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Influence of a Single Viral Epitope on T Cell Response and Disease After Infection of Mice with Respiratory Syncytial Virus

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CTL are important for virus clearance but also contribute to immunopathology after the infection of BALB/c mice with respiratory syncytial virus (RSV). The pulmonary immune response to RSV is dominated by a CTL population directed against the CTL epitope M2-1 82–90. Infection with a virus carrying an M2-1 N89A mutation introduced by reverse genetics failed to activate this immunodominant CTL population, leading to a significant decrease in the overall antiviral CTL response. There was no compensatory increase in responses to the mutated epitope, to the subdominant epitope F 85–93, or to yet undefined minor epitopes in the N or the P protein. However, there was some increase in the response to the subdominant epitope M2-1 127–135, which is located in the same protein and presented by the same H-2Kd MHC molecule. Infection with the mutant virus reversed the oligoclonality of the T cell response elicited by the wild-type virus. These changes in the pattern and composition of the antiviral CTL response only slightly impaired virus clearance but significantly reduced RSV-induced weight loss. These data illustrate how T cell epitope mutations can influence the virus-host relationship and determine disease after an acute respiratory virus infection. The Journal of Immunology, 2007, 179: 8264–8273.

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R espiratory syncytial virus (RSV) is the major pathogen causing respiratory disease in infants and young children (1–3). The clinical sequelae of RSV infection range from mild upper airway disease to severe bronchiolitis or pneumonia that can sometimes be lethal. The factors determining the individual outcome of infection are poorly defined, but it is likely that both viral and host factors contribute. Control of primary RSV infection is dependent on the generation of an antiviral T cell response. Infants with congenital T cell deficiencies are not able to eliminate the virus (4, 5), and depletion of T cells leads to persistent infection in BALB/c mice (6). However, the antiviral T cell response also contributes to disease. T cell reconstitution of T cell-deficient infants persistently infected with RSV can exacerbate lung disease (Ref. 7 and our own unpublished observations), and the transfection of virus-specific T cells into RSV-infected mice aggravates disease (8). Both CD4+ and CD8+ T cells can eliminate virus and cause immunopathology independently (9), but CD8+ T cells appear to be more effective.

CTL recognize viral Ags that are presented as peptide fragments by MHC class I molecules on the surface of infected cells (10). In the course of a viral infection, multiple viral peptides are presented and activate epitope-specific CTL populations varying in size and clonal composition. The hierarchy of these CTL responses is determined by the efficiency of Ag presentation and the composition of the peripheral T cell pool (11–14). Immunodominant epitopes stimulating the largest number of antiviral CTL usually offer the best level of protection against the infecting virus. However, they may also make the most important contribution to T cell-mediated immunopathology.

One possibility to better understand this delicate balance between the viral and host parameters that determine the clinical outcome of viral infections is the experimental analysis of infection with viruses carrying mutations in immunodominant T cell epitopes. Studies with in vivo derived or genetically engineered viral T cell epitope mutants have been performed in the mouse models of infection with lymphocytic choriomeningitis virus (LCMV) (15–17), influenza (13, 18–20), and HSV (21). In none of these models did the epitope mutation have a significant influence on the kinetics of virus control, and the impact on disease was variable. Although disease was attenuated after infection with an LCMV epitope mutant (16), infection with an influenza epitope mutant enhanced disease (18). Thus, the contribution of immunodominant CTL populations to viral diseases is not easily predictable and may differ for individual viruses.

The RSV-specific CTL response in BALB/c mice is highly focused on the immunodominant H-2Kd restricted epitope M2-1 82–90 (22–24). At the acute phase of RSV infection, ~10–30% of total pulmonary CTL and ~80% of RSV-specific CTL are directed...
against this epitope. In this study, we used reverse genetics to introduce a single amino acid change into this immunodominant CTL epitope and analyzed the consequences of this mutation for epitope-specific and overall RSV-specific CTL responses as well as for virus clearance and disease. We found that lack of the immunodominant CTL population led to a reduced overall CTL response to RSV and only minor compensatory increases in responses to other subdominant epitopes. The consequences of these changes in the CTL response were reduced disease after the primary infection of BALB/c mice accompanied by a slight delay in virus elimination.

