

Complement Component 3 Is Required for Optimal Expansion of CD8 T Cells During a Systemic Viral Infection¹

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In addition to its established role in innate immune mechanisms, complement component C3 is also of critical importance in B cell activation and T cell-dependent Ab responses. In this study, we have examined the requirement for C3 in the generation of primary CD8 T cell responses to an acute systemic viral infection. We compared Ag-specific CD8 T cell responses to lymphocytic choriomeningitis virus (LCMV) between wild-type (+/+) and C3-deficient (C3^{-/-}) mice on both 129/B6 and B6 backgrounds. These studies revealed that C3 activity is required for optimal expansion of LCMV-specific effector CD8 T cells in an epitope-dependent fashion, which is influenced by the genetic background of the mice. Studies in complement receptor 1/2 (CR1/CR2)-deficient mice showed that regulation of LCMV-specific CD8 T cell responses by C3 is not dependent upon CR1/CR2. These findings may have implications in vaccine development, therapy of autoimmune diseases, and prevention of graft rejection. *The Journal of Immunology*, 2003, 170: 788–794.

Complement proteins form an integral component of the innate immune system. The complement system comprises more than 30 proteins that interact in a cascade of enzymatic reactions, culminating in the generation and release of proinflammatory peptides, opsonization, and formation of the membrane attack complex (1–3). Complement component 3 is of vital importance in the cascade of complement activation, and it has been known for a long time that C3 deficiencies in humans and animals render them highly susceptible to several bacterial infections (4, 5). In addition, complement proteins may play a protective role in viral infections (6–8). Although the primary source of C3 is the liver, cells like macrophages, endothelial cells, and keratinocytes can synthesize C3 (9). Upon activation, C3 undergoes cleavage to generate C3a, C3b, iC3b, and C3d, which have potent biological activities (4). These C3 cleavage products modulate Ab responses via complement receptors (CR)³ 1 (CR1 or CD35) and 2 (CR2 or CD21), which are expressed on B cells, follicular dendritic cells, and some T cells (9–13). Coligation of the B cell Ag receptor with the CD21/CD19 receptor complex reduces the activation threshold of B cells (12, 13). Attachment of C3-Ag complexes to CRs promotes Ag uptake and processing by B cells and macrophages (14, 15). Further, Ag retention by follicular dendritic

cells via CRs is important in the generation and maintenance of memory B cells (9).

In recent times, there has been a surge in studying the role of complement proteins in regulating T cell responses. Recent reports have provided strong evidence that C3 might be important in the elicitation of T cell responses during autoimmune myocarditis, influenza infection, and acute renal graft rejection in mice (16–18). However, the role of C3 in the activation and expansion of T cells during a systemic infection has not been examined. To address this issue, we determined the role of C3 in the generation of Ag-specific CD8 T cell responses following an acute infection with lymphocytic choriomeningitis virus (LCMV) in wild-type (+/+), C3-deficient (C3^{-/-}), and CR1/CR2-deficient (CR1/CR2^{-/-}) mice. Our studies provide strong evidence that C3 promotes CD8 T cell responses in an epitope-specific manner, which is influenced by the genetic background of mice.

Materials and Methods

Mice

The C3^{-/-} mice on the 129/C57BL6 background (C3^{-/-} 129/B6) were kindly provided by Dr. H. R. Colten (Washington University, St. Louis, MO) (19). The control wild-type 129/B6 mice (+/+ 129/B6) were backcrossed similar to the C3^{-/-} 129/B6 mice. The C3^{-/-} mice on the C57BL/6 (B6) background were generated by backcrossing the C3^{-/-} 129/B6 to the C57BL/6 (10 generations). The generation and characterization of CR1/CR2^{-/-} mice has been described previously (20). Wild-type B6 mice were purchased from the National Cancer Institute (Bethesda, MD). All animal experiments were performed as per institutional animal care guidelines.

