old female BALB/c mice were purchased from Charles River Breeding Laboratories (Shizuoka, Japan). Mice were housed in a pathogen-free environment in an internally approved vivarium at the Institute.

Viruses and infection of mice

The A2 strain of RSV was a gift from P. L. Collins (National Institutes of Health, Bethesda, MD). RSV stock was grown on HEp-2 cells (American Type Culture Collection, Manassas, VA) and titered for infectivity. For the construction of recombinant replication-defective adenoviruses (rAd),

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[3] Abbreviations used in this paper: RSV, respiratory syncytial virus; rAd, recombinant replication-defective adenovirus; i.n., intranasally; rAd-IL-2, recombinant defective adenovirus expressing human IL-2; rAd-LacZ, recombinant defective adenovirus expressing β-galactosidase.

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pShuttle-CMV and pAd-Easy vectors were used (a kind gift of Dr. Vogelstein, Johns Hopkins University, Baltimore, MD) (19). Replication-defective rAd expressing β-galactosidase (rAd-LacZ) or human IL-2 (rAd-IL-2) were produced and titered with 293 cells (American Type Culture Collection). Mice were lightly anesthetized with a 2:1 mixture of ether and chloroform, and intranasally (i.n.) inoculated with 1×10^6 PFU of RSV in 50 μl and 2×10^7 PFU of rAd later at indicated time points. At various times after infection, infected mice were sacrificed for the analysis. For measurement of mouse weights and illness scores, mice were monitored daily. Illness was scored using a standard scale: 0 = healthy; 1 = slightly ruffled fur; 2 = ruffled fur but active; 3 = ruffled fur and inactive; 4 = ruffled fur, inactive, and hunched; and 5 = dead.

Preparation of lung lymphocytes

The lungs were perfused with 5 ml of PBS containing 10 U/ml heparin (Sigma-Aldrich, St. Louis, MO) through the right ventricle using a syringe fitted with a 25-gauge needle. The lungs were removed and placed into RPMI 1640 medium supplemented with glutamine, gentamicin, penicillin G, and 10% FBS (HyClone Laboratories, Logan, UT). The tissue was then processed through a steel screen to obtain single-cell suspension and particulate matter was removed by passing through a Falcon cell strainer (BD Labware, Franklin Lakes, NJ). Cells were counted and resuspended at the given cell concentrations for the appropriate in vitro assay.

Flow cytometry and tetramer staining

MHC class I-peptide tetramers were produced as described previously (20). Freshly explanted lung lymphocytes were purified by density gradient centrifugation and cells (1×10^6) were stained in PBS/3% (w/v) FBS/0.09% (w/v) NaN_3 using fluorochrome-conjugated Abs and MHC class I tetramers. Influenza NP-specific H-2K^d tetramer loaded with NP (147–155) peptide was used as negative control. The Abs used were anti-CD8 (clone 53-6.7), anti-CD11a (clone 2D7), anti-CD44 (clone IM7), and anti-CD62L (clone MEL-14). All Abs were purchased from BD PharMingen (San Diego, CA). After staining, cells were fixed in PBS/2% (w/v) paraformaldehyde, and events were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). To enumerate the number of cytokine-producing cells, intracellular cytokine staining was performed as previously described (21). In brief, 2×10^6 freshly explanted lung lymphocytes were cultured in culture tube. Cells were left untreated or stimulated with M2/82–90 peptide (SYIGSINNI), and incubated for 5 h at 37°C in 5% CO_2. Brefeldin A (5 μg/ml; Sigma-Aldrich) was added for the duration of the culture period to facilitate intracellular cytokine accumulation. The Abs used were anti-IFN-γ (clone XMG1.2) or its control isotype Ab (rat IgG1). Dead cells were excluded on the basis of forward and side light scatter. Data were analyzed using CellQuest (BD Biosciences) and FlowJo software (Tree Star, San Carlos, CA).

Anti-RSV Ab ELISA

Peripheral blood was collected before and 40 days after RSV infection from the eye capillary bed. RSV A2 Ag (US Biological, Swampscott, MA) was diluted in PBS to 10 μg/ml, and then coated on microtiter plates. Pre- or postimmune sera were 2-fold serially diluted in PBS containing 2% BSA from 1/50 to 1/6400 and added to each well. After stringent washing with PBS containing 0.05% Tween 20, anti-mouse Ig Ab (clone 187.1; BD PharMingen) conjugated to HRP was used as detection Ab. The absorbance from preimmune sera was below 0.1 for all mice.