Materials and Methods

Plasmid construction and recovery of recombinant RSV

The parental RSV strain A2 antigenic cDNA used for constructing the M2-1 82–90 variants was pD53 BsiWI, which had three changes from the original wild-type antigenic cDNA: (25) 1) the SH gene was modified by the deletion of 112 nucleotides from the distal noncoding region and the introduction of silent substitutions in the last 10 nucleotides of the open reading frame to stabilize the complete antigenic cDNA in bacteria (26); 2) a BsiWI site was created in the trailer region 9–14 nt downstream of the L gene signal by three nucleotide substitutions (R. Fears and P. L. Collins, unpublished results); and 3) the hammerhead ribozyme was replaced by a hepatitis delta ribozyme (R. Fears and P. L. Collins, unpublished results). To facilitate exchange of the M2 gene, this cDNA was modified to contain a unique KpnI site in the intergenic region preceding the M2 gene at nt 7461–7466 relative to the 5′ end of the encoded antigenic cDNA. Insertion of the KpnI site was conducted in a subclone of the complete antigenic plasmid containing the G, F, and M2 genes (pGFM2; Ref. 25) and involved nucleotide substitutions at positions 7461 (C→G) and 7462 (T→G) such that the sequence of the intergenic region was now 7440–CACAATTTCGATCCAGATTAAAGGTACATCTGATAA AAATG AAAACT-7485 (cDNA) with the changed nucleotides in bold letters and the KpnI site underlined. The resulting subgenomic plasmid was designated pGFM2 KpnI. The fragment containing the KpnI site was reintroduced into the SBal-BamHI window of pD53/BsiWI, resulting in pD53/KpnI. The subgenomic clone pGF2M KpnI was also used for modification of the M2 gene. The 2R mutation (Y83R) was introduced by substitution of codon 249-TAT-251 (positions relative to the gene) with CGA using the QuickChange mutagenesis kit (Stratagene) following the manufacturer’s instructions. The 8A mutation (N89A) was introduced accordingly by substitution of codon 267-AAT-269 with GCG. The modified M2 gene was inserted into the KpnI-BamHI window of pD53/KpnI, thereby replacing the wild-type M2 gene and generating plasmid pD53/KpnI 8A. The primer sequences used for mutagenesis are available from the authors upon request. All regions of the subgenomic and final full-length cDNA clones that had been amplified by PCR were confirmed completely by sequence analysis. Transfections and recovery of recombinant RSV (rRSV KpnI and rRSV 8A) were performed as described previously (25).

Mice and viral infection

Specific pathogen-free BALB/c mice were obtained from Charles River and used at 6–12 wk of age. Mice were kept in an individual ventilated cage unit (BioZone). All animal experiments were performed in accordance with the local animal care commission. HRV A2 was grown on HEP-2 cells and kept at −80°C. RSV titers from lung homogenates were determined as described previously (8). Recombinant vaccinia virus (rVACV) expressing the M2-1 protein, the F protein, the N protein, or the P protein of RSV (rVACV M2-1, rVACV F, rVACV N, and rVACV P) were described (27). Vaccinia virus expressing the NP protein of LCMV (rVACV NP) was provided by Dr. Rolf Zinkernagel (Institute for Experimental Immunology, Zürich, Switzerland). All vaccinia viruses were propagated in BSC-40 cells and stored at 80°C. RSV titers from lung homogenates were determined as described previously (8). Recombinant vaccinia virus expressing the M2-1 protein, the F protein, the N protein, or the P protein of RSV (rVACV M2-1, rVACV F, rVACV N, and rVACV P) were described (27). Vaccinia virus expressing the NP protein of LCMV (rVACV NP) was provided by Dr. Rolf Zinkernagel (Institute for Experimental Immunology, Zürich, Switzerland). All vaccinia viruses were propagated in BSC-40 cells and stored at −80°C until use.

RMA-S stabilization assay

The ability of peptides to bind to and stabilize H-2Kd was measured by determining the expression of class I molecules on the surface of RMA-S-Kd cells. RMA-S-Kd cells (provided by T. J. Braciale, Beime B. Carter Center for Immunology Research, University of Virginia Health System, Charlottesville, VA) were cultured at 26°C overnight and then incubated with the indicated concentrations of peptide for 1.5 h. The cells were then incubated at 37°C for another 2.5 h, washed, stained with a mAb to H-2Kd (clone SF1-1; BD Pharmingen) and analyzed by flow cytometry.