Virus

Mice were infected with 2×10^5 PFU of the Armstrong strain of LCMV (LCMV-Arm) by i.p. injection (21). Infectious LCMV in the tissues was quantitated by a plaque assay using Vero cell monolayers as described elsewhere (21).

Cytotoxicity assay

The MHC class I-restricted cytotoxic activity in the spleen was measured by a ⁵¹Cr-release assay using MC57G target cells as described previously (21, 22).

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³ Abbreviations used in this paper: CR, complement receptor; LCMV, lymphocytic choriomeningitis virus; NP, nucleoprotein; PI, postinfection.

Intracellular cytokine staining

The number of LCMV-specific cytokine-producing CD8 T cells in the spleen was quantitated by staining for intracellular IFN- γ as described previously (22). Briefly, splenocytes were cultured for 5 h with or without the LCMV CTL epitope peptides in the presence of brefeldin A. After culture, cells were stained for cell surface CD8 and intracellular IFN- γ using the Cytofix/Cytoperm kit (BD PharMingen, La Jolla, CA). The number of IFN- γ -producing CD8 T cells was determined by flow cytometry using a FACScan or FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). The flow cytometry data were analyzed using CellQuest software (BD Biosciences).

Quantitation of LCMV-specific CD8 T cells using MHC I tetramers

The production and use of MHC I tetramers (D^b) specific to the two immunodominant epitopes present in the LCMV nucleoprotein (NP396-404) and glycoprotein (gp33-41) have been described elsewhere (22). Single cell suspensions of splenocytes were stained with allophycocyanin-labeled MHC I tetramers, PE-labeled anti-CD8 Abs, and FITC-labeled anti-CD44 Abs at 4°C for 1 h. After staining, cells were fixed in 2% paraformaldehyde and samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences). All Abs were purchased from BD PharMingen.

Flow cytometry

To examine the expression of complement receptors on LCMV-specific CD8 T cells, single cell suspensions of spleen were costained with MHC class I tetramers (specific to nucleoprotein (NP) 396-404) and FITC-labeled anti-CR1/2 (7G6, 7E9), anti-CR3 (Mac-1), or anti-C5aR Abs and analyzed by flow cytometry. To determine the expression of MHC I (D^b) on macrophages and B lymphocytes, splenocytes were stained with anti-CD8 (BD PharMingen), anti-Mac-1 (BD PharMingen), anti-CD19 (Caltag Laboratories, Burlingame, CA), and anti- D^b (BD PharMingen) Abs. Macrophages and B cells were identified based on being Mac-1-positive CD8-negative and CD19-positive, respectively.

Statistical analysis

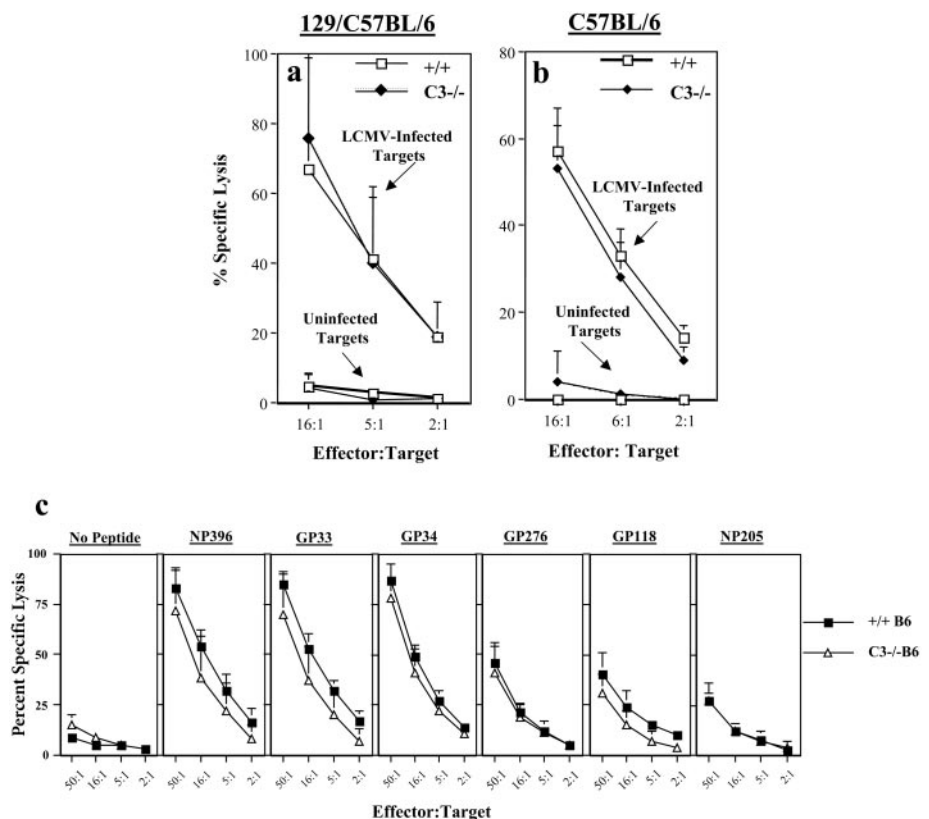
Data were analyzed using the commercially available statistical software (Systat version 8.0, Chicago, IL). Groups were compared by the Student's *t* test and significance was defined at $p \leq 0.05$.