Results

We previously reported (13) that activated CD8 T cells infiltrating the lungs of mice in response to intranasal RSV infection were defective in the expression of effector activity. This deficit, which was expressed as a failure of ~50% of the activated CD8 T cells to produce IFN-γ in response to antigenic stimulation, was reversed by exposure of the lung infiltrating CD8 T cells to IL-2 in vitro. We now wanted to test the hypothesis that in vivo IL-2 expression may reverse immunosuppressive effects of RSV on CD8 T cell function. Mice were infected with RSV, and 2 days later recombinant defective adenovirus expressing human IL-2 (rAd-IL-2) or β-galactosidase (rAd-LacZ) as a control was i.n. delivered. Initially, we chose 2 days after RSV infection as a time point for rAd delivery since peak expression of the cytokine by replication-defective adenovirus is shown to be within 48 h in cell culture system (data not shown) and IL-2 expression in this circumstance might coincide with peak virus replication and subsequent virus-specific CD8 T cell development. As shown in Fig. 1*A*, at the peak of CD8 T cell responses to RSV in the lung, the frequency of RSV-specific CD8 T cells as measured by H-2K^d tetramer complexes loaded with M2/82–90 epitope (designated hereafter as M2Tet) was not affected by IL-2 expression. However, intranasal administration of the replication-defective rAd-IL-2 but not the control adenovirus significantly increases the frequency of IFN-γ-producing CD8$^+$ T cells at the peak of primary RSV infection. The ratio of IFN-γ-producing cells to M2Tet$^+$ cells (as means of functional frequency vs phenotype frequency) in the rAd-IL-2 group reached ~80%, while ~57.3% of M2Tet$^+$ cells responded to antigenic stimulation in the rAd-LacZ group (Fig. 1*A*). We did not observe any difference in the expression of α and β subunits of IL-2R on virus-specific CD8 T cells between rAd-IL-2-treated and control group (data not shown), suggesting that effect of IL-2 expression in vivo was unrelated to the ability of the

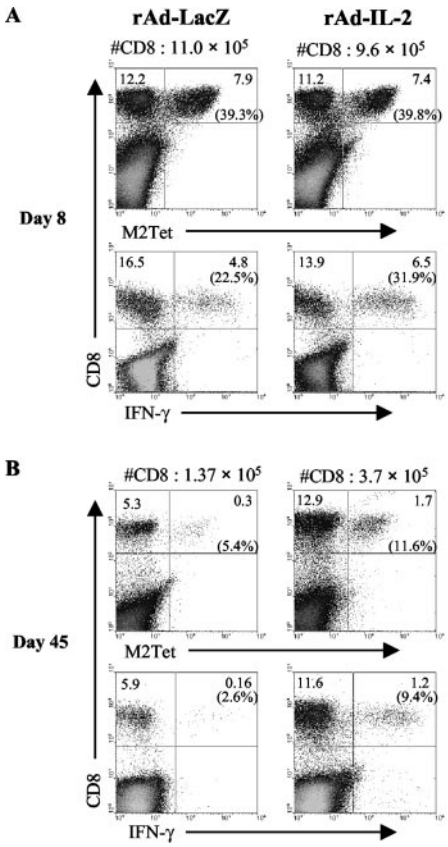

FIGURE 1. IL-2 delivery by rAd during primary RSV infection increases effector functions of RSV-specific CD8 T cells and memory T cells in the lung. *A*, Mice were infected with 1×10^6 PFU of RSV and 2 days later with 2×10^7 PFU of rAd-LacZ or rAd-IL-2. At day 8 after RSV infection, lung lymphocytes were prepared and stained with M2Tet. The numbers in each quadrant indicate the percentage of gated lymphocytes and the number in parenthesis represents the percentage of M2Tet$^+$ cells among total CD8 T cells. The numbers of total CD8$^+$ cells (#CD8) were calculated by multiplying the percentage of CD8$^+$ T cells with the total number of mononuclear cells and are indicated on top of each panel. The same batches of lung lymphocytes were stimulated with M2 peptide in the presence of brefeldin A, and then stained for intracellular IFN-γ. The number in parenthesis represents the percentage of IFN-γ-producing cells among total CD8$^+$ T cells. *B*, Lung lymphocytes were prepared from the same group of mice shown in *A* at day 45 after RSV infection and stained with M2Tet. Representative results from one of three independent experiments with four mice per group are shown.