Flow cytometry

Pulmonary cells were isolated by performing a bronchoalveolar lavage (BAL) as described (28). Surface staining was performed for 30 min at 4°C using the following Abs: CD3-allophycocyanin (clone 145-2C11), CD8-PE-Cy5 (clone 53-6.7), and/or Vj-chains-FITC (all from BD Pharmingen, San Diego). Staining for RSV-specific T cells with MHC Kd was performed by the National Institutes of Health tetramer facility, Emory University, Atlanta, GA) and used at 6–12 wk of age. Mice were kept in an individual ventilated cage unit (BioZone). All animal experiments were performed in accordance with the local animal care commission. HRV A2 was grown on HEP-2 cells and kept at −80°C. RSV titers from lung homogenates were determined as described previously (8). Recombinant vaccinia virus expressing the M2-1 protein, the F protein, the N protein, or the P protein of RSV (rVACV M2-1, rVACV F, rVACV N, and rVACV P) were described (27). Vaccinia virus expressing the NP protein of LCMV (rVACV NP) was provided by Dr. Rolf Zinkernagel (Institute for Experimental Immunology, Zürich, Switzerland). All vaccinia viruses were propagated in BSC-40 cells and stored at −80°C until use.

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induced K^4 surface expression similar to that of the wild-type peptide (Fig. 1B). To test for the ability of p82-specific CTL to recognize the mutant epitopes, BAL cells of RSV-infected mice were incubated with the various peptides and analyzed for intracellular IFN-γ production. The 8A and the 2R peptides did not elicit IFN-γ production above background, while the other peptides induced variable residual IFN-γ production (Fig. 1C). Recognition of the peptides was also analyzed in a cytotoxicity assay using target cells labeled with the various epitopes. For this, spleen cells from RSV-immune mice were restimulated for 5 days with the p82 wild-type peptide and used as effector cells in a 51Cr release assay against target cells labeled with the various mutant peptides. The results confirmed the IFN-γ data showing lack of recognition of the 2R and the 8A epitopes (Fig. 1D). Taken together, the results identified the 2R and the 8A substitutions as a “binding mutation” and a “recognition mutation,” respectively. Hence, both substitutions were chosen for introduction into the RSV genome.

**Generation of recombinant RSV with a mutation in the immunodominant CTL epitope**

To generate rRSV containing one of these two mutations, codons 249-TAT-251 (amino acid Y83) or 267-AAT-269 (amino acid N89) of the M2 gene were replaced by nucleotide triplets coding for arginine (CGA) and alanine (GCC), respectively (Fig. 2A). Transfection of the corresponding full-length antigenic cDNA plasmids should result in viruses rRSV 2R and rRSV 8A upon recovery. The parental virus of these mutants was rRSV KpnI, which contained a unique KpnI restriction site in the F-M2 intergenic region. Upon transfection in HEp-2 cells, rRSV KpnI and rRSV 8A were readily recovered and the substitution was confirmed in the viral RNA of the final virus stocks by RT-PCR amplification and sequencing. However, we were not able to recover rRSV 2R. Because reintroduction of wild-type M2 into the plasmid backbone permitted successful recovery, we conclude that the 2R mutation is deleterious for the function of the M2-1 protein (data not shown).

The in vitro replication efficiency of rRSV KpnI and rRSV 8A was compared with that of the biological parent RSV A2 in a multicycle time course in HEp-2 cells. Cell monolayers were infected in duplicate at a MOI of 0.01 PFU/cell, and cells and medium supernatants were harvested at 8 to 24 h intervals and stored for subsequent viral titration by plaque assay. As shown in Fig. 2B, the kinetics and viral yields of RSV A2, rRSV KpnI, and rRSV 8A were essentially the same, reaching titers of up to 10^7 PFU/ml by day 4.

To compare replication of the recombinant viruses in vivo, BALB/c mice were intranasally inoculated with 10^6 PFU of rRSV KpnI and rRSV 8A, respectively, and pulmonary virus titers were determined by plaque assay 4 days later. In two of four experiments, the 8A virus replicated to similar titers as those of its rRSV KpnI parent, while in two other experiments it replicated less efficiently. When the data were pooled from all 19 mice (Fig. 2C) the difference reached statistical significance, indicating slight attenuation of the 8A virus in vivo. There was no difference in the number of macrophages, granulocytes, and lymphocytes recruited to the BAL on day 3 after infection, and there were similar levels of NK cytotoxicity (data not shown). Thus, substitution of asparagine 89 of the M2-1 protein with alanine slightly reduced the replication efficiency in vivo but did not appear to affect the early immune response as evaluated by the leukocyte content of the BAL.