FIGURE 1. MHC class I-restricted cytotoxic T cell response in C3-deficient mice. *a*, Groups of $+/+$ 129/B6 and $C3^{-/-}$ 129/B6 mice were infected with LCMV-Arm. On day 8 postinfection, virus-specific cytotoxic activity in the spleens was measured directly ex vivo by a ^{51}Cr -release assay using syngeneic LCMV-infected and uninfected MC57G cells as target cells. *b*, Eight days after LCMV-Arm infection, cytotoxic activity in the spleens of $+/+$ B6 and $C3^{-/-}$ B6 mice was measured as described for *a*. *c*, On day 8 postinfection with LCMV-Arm, the splenocytes from $+/+$ B6 and $C3^{-/-}$ B6 mice were tested for cytotoxic activity directly ex vivo using ^{51}Cr -labeled MC57G cells pulsed with the indicated LCMV epitope peptides (NP396, gp33, gp34, gp276, gp118, and NP205) as target cells. Data in *a-c* are the mean of four mice per group.

Results

Primary CD8 T cell responses in C3-deficient mice

C3 levels and/or activity can vary between inbred strains of mice (23-25). Our studies have also shown that serum levels of C3 in 129/B6 mice are significantly lower than in B6 mice (data not shown). Because the regulatory role of C3 on T cell responses may depend upon the genetic background of the mice, we compared Ag-specific CD8 T cell responses to LCMV infection between wild-type ($+/+$) and $C3^{-/-}$ mice on both 129/B6 and B6 backgrounds. It is worth mentioning that the MHC haplotype of both 129/B6 and B6 mice is H-2^b. Groups of $+/+$ 129/B6, $C3^{-/-}$ 129/B6, $+/+$ B6, and $C3^{-/-}$ B6 mice were infected with LCMV and on day 8 postinfection (PI), we measured MHC class I-restricted cytotoxicity in the spleens directly ex vivo. As shown in Fig. 1*a*, the spleens of LCMV-infected $+/+$ 129/B6 exhibited potent cytotoxic activity on virus-infected targets. The cytotoxic activity in the spleens of $C3^{-/-}$ 129/B6 mice was comparable to that of $+/+$ 129/B6 mice. Similarly, the LCMV-specific cytotoxic activities in the spleens of $+/+$ B6 and $C3^{-/-}$ B6 mice were alike (Fig. 1*b*). The CTL assays performed on LCMV-infected targets did not reveal any epitope-specific differences in the cytotoxic activity of CD8 T cells between $+/+$ and $C3^{-/-}$ mice. To address this issue, groups of $+/+$ B6 and $C3^{-/-}$ B6 mice were infected with LCMV-Arm, and MHC I-restricted cytotoxic activity against various LCMV CTL epitopes was quantitated using peptide-pulsed target cells (Fig. 1*c*). As shown in Fig. 1*c*, splenocytes from $+/+$ B6 mice exhibited specific cytotoxic activity against multiple epitopes, and the relative levels of cytotoxicity clearly demonstrate the immunodominance hierarchy of the anti-LCMV CTL response in C57BL/6 mice (22). Fig. 1*c* also shows that the splenocytes from LCMV-infected $C3^{-/-}$ B6 mice showed potent killing against all the LCMV CTL epitopes in a manner consistent with