$CD8^+$ T cells to signal through the IL-2 receptor. These results demonstrate that the impaired CD8 T cell effector activity caused by RSV could be partially recovered by in vivo expression of IL-2 during primary infection, which is consistent with the previous report showing that RSV-induced functional defect of effector CD8 T cells is restored by ex vivo IL-2 treatment (13).

Although IL-2 delivery by rAd did not increase the number of RSV M2-specific T cells, it influences the effector functions of virus-specific $CD8^+$ T cells. Since RSV-induced immune dysregulation was also reported to impair the development of memory T cell responses in the lung (13), it was of interest to determine whether IL-2-mediated restoration of effector CD8 T cell function in vivo also enhanced the development of pulmonary CD8 T cell memory. To investigate this possibility, we examined the frequency and absolute number of RSV M2-specific memory CD8 T cells in the lungs of mice at day 45 after coinfection with RSV and either the rAd-IL-2 or control rAd-LacZ virus. In the rAd-LacZ group of mice, only ~5.4% of total CD8 T cells in the lung was M2-specific determined by M2Tet staining (Fig. 1*B*). In contrast, rAd-IL-2-treated group of mice showed >2-fold increase in the percentage of M2-specific memory cells (~11.6% of total CD8 T cells in the lung). This increased frequency of M2-specific memory cells was detectable despite the higher overall number of total residual $CD8^+$ T cells detected in the rAd-IL-2-treated group (Fig. 1*B*; 5.6% in the rAd-LacZ group vs 14.6%). Importantly, the absolute number of RSV M2-specific memory T cells in rAd-IL-2 group at this time after primary infection was substantially higher, i.e., ~7-fold more than that of the rAd-LacZ group; (1.7% vs 0.3%, respectively), indicating that IL-2 delivery by rAd during primary RSV infection significantly enhances both the frequency and the residual number of memory CD8 T cells in the lung. It is also interesting to note that the ratio of IFN-γ-producing cells to M2Tet-positive cells was still higher in rAd-IL-2-infected mice at this time point (i.e., ~48.1% vs ~81%), indicating that recovered effector activity of CD8 T cells by IL-2 expression is maintained through memory phase. In this setting, it should be noted that differences in the frequency and number of memory $CD8^+$ T cells was unrelated to the extent of adenovirus replication in the lungs as both recombinant viruses are replication-defective.

Next, we examined whether the timing of IL-2 expression in the lung has any differential effects on CD8 T cell effector activity or memory duration by varying the time point of rAd administration after primary RSV infection. Mice were infected with RSV, and then rAd-IL-2 was i.n. delivered at indicated time points as shown in Table I. The timing of IL-2 delivery after RSV infection did not significantly affect the frequency of M2-specific CD8 T cells in the lung at peak primary response, i.e., day 8. The ratio of IFN-γ-producing cells to M2Tet-positive cells was slightly higher in the group that received rAd-IL-2 two days after RSV infection than other groups. In contrast, the frequency of M2-specific memory CD8 T cells in the lung at day 45 was significantly increased in day 2 and day 3 groups (~13.3% and ~9.4% $M2Tet^+/CD8^+$, respectively) compared with control group (Table I). When rAd-IL-2 was delivered at day 1 after RSV infection, there was a marginal increase in the number of M2-specific memory CD8 T cells compared with control group (~6.9% vs ~5.3% $M2Tet^+$, respectively). Considering the differences in the total number of residual $CD8^+$ T cells in each group of mice, the absolute numbers of $M2Tet^+$ cells in day 1, day 2, and day 3 groups were even greater than that of control mice. However, rAd-IL-2 delivery at day 5 after RSV infection failed to increase the frequency of either M2-specific memory CD8 T cells or total CD8 T cells in the lung, suggesting that the timing of expression of IL-2 during primary RSV infection may be critical for the reversal of RSV-induced suppression of memory T cell development but less critical for the reversal of RSV-mediated suppression of CD8 T cell effector activity.