**The mutant virus abrogates activation and expansion of the immunodominant CTL population**

To analyze the effect of the 8A mutation on the activation of p82-specific CTL in vivo, BALB/c mice were infected with rRSV KpnI and rRSV 8A, respectively, and the CTL response in the BAL was determined on day 7 after infection. There was no difference in the absolute number of total CD8^+ T cells eluted by BAL from mice in the two experimental groups (Fig. 2D). The percentage of CTL with specificity for the p82/K^4 complex was quantified by tetramer staining. As expected, 10–30% of all CTL in the BAL of mice infected with rRSV KpnI were specific for this single epitope (Fig. 3A). In contrast, tetramer-binding CTL were not detectable in the BAL from mice infected with rRSV 8A, indicating that the 8A virus was not able to activate this dominant CTL population in vivo (Fig. 3, A and B). These data were confirmed in functional assays. Whereas 10–30% of CTL from mice infected with rRSV KpnI produced IFN-γ upon stimulation with the p82 peptide, this response was absent from mice infected with rRSV 8A (Fig. 3, C and D). Furthermore, cytotoxic activity against target cells labeled with p82 peptide was completely abolished in BAL cells from mice infected with rRSV 8A (Fig. 3E). This was not simply due to a reduced number of CTL in the BAL (Fig. 2D), and the difference remained similar when the data were analyzed for
individual mice on the basis of a CTL to target ratio (Fig. 3E, right panel). Thus, infection with rRSV 8A does not activate the p82-specific CTL population that is immunodominant after infection with rRSV KpnI.

**CTL directed against the 8A peptide do not compensate for the loss of the immunodominant CTL population**

Because the p82-8A peptide was still able to bind to and stabilize Kd (Fig. 1B), it was possible that rRSV 8A-infected mice generated a response to the p82-8A peptide that could compensate for the loss of the p82-specific CTL population. To address this question, IFN-γ production by BAL CD8+ T cells from mice infected with rRSV 8A or rRSV KpnI was analyzed after short-term stimulation with the mutant p82-8A peptide. Between 1 and 4% of CTL from both groups of mice produced IFN-γ (Fig. 3F). Also, CTL from both groups of mice were able to lyse target cells loaded with p82-8A peptide ex vivo. Lysis was slightly more efficient using BAL cells from rRSV 8A-infected mice (Fig. 3G), but this remained far below the level of lysis of p82-labeled targets by CTL from rRSV KpnI-infected mice (Fig. 3E). Thus, no significant CTL population specific for the p82-8A peptide was generated in the absence of the immunodominant CTL population.

**Loss of the immunodominant CTL population is partially compensated by CTL directed against subdominant epitopes**

The RSV-F protein contains a subdominant epitope, F p85–93 (32), and two minor epitopes, F p92–106 (33) and F p249–258 (34), which also are presented by H-2Kd. To analyze whether lack of the dominant CTL population is compensated by the CTL response to F p85, the specific IFN-γ production and cytotoxic activity were determined following infection with rRSV KpnI or rRSV 8A. After infection with either of the two viruses, ~3% of CTL produced IFN-γ in response to F p85 peptide stimulation (Fig. 4A) and there was also no difference in cytotoxic activity against target cells labeled with F p85 peptide (Fig. 4B). To enhance the sensitivity of detection, we increased the frequency of F-specific precursor CTL by priming mice with rVACV expressing the RSV-F protein followed by challenge with rRSV KpnI or rRSV 8A 3 wk later. The secondary F-specific CTL response was analyzed with respect to F p85-specific IFN-γ production and cytotoxicity. In this setting also no difference was observed between mice challenged with either virus (Fig. 4, C and D). Thus, loss of the immunodominant p82-specific CTL population is not compensated by an increase in the response to the subdominant F p85 epitope.

To identify additional subdominant CTL epitopes, we screened RSV proteins with algorithms for epitope prediction (SYFPEITHY (35) and BIMAS (36)). Among the peptides not yet characterized, the top scoring epitope was also located in the M2-1 protein (M2-1 127–135; p127). While this article was in preparation, M2-1 p127 was independently described by Crowe and colleagues who found it to be presented by H-2Kd (37). Analysis of the M2-1 p127-specific CTL-response from mice infected with rRSV KpnI or rRSV 8A, respectively, revealed a similar IFN-γ production after
primary infection (Fig. 5A). However, the cytotoxic activity of CTL from mice infected with the 8A mutant appeared somewhat increased (Fig. 5B). This difference was more pronounced after secondary infection of rVACV M2-primed mice (Fig. 5, C and D), indicating that the response to the M2-1 p127 epitope is slightly up-regulated in the absence of the immunodominant CTL population.