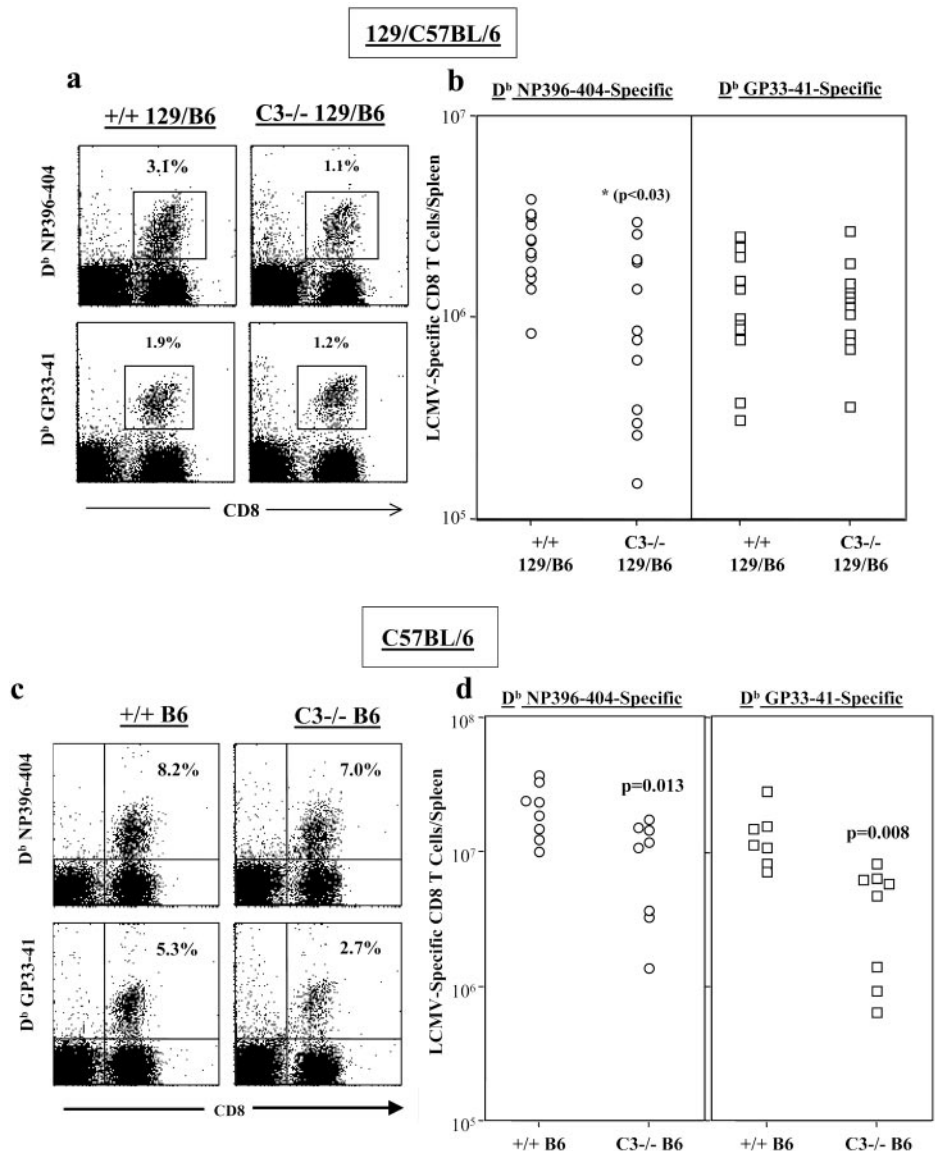


the epitope hierarchy of the LCMV-specific CTL response. However, splenocytes from LCMV-infected $C3^{-/-}$ B6 mice showed a clear tendency of lower cytotoxic activity (<2-fold) against most of the epitopes, as compared with $+/+$ B6 mice (Fig. 1c). Studies to compare CTL activity against several LCMV epitopes between $+/+$ 129/B6 and $C3^{-/-}$ B6 mice yielded similar results (data not shown). Taken together, these data show that C3 deficiency did not significantly ($p < 0.05$) affect the generation of MHC class I-restricted cell-mediated cytotoxic activity during an acute LCMV infection.

Next, we examined the role of C3 in the expansion of CD8 T cells specific to two immunodominant epitopes, NP396–404 and gp33–41, presented by D^b . LCMV-specific CD8 T cells were visualized by staining splenocytes with MHC I tetramers (D^b) complexed with NP396–404 or gp33–41 peptides. As illustrated in Fig. 2a, LCMV-specific CD8 T cells were readily detected in the spleens of both $+/+$ 129/B6 and $C3^{-/-}$ 129/B6 mice. However, data in Fig. 2a show that the relative proportion of NP396–404-specific CD8 T cells in the spleens of $C3^{-/-}$ 129/B6 mice was substantially lower, in comparison to $+/+$ 129/B6 mice. Interestingly, the percentages of gp33–41-specific CD8 T cells in the spleen were comparable between $+/+$ 129/B6 and $C3^{-/-}$ 129/B6