Although IL-2 expression did not increase the number of primary RSV-specific CD8 T cells in the lung, IL-2 expression in the lungs did enhance the effector activity of lung infiltrating CD8 T cells and enhance the formation of residual memory CD8 T cells in lungs. In view of the effect of IL-2 on memory CD8 T cell frequency/numbers, in vivo exposure to IL-2 during primary RSV infection may enhance resistance to or recovery from subsequent RSV challenge. To determine whether IL-2 delivery increased capacity of RSV-specific memory T cells to control challenge infection, we measured virus replication, weight loss, and illness score after RSV challenge of mice previously coinfected with RSV and either the rAd-IL-2 or control adenovirus vector. Replication of challenged RSV inoculum was almost undetectable in the lungs of all animals (mean titer of <2.0 $\log_{10}$ PFU/g; data not shown). However, RSV-immune mice coinfected with the rAd-IL-2 showed significantly reduced weight loss and illness scores after RSV challenge than the RSV/rAd-LacZ group (Fig. 2, *A* and *B*). The rAd-IL-2 group demonstrated reversal of weight loss starting from day 2 postchallenge while rAd-LacZ-delivered group showed continuous weight loss until day 4 postchallenge ($p < 0.01$, days 3–6 p.i.). We also monitored the frequency of secondary RSV-specific CD8 T cells in the lung during challenge infection (Fig. 2*C*). As expected, the number of M2-specific CD8 T cells was

Table I. *Optimal time point of rAd-IL-2 delivery during RSV infection*[a]

	Days Post RSV Infection			
	Day 8		Day 45	
rAd-IL-2 Delivery	Percentage of $M2Tet^+/CD8^+$	Ratio of the percentage $IFN\text{-}\gamma^+$ to the percentage $M2Tet^+$ [b]	Percentage of $M2Tet^+/CD8^+$	No. of total $CD8^+$ ($\times 10^4$)[c]
Day 1	32.6	76.5	6.91	12.6
Day 2	38.6	80.5	13.28	30.4
Day 3	38.1	72.0	9.43	10.6
Day 5	39.9	78.3	5.61	9.8
No	39.2	50.5	5.33	8.8

[a] Mice were infected i.n. with 1×10^6 PFU RSV and at indicated day after RSV infection 2×10^7 PFU rAd-IL-2 were i.n. administered. Lung lymphocytes were prepared at day 8 and day 45 after RSV infection and analyzed for M2Tet staining and IFN-γ production after peptide stimulation.

[b] Percentages of IFN-γ-producing cells among $M2Tet^+$ cells are calculated by dividing the percentage peptide-stimulated $IFN\text{-}\gamma^+/CD8^+$ by the percentage of $M2Tet^+/CD8^+$ in each group.

[c] The numbers of total $CD8^+$ cells were calculated by multiplying the percentage of $CD8^+$ T cells with the total number of mononuclear cells.