FIGURE 3. CTL responses against the wild-type and the mutant peptide epitope. BALB/c mice were infected intranasally with 1 × 10^6 PFU rRSV 8A or rRSV Kpn I. Seven days later, BAL cells were stained with p82 tetramer (A and B) and INF-γ production was quantified after restimulation with p82 (C and D) or p82-8A (F) peptide. INF-γ production by CD8^+ BAL cells incubated with medium was <1%. A and C. Representative FACS plots; numbers in the upper right quadrants indicate the percentage of tet^+ or INF-γ^+ cells among total CD8^+ T cells, gated on CD3^+ T cells. B and D. Pooled data from two or three independent experiments with 3–5 mice/group. E and G. Cytotoxic T cell activity was determined on p82 (E), p82-8A (G), or unlabeled control (ctrl) target cells using effector cells from rRSV Kpn I and rRSV 8A infected mice (E and G, left panel). The CTL:target ratio (CTL:T-ratio) was determined by a combination of microscopic cell counts and flow cytometry (E and G, right panels). Mean and SD from four mice per group are shown. Experiments were performed three times with 3–5 mice per group with similar results.

FIGURE 4. The subdominant F p85 epitope does not compensate for the loss of the immunodominant p82 epitope. A and B. BALB/c mice were infected with 1 × 10^6 PFU of rRSV 8AV or rRSV Kpn I. F p85-specific INF-γ production by BAL CTL was determined by flow cytometry (A). Cytotoxic T cell activity was determined on F p85-labeled or unlabeled control (ctrl) target cells (B) using effector cells from rRSV Kpn I- and rRSV 2A-infected mice. C and D. BALB/c mice were immunized with 2 × 10^6 PFU rVACV F and challenged with 1 × 10^6 PFU rRSV 8A or rRSV Kpn I 3 wk later. Seven days after challenge infection, F p85-specific INF-γ production by BAL CTL was determined by flow cytometry. C. INF-γ production by CD8^+ BAL cells incubated with medium was <1%. D. Cytotoxic T cell activity was determined on F p85 or unlabeled control (ctrl) target cells using effector cells from rRSV Kpn I- and rRSV 8A-infected mice. Mean and SD from four mice per group are shown. Experiments were performed twice with 3–5 mice/group.

FIGURE 5. The subdominant p127 epitope partially compensates for the loss of the immunodominant p82 epitope. The experiments were performed as described in Fig. 4 using p127 for restimulation of BAL cells and for labeling of target cells. The experiments were performed twice with similar results with 3–5 mice/group.
Minor CTL responses have also been demonstrated against the N and the P protein (38), but the exact epitopes have not been identified. To detect possible changes in the CTL response to these undefined epitopes, we primed mice with rVACV encoding N or P. These mice generate N- or P-specific CTL, which are amplified upon subsequent RSV infection and then should contribute to the restriction of RSV replication. In the case of increased activation of N- or P-specific CTL following challenge with the 8A mutant, replication of rRSV 8A should be reduced compared with rRSV KpnI. Mice primed with rVACV expressing the RSV-M2 or the LCMV-NP proteins were used as positive and negative control, respectively.

In these experiments, priming with rVACV LCMV-NP did not confer any protection from the replication of rRSV 8A and rRSV KpnI, whereas virus was rapidly eliminated in mice primed with rVACV M2 (Fig. 6). This demonstrated that the response to p127 (and potential other epitopes) was sufficient to completely restrict RSV replication even in the absence of reactivated p82-specific CTL. Mice primed with rVACV N were only partially protected from RSV replication, whereas priming with rVACV P did not provide any protection. Replication of rRSV 8A was even slightly higher than of rRSV KpnI (Fig. 6) suggesting that the N-specific
CTL response was not significantly enhanced in the absence of the immunodominant CTL epitope.