mice. The absolute numbers of NP396–404- and gp33–41-specific CD8 T cells in the spleens of $+/+$ 129/B6 and $C3^{-/-}$ 129/B6 mice are shown in Fig. 2b. The spleens of $+/+$ 129/B6 mice contained $2.4 \pm 0.8 \times 10^6$ NP396–404-specific CD8 T cells. The number of NP396–404-specific CD8 T cells in the spleens of $C3^{-/-}$ 129/B6 mice was 2–16-fold lower, as compared with $+/+$ 129/B6 mice (Fig. 2b). The total number of gp33–41-specific CD8 T cells in the spleens of $C3^{-/-}$ 129/B6 mice was similar to those of $+/+$ 129/B6 mice. Data in Fig. 2c illustrate the effect of C3 deficiency on the expansion of LCMV-specific CD8 T cells in mice on the B6 background. As shown in Fig. 2c, the frequencies of NP396–404-specific CD8 T cells in the spleens of $C3^{-/-}$ B6 mice were slightly lower than in $+/+$ B6 mice. However, the percentages of gp33–41-specific CD8 T cells in $C3^{-/-}$ mice were reduced by ~50%, as compared with $+/+$ B6 mice. Comparison of the total number of LCMV-specific CD8 T cells in the spleens between $+/+$ B6 and $C3^{-/-}$ B6 mice revealed that C3 deficiency reduced the expansion of NP396–404-specific CD8 T cells by 2-fold (Fig. 2d). C3 deficiency had a more pronounced effect on the expansion of gp33–41-specific CD8 T cells in B6 mice; the spleens of $C3^{-/-}$ B6 mice contained an ~4-fold lower number of gp33–41-specific CD8 T cells, as compared with those of $+/+$ B6