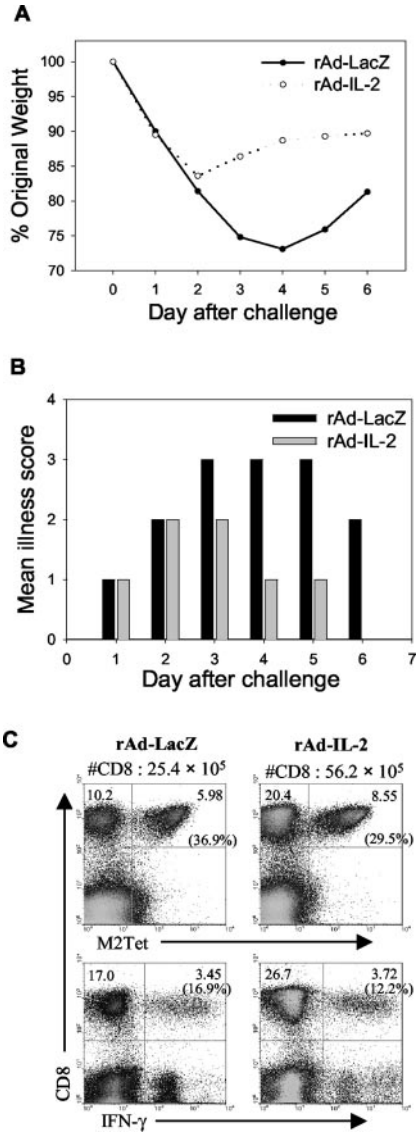

FIGURE 2. IL-2 delivery by rAd during primary RSV infection reduces RSV-induced disease and increases secondary CD8 T cell responses during RSV challenge. *A*, Mice were infected with RSV and 2 days later with rAd as shown in Fig. 1. At day 45, mice were challenged with 1×10^6 PFU of RSV and weight loss was monitored daily following RSV challenge. The percentage of weight loss was calculated based on the original weight of each mice before challenge. *B*, Mean illness scores from four individual mice per group are shown. *C*, At day 6 after RSV challenge, lung lymphocytes were prepared and stained with M2Tet or stimulated with M2 peptide and stained for IFN-γ. The numbers of total $CD8^+$ cells are indicated on top of the panel. The numbers in each quadrant indicate the percentage of gated lymphocytes and the number in parenthesis represents the percentage of M2Tet-positive or IFN-γ-positive cells among total $CD8^+$ T cells. Representative results from one of two independent experiments with four mice per group are shown.

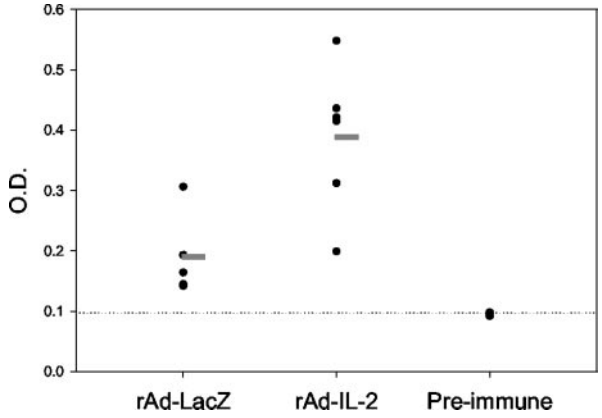

FIGURE 3. IL-2 delivery by rAd during primary RSV infection increases anti-RSV Ab responses. Mice were infected with RSV and rAd as described in Fig. 1. and serum samples were prepared at day 40 after infection and examined by ELISA for total Ig anti-RSV Ab titers. The results shown are the absorbance value for serum at dilution of 1/400 from individual six mice per group. The horizontal bar represents the mean absorbance of each group. ($p < 0.01$). The dotted line indicates threshold level of detection determined by preimmune serum samples.

greater in IL-2-delivered group of mice than that of control group at day 6 postchallenge, suggesting that increased memory CD8 T cell responses in the lung might have provided better protection from pathogenesis of RSV-induced disease. The number of CD8 T cells that are not M2-specific was also higher in the lung of rAd-IL-2-infected mice than that of control group (Fig. 2*C*; 20.4% vs 10.2%), suggesting that other RSV Ag-specific CD8 T cells, for example, F-specific cells might also have increased. Importantly, the enhancement of memory CD8 T cell response by IL-2 did not reverse the suppressive effect of secondary RSV infection, since the ratio of IFN-γ-producing cells to $M2Tet^+$ cells was similar between two groups at the peak of challenge infection (~41.4% in rAd-IL-2 group vs ~45.8% in control group, respectively). This finding is consistent with the previous observation that RSV infection inhibits the expression of effector activity of lung-infiltrating CD8 T cells in both the primary and secondary response to intranasal RSV infection (13). Thus, while in vivo IL-2 expression during primary infection increases the duration and the magnitude of the memory CD8 T cell response, the effector CD8 T cells responding to challenge infection are still susceptible to RSV-induced immune dysregulation.

IL-2 is known to stimulate Ab secretion by activated B cells (22, 23). Thus, it is possible that IL-2 delivery during primary RSV infection also augments Ab response against RSV. To determine the effect of IL-2 expression on the anti-RSV Ab response, the serum samples were collected on day 0 (immediately before infection) and day 40 after RSV infection and analyzed. Mice infected with rAd-IL-2 did develop higher titer of total Ig anti-RSV Ab, i.e., ~2-fold increase in titer on day 40 p.i. compared with rAd-LacZ-infected mice (Fig. 3; 0.19 vs 0.38 mean OD value, $p < 0.01$), as determined by ELISA with purified RSV A2 as Ag. These results indicate that IL-2 priming during primary infection enhanced Ab responses to the virus, which might also contribute to better protective immunity and reduced illness.