**Lack of the dominant epitope leads to a reduction of the overall RSV-specific CTL response**

The experiments described above analyzed the CTL response to single peptides but did not provide information on whether the overall CTL response to RSV was compromised in the absence of the immunodominant CTL population. To address this question, we determined the response of CTL from RSV-infected mice to stimulation with RSV-infected target cells in vitro. Infected cells should present the full spectrum of viral epitopes, including any unknown T cell epitopes, and should present these in physiologically relevant concentrations. BAL cells from BALB/c mice that had been infected with rRSV KpnI or rRSV 8A 7 days previously were stimulated with RAW 264.7 macrophage-like cells infected with either of the two recombinant viruses, and the percentage of CTL producing IFN-γ was determined. Approximately 25% of CTL from the BAL of mice infected with rRSV KpnI produced intracellular IFN-γ in response to stimulation with rRSV KpnI-infected cells, while only 15% of CTL produced IFN-γ if the mice and stimulators had been infected with rRSV 8A (Fig. 7, A and B). Thus, substitution of a single amino acid in the immunodominant p82 epitope significantly reduced the overall RSV-specific CTL response to RSV, confirming and extending the data obtained in the peptide experiments. Notably, CTL from mice that had been infected with either recombinant virus and restimulated with the other recombinant virus showed a slightly lower response compared with CTL from mice infected and restimulated with rRSV 8A. This may indicate a small response specifically directed against the 8A mutant.

To exclude the possibility that the observed differences in the percentage of RSV-specific CTL after infection with the two viruses were the result of a delayed kinetic of CTL activation, we analyzed mice 6, 7, and 8 days after infection. We found a similar kinetic of the virus-specific T cell response following infection with either of the two viruses (Fig. 7C). The overall RSV-specific CTL response against rRSV 8A remained 70–85% of that against rRSV KpnI. We did not perform additional experiments extending beyond day 8 after infection because days 7 and 8 were the time points of virus elimination and maximal disease, the two relevant biological end points of our study (see below).

**Loss of the dominant CTL population reverses the bias in the TCR repertoire**

To analyze the diversity of pulmonary CTL in response to RSV infection, we determined the V β-chain usage among CTL from BAL of RSV-infected mice and compared it to the V β-chain usage of CTL obtained from the spleens of naive mice. Seven days after infection with rRSV KpnI, the V β-chain usage among pulmonary CTL was highly biased toward the use of Vβ8.1/8.2 chains. The proportion of CTL that used one of these two chains was ~30% increased compared with the CTL from spleens of naive BALB/c mice (Fig. 8A). There was significantly less change (<5%) in the frequency of CTL using other chains, indicating an oligoclonal pulmonary CTL response to RSV infection in BALB/c mice. Analysis of V β-chain usage among p82 tetramer-binding CTL revealed the use of Vβ8.1/8.2 chains in almost 80% of these cells, indicating that this immunodominant CTL population significantly contributed to the skewed V β repertoire (Fig. 8B). After infection with rRSV 8A, the V β skewing was completely reversed. Moreover, no significant increase in the use of any other V β-chain was detected (Fig. 8C). These data further confirm the previous observation that loss of the immunodominant CTL population is not compensated by a CTL population of different epitope specificity.

**The immunodominant CTL population significantly contributes to immunopathology but is largely dispensable for virus clearance**

The biological consequences of the T cell epitope mutation were further evaluated by monitoring weight loss as a parameter for RSV-induced pathology. Following infection with rRSV KpnI, mice significantly lost weight from day 5 to day 7 that paralleled...
FIGURE 9. Reduced weight loss but little delay in virus clearance after infection with the mutant virus was found. BALB/c mice were infected intranasally with 1 × 10⁶ PFU of rRSV Kpn I or rRSV 8A. A, Weight was monitored for 7 days. The data show mean and SD from 48 mice per group obtained in nine independent experiments. *, p < 0.05. B, Mice were sacrificed on days 6, 7, and 8 and RSV titers in the lungs were determined. Data were pooled from two independent experiments with 3–4 mice per group and time point. C and D, Groups of four mice were depleted of CD8+ T cells (days −1 and day 0) and were infected with 1 × 10⁶ PFU of rRSV Kpn I. Mice were weighed daily (C) and pulmonary RSV titers were determined on day 7 after infection.