FIGURE 2. Expansion of LCMV-specific CD8 T cells in C3-deficient mice. *a* and *b*, On the eighth day after infection with LCMV-Arm, virus-specific CD8 T cells in the spleens of $+/+$ 129/B6 and $C3^{-/-}$ 129/B6 mice were quantitated by staining with anti-CD8 Abs and MHC I tetramers (D^b). The numbers in *a* denote percentages of LCMV-specific CD8 T cells among splenocytes. Each data point in *b* represents the number of LCMV-specific CD8 T cells of an individual mouse. *c* and *d*, On day 8 postinfection, the number of LCMV-specific CD8 T cells in the spleens of $+/+$ B6 and $C3^{-/-}$ B6 mice was quantitated by staining with anti-CD8 Abs and MHC I tetramers. The numbers in *c* represent percentages of LCMV-specific CD8 T cells of splenocytes. Each data point in *d* represents the number of LCMV-specific CD8 T cells in an individual mouse. The flow cytometry plots in *a* and *c* are gated on total viable splenocytes based on forward and side scatter properties.



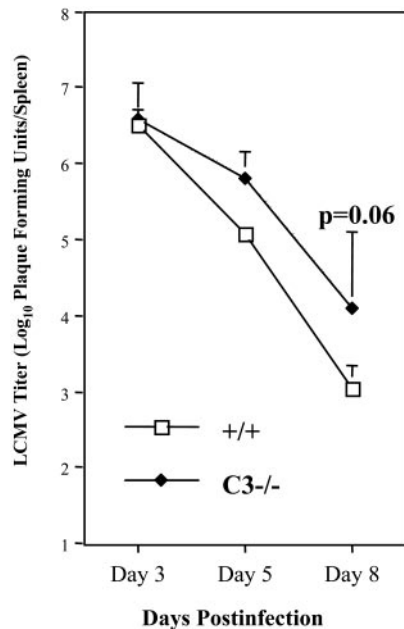


FIGURE 3. Clearance of LCMV in C3-deficient mice. Groups of +/+ 129/B6 and C3^{-/-} 129/B6 mice were infected with LCMV-Arm, and levels of infectious LCMV in the spleens were determined by plaque assay on Vero cells. The data are the means of three to four mice per group at each time point.

mice (Fig. 2*d*). This reflects a ~75% reduction in the expansion of gp33–41-specific CD8 T cells in C3^{-/-} B6 mice, as compared with +/+ B6 mice. These data show that in C57BL/6 mice, the expansion of gp33–41-specific CD8 T cells seem to be more C3-dependent, as compared with NP396–404-specific CD8 T cells. This is in striking contrast to 129/B6 mice, where C3 deficiency resulted in selective diminution in the expansion of NP396–404-specific CD8 T cells (Fig. 2, *a* and *b*). In summary, these data suggest that C3 1) promotes expansion of CD8 T cells and 2) may regulate epitope selection during T cell responses.

Resolution of an LCMV infection is critically dependent upon the function of CD8 T cells (26, 27). The relative importance of the CD8 T cell responses to NP396–404 and gp33–41 epitopes in the clearance of an acute infection with LCMV-Arm is not known. However, a chronic infection with a highly virulent strain of LCMV (LCMV-clone 13) is associated with the deletion of NP396–404-specific CD8 T cells in mice (28). It was of interest to determine whether reduced CD8 T cell responses in C3^{-/-} mice affected the clearance of LCMV. We quantitated infectious LCMV in the spleens of +/+ 129/B6 and C3^{-/-} 129/B6 mice on various days PI. As shown in Fig. 3, LCMV replicated to high levels in the spleens of both +/+ 129/B6 and C3^{-/-} 129/B6 mice. Consistent with lower CD8 T cell responses, C3^{-/-} 129/B6 mice showed a delay in resolving an acute LCMV infection; on day 8 PI, the viral

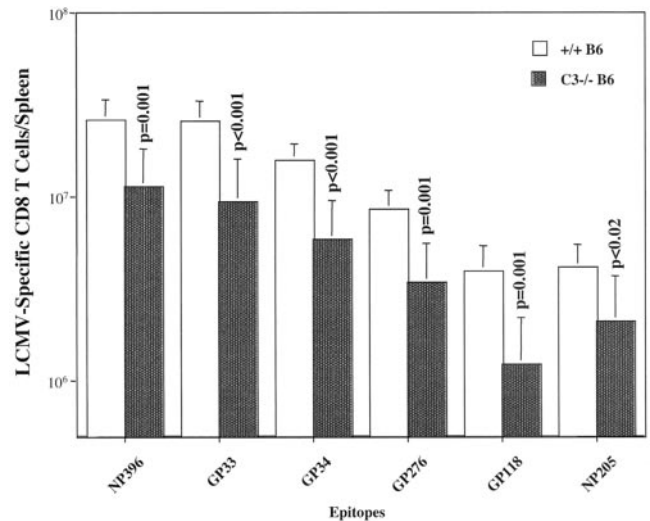


FIGURE 4. Effect of C3 deficiency on CD8 T cell responses to dominant and subdominant epitopes. Eight days following infection with LCMV-Arm, splenocytes from +/+ B6 and C3^{-/-} B6 mice were stimulated with LCMV CTL epitope peptides *in vitro* for 5 h. Following *in vitro* stimulation, the number of CD8 T cells specific to various LCMV CTL epitopes was determined by staining for intracellular IFN- γ . Data are the means of eight mice per group.

load in the spleens of C3^{-/-} 129/B6 mice was ~8- to 10-fold higher, as compared with +/+ 129/B6 mice (Fig. 3). However, in mice on the B6 background, LCMV clearance was minimally affected by C3 deficiency; on day 8 PI, extremely low levels of LCMV were detected in the spleens of both +/+ B6 and C3^{-/-} B6 (data not shown).

A previous study has shown that complement activity is required for T cells to produce IFN- γ and elicit contact sensitivity in mice (29, 30). We next used intracellular cytokine staining to determine whether C3 deficiency affected the: 1) CD8 T cell responses to subdominant epitopes and 2) ability of CD8 T cells to produce IFN- γ . As shown in Table I and Fig. 4, the anti-LCMV CD8 T cell response in the +/+ B6 mice exhibited a distinct epitope hierarchy. This CD8 T cell epitope hierarchy was not affected by C3 deficiency. However, the frequencies of LCMV-specific CD8 T cells in C3^{-/-} B6 mice were substantially reduced, as compared with +/+ B6 mice. The differences were more dramatic when absolute numbers of LCMV-specific CD8 T cells were compared between LCMV-infected +/+ B6 and C3^{-/-} B6 mice (Fig. 4). The total number CD8 T cells specific to the epitopes in the viral glycoprotein was significantly reduced in C3^{-/-} B6 mice, as compared with +/+ B6 mice; CD8 T cell responses to the epitopes in the viral NP tended to be less affected by C3 deficiency (Fig. 4). In comparison to +/+ B6 mice, the expansion of CD8 T cells specific to the epitopes in the viral NP and glycoprotein was reduced by ~50% and ~66%, respectively, in the C3^{-/-} B6 mice.

Table I. Effect of C3 deficiency on the frequencies of LCMV-specific CD8 T cells in the spleen^a

Groups	LCMV CTL Epitopes					
	NP396	gp33	gp34	gp276	gp118	NP205
+/+ B6	11.2 ± 2.3	11.2 ± 2.7	6.9 ± 1.6	3.6 ± 0.4	1.7 ± 0.8	1.8 ± 0.5
C3 ^{-/-} B6	8.0 ± 3.6	6.6 ± 4.3	4.1 ± 2.2	2.4 ± 1.4	0.8 ± 0.7	1.5 ± 1.1

Groups of +/+ and C3^{-/-} mice on the C57BL/6 background were infected with LCMV-Arm. Eight days following infection, the frequencies of CD8 T cells specific to the indicated LCMV CTL epitopes were determined by intracellular cytokine staining as described in *Materials and Methods*. The numbers in the table represent percentages of epitope-specific CD8 T cells of total splenocytes ± SD. The data are the mean of eight mice per group.