Discussion

In this study, we have shown that IL-2 expression during primary RSV infection restores effector function of M2-specific CD8 T cells in the lung and subsequently enhances the development of memory CD8 T cells and Ab responses. These findings have important implications for RSV vaccine development. As previously reported, RSV-induced immunosuppression might be the important cause of the absence of durable long-lived immunity to RSV and frequent reinfection events in the human population (13). In this regard, it is likely that the use of IL-2 together with live-attenuated vaccines augments both generation of RSV-specific memory T cells in the lung parenchyma and Abs, which might be helpful for prevention and control of subsequent RSV infection.

Our previous results suggest that RSV infection results in defective TCR-mediated signaling and expression of effector activity even though activation and proliferation of virus-specific CD8 T cells is normal (13). In virus infection models, there are several reports showing that Ag-specific CD8 T cells are functionally inactive while they could be still detectable using MHC I tetramers. For example, Zajac et al. (24) have reported that LCMV-specific CD8 T cells fail to secret IFN-γ in response to Ag. In human cases, it has been reported that HIV-1 or HCV-infected individuals harbor high frequencies of virus-specific CD8 T cells which are defective in IFN-γ production in response to peptide stimulation (25, 26). The nonresponsiveness of virus-specific CD8 T cells may be one of the viral evasion strategies to escape host immune system, but this could be hardly explained by a single mechanism. However, one intriguing observation has been recently reported that IL-2 treatment restored Ag-specific CD8 T cell responses in SIV-infected monkey model, resulting in the quantitative correlation between tetramer recognition and Ag-responsive IFN-γ secretion (27). This is consistent with our previous and present data showing that the presence of IL-2 both in vitro and in vivo restores IFN-γ response from defective virus-specific CD8 T cells. Thus, the impaired CD8 T cell response may be a result of depletion of cofactors such as IL-2 which is necessary for the optimal activation and differentiation of Ag-specific effector CD8 T cells. In the previous study, it has been shown that the defect exhibited by RSV-specific CD8 T cells is reversed when purified CD8 T cells from the lung are cultured with IL-2 (13). In this regard, it is likely that supplemental IL-2 expression and IL-2 receptor engagement on CD8 T cells directly compensate the deficit in TCR-mediated triggering of effector function rather than have influence on other types of immune cells. In support of this hypothesis, we recently observed that RSV G-specific CD4 T cell and NK-cell responses are unaffected by IL-2 expression during primary RSV infection (our unpublished results).

The previous study reported that recombinant RSV containing the coding region of murine IL-2 exhibited increased CD4 T cell response and modest attenuation of virus replication in BALB/c mice whereas the Ab response and the level of resistance to reinfection were not significantly different from those of wt RSV (28). In our study, replication of challenged virus was almost undetectable in animals which had been infected with RSV plus either rAd-LacZ or rAd-IL-2 (data not shown), showing that the level of resistance to reinfection is indistinguishable between two groups. However, weight loss and virus-induced illness was significantly reduced in mice that received rAd-IL-2 (Fig. 2). Many studies have described that RSV-specific CD4 T cells that produce Th2 cytokines such as IL-5 and IL-13 are mainly involved in pulmonary eosinophilia, enhanced illness and weight loss (29–34). In addition, it was suggested that CD8 T cells play an important role in the regulation of the differentiation and activation of Th2 CD4 T cells during RSV infection (7, 8). Thus, it is possible that moderately increased CD8 T cells in the lungs of rAd-IL-2-infected mice might have down-regulated Th2-like CD4 T cell response, leading to reduced weight loss and illness during challenge infection. Alternatively, increased memory CD8 T cells in the lung and Abs of rAd-IL-2-infected mice might have cleared challenge infection more effectively and attenuated recruitment of other inflammatory cells into the lung that are associated with weight loss and illness (35).

In summary, we show that supplementary expression of IL-2 overcomes RSV-induced immunosuppression and enhances RSV immunity to reinfection. This information will be important to elucidate how to induce beneficial virus-specific immune responses in future development of RSV vaccines.

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