The consequences of T cell epitope mutations for virus-specific T cell responses have been previously addressed in mouse models of infection with LCMV (15–17, 39), influenza (13, 18–20), and HSV (21). A comparison of these data to the results obtained in our study reveals that the consequences of a mutation in the major CTL epitope for the virus-specific CTL response are variable and not easy to predict (Table I). Despite the loss of the immunodominant CTL population, the overall CTL response was fully compensated after infection with mutant HSV (21) or influenza viruses (18, 20), but not with LCMV (15, 17). It was speculated that this could be due to the fact that the former are localized infections in contrast to the systemic infection with LCMV (21). This hypothesis does not hold, as the same LCMV infection has opposite effects in different mice strains (Table I). In addition, our observation of a significant decrease in the overall CTL response after localized infection with the RSV epitope mutant argues against this possibility. Another explanation could be that the ability to compensate may be related to the size of the overall virus-specific CTL response. After infection with HSV or influenza, 5–15% of all CD8+ T cells are virus-specific, whereas RSV induces a stronger response (25–30% of total BAL CTL are RSV-specific) and up to 30% of splenic CTL recognize viral epitopes during LCMV infection. Because the proportion of virus-specific CTL directed against the immunodominant epitope is comparable in all of these infections (~60–80%), this means that 2–3-fold more CTL with specificity for subdominant epitopes have to be generated for a compensatory response after RSV or LCMV infection compared with HSV or influenza virus infection.
addition, host variables could contribute to the observed differences. Although the loss of the immunodominant CTL population was compensated in all three infection models involving C57BL/6 mice, there was no overall compensation in the two viral infection models using BALB/c mice (Table I). These strain-specific differences could be related to differences in the spectrum and diversity of the overall CTL repertoire.

In none of the models could the overall ability to compensate for the loss of the immunodominant CTL population be easily predicted by the analysis of responses to the mutant neo-epitope or to defined subdominant epitopes. In the influenza model, it was shown that epitope mutations can lead to significant changes in the structure of the mutant peptide MHC-complex (pMHC) compared with the wild-type pMHC (19, 40). This complex may not be recognized with sufficient avidity by T cells available in the repertoire. Neither in the present study nor in the other viral models did the absence of the immunodominant CTL population lead to increases in T cell responses directed against defined subdominant epitopes to provide a significant level of overall compensation (15, 16, 18, 20, 21). We observed no increase in the response to a subdominant epitope in the F protein and a minor increase in response to the M2-1 p127 epitope that was clearly detectable only after secondary infection. Because this epitope is derived from the same protein as the immunodominant RSV epitope, this may represent an example of intramolecular competition between epitopes at the level of the T cell response. This does not appear to be a general rule, however, because intramolecular competition was not observed in the LCMV or Listeria models (41). Although we failed to detect compensatory responses against several epitopes in all RSV proteins that have previously been shown to elicit CTL responses (38), loss of 80% of the virus-specific CTL only led to a 20–30% loss in overall RSV-specific CTL activity. This suggests some increase in the response to yet undefined epitopes or minor epitopes not investigated in this study such as F p92–106 (33) or F p249–258 (34). However, this does not occur to an extent allowing full compensation of the total response such as that observed in the HSV (20, 21) and influenza models (20).

Lack of the immunodominant CTL population not only diminished the overall CTL response to RSV but also had a significant impact on the T cell repertoire. Although rRSV Kpnl infection induced a large predominance of Vβ 8.1/8.2-expressing T cells, no dominating Vβ population was detected after infection with the 8A virus. This is in contrast to influenza infection, where prominent usage of Vβ 7 chains after wild-type infection was replaced by usage of Vβ 4 chains directed against the mutant virus (19). This difference may reflect a limited plasticity of the RSV-specific T cell repertoire of BALB/c mice, which is not able to compensate for the loss of the immunodominant CTL population as efficiently as can the influenza-specific T cell repertoire of C57BL/6 mice.

An important question is whether changes in the strength and composition of the antiviral T cell response have an influence on virus control and disease. Elimination of the rRSV 8A mutant was only minimally delayed, demonstrating that the immunodominant CTL population was largely dispensable for virus elimination. Virus elimination was more delayed in the LCMV system (16), while no impairment in virus control was observed after infection with an HSV-1 mutant (21). More significantly, in contrast to mice infected with rRSV Kpnl, mice infected with the 8A mutant did not experience any weight loss. This confirms that CD8+ T cells not only contribute to virus clearance but also mediate immunopathology in the RSV mouse model (6, 8, 30). Our observation of reduced pathology after infection with the mutant virus is in interesting contrast to the influenza model, where infection with the CTL epitope mutant led to enhanced disease (18). This could reflect enhanced viral cytopathogenicity due to a different kinetic in virus control. Thus, a single viral epitope mutation leading to a failure to activate the immunodominant CTL population can have significant but variable consequences for the host. It can cause enhanced disease in situations where viral cytopathogenicity dominates disease but may also attenuate disease if there is a significant component of CTL-mediated immunopathology.