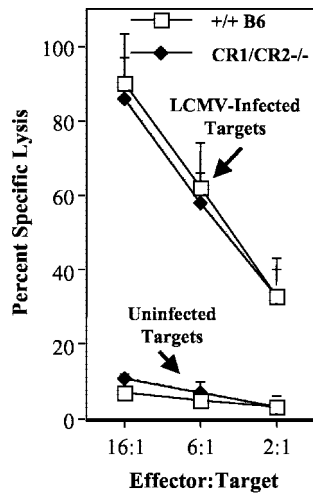


FIGURE 5. LCMV-specific MHC class I-restricted cytotoxic activity in CR1/CR2-deficient mice. Groups of +/+ B6 and CR1/CR2^{-/-} mice were infected with LCMV-Arm. Eight days after infection, the cytotoxic activity in the spleens was determined directly ex vivo by ⁵¹Cr-release assay using LCMV-infected and uninfected MC57G cells as target cells. Data are the mean of five mice per group.

It is worth pointing out that the gp33–41 peptide is presented by both D^b and K^b MHC I molecules. Although both D^b- and K^b-restricted gp33–41-specific CD8 T cells are detected by intracellular cytokine staining, only D^b-restricted gp33–41-specific CD8 T cells bind MHC class I tetramers (31). This explains why intracellular cytokine data for gp33–41-specific CD8 T cells did not exactly mirror the numbers obtained by MHC I tetramer staining (as compared with +/+ mice, C3-deficient mice had 4-fold and 3-fold fewer gp33–41-specific CD8 T cells as measured by MHC I tetramers and intracellular cytokine staining, respectively; Figs. 2d and 4). Intracellular cytokine staining was also used to compare LCMV-specific CD8 T cell responses to multiple epitopes between +/+ 129/B6 and C3^{-/-} 129/B6 mice. These studies showed that NP396–404-specific CD8 T cell responses were selectively inhibited in C3^{-/-} 129/B6 mice as compared with +/+ 129/B6 mice (data not shown).

Primary T cell responses in CR1/CR2-deficient mice

Active cleavage products of C3 modulate lymphocyte function via binding to CR1 and 2 (9). It has been shown previously that CR1 and CR2 are expressed on T cells and may be involved in the

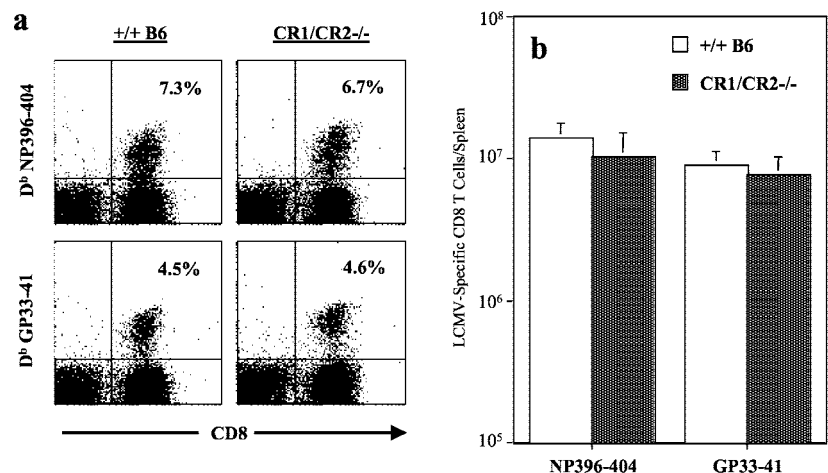
genesis of T cell-dependent autoimmune myocarditis in mice (16). Data presented in Figs. 2 and 4 showed that C3 activity might be important for optimal proliferation of LCMV-specific CD8 T cells. We determined whether C3 regulates LCMV-specific CD8 T cell responses via CR1 and CR2. To address this issue, we compared the generation of LCMV-specific CD8 T cell responses between +/+ B6 and CR1/CR2^{-/-} mice. Eight days following LCMV infection, we assessed MHC class I-restricted cytotoxic activity in the spleens of +/+ B6 and CR1