Are there implications of our results for human RSV disease? A recent study has generally questioned the role of CTL in the pathogenesis of human RSV infection (42). Analysis of lung tissue from human infants with lethal RSV infection revealed very few CD8+ T cells in nasal infiltrates. Combined with the observation that there was little evidence for T cell cytokines in nasal secretions (42) and previous data that only few CTL can be recovered by BAL from infants with bronchiolitis (42), it was concluded that in humans T cells play a minor role in virus elimination and immunopathology. Instead, it was postulated that virus elimination is mainly mediated by neutrophils and macrophages and that RSV-induced pathology is largely due to viral cytopathogenicity (42). This interpretation neglects evidence from infants with primary immunodeficiencies. Although particularly severe or prolonged RSV infections have not been reported in infants with congenital deficiencies in neutrophils or macrophage function, it is well known that children with T cell deficiencies are highly susceptible (4, 5). These infants may shed high titers of virus for weeks, frequently with moderate disease. Interestingly, T cell reconstitution of these infants after bone marrow transplantation often severely aggravates lung disease (Ref. 7 and our unpublished observations) that sometimes responds to aggressive immunosuppressive therapy. These observations argue strongly for a role for T cells in controlling viral replication but also for contributing to disease during RSV infection of human infants. We therefore think that our findings illustrate a concept that is also valid in humans.

In summary, our data illustrate the delicate balance between the viral and host parameters that together determine the outcome of a viral infection. A single amino acid change in a viral T cell epitope can influence the size and composition of the CTL response to an extent that can have a significant impact on the clinical course of the disease.

Table I. Summary of experiments using viruses and bacteria with mutations in the immunodominant CTL epitope sequence

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Mouse Strain</th>
<th>CTL Epitope</th>
<th>Percentage of Total CTL (%)</th>
<th>Percentage of Virus-specific CTL (%)</th>
<th>Epitope Mutant</th>
<th>Effect on Overall CTL Response</th>
<th>Compensation by Other Epitopes</th>
<th>Virus Control</th>
<th>Disease</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCMV</td>
<td>C57BL/6</td>
<td>Gp33</td>
<td>30–50</td>
<td>60</td>
<td>V35L</td>
<td>Not reduced</td>
<td>Partial</td>
<td>Not Impaired</td>
<td>Not reduced</td>
<td>39</td>
</tr>
<tr>
<td>HSV</td>
<td>BALB/c</td>
<td>Np118</td>
<td>40–50</td>
<td>70</td>
<td>Q120R</td>
<td>Reduced</td>
<td>Partial</td>
<td>Impaired</td>
<td>Not reduced</td>
<td>15</td>
</tr>
<tr>
<td>Influenza</td>
<td>C57BL/6</td>
<td>PA224</td>
<td>10–15</td>
<td>70</td>
<td>R230A</td>
<td>Reduced 5–10%</td>
<td>None</td>
<td>Not impaired</td>
<td>Not reduced</td>
<td>21</td>
</tr>
<tr>
<td>Listeria</td>
<td>BALB/c</td>
<td>LLO91</td>
<td>17</td>
<td>70</td>
<td>Y92S</td>
<td>Reduced 30%</td>
<td>None</td>
<td>Mildly impaired</td>
<td>Not reduced</td>
<td>41</td>
</tr>
<tr>
<td>RSV</td>
<td>BALB/c</td>
<td>M2-1 82–90</td>
<td>10–30</td>
<td>80</td>
<td>N89A</td>
<td>Not reported</td>
<td>None</td>
<td>Not Impaired</td>
<td>Attenuated</td>
<td></td>
</tr>
</tbody>
</table>

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By usage of V/H9252 dominating V/H9252 general rule, however, because intramolecular competition was not present an example of intramolecular competition between epitopes same protein as the immunodominant RSV epitope, this may represent an example of intramolecular competition between epitopes at the level of the T cell response. This does not appear to be a general rule, however, because intramolecular competition was not observed in the LCMV or Listeria models (41). Although we failed to detect compensatory responses against several epitopes in all RSV proteins that have previously been shown to elicit CTL responses (38), loss of 80% of the virus-specific CTL only led to a 20–30% loss in overall RSV-specific CTL activity. This suggests some increase in the response to yet undefined epitopes or minor epitopes not investigated in this study such as F p92–106 (33) or F p249–258 (34). However, this does not occur to an extent allowing full compensation of the total response such as that observed in the HSV (20, 21) and influenza models (20).

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Disclosures

The authors have no financial conflict of interest.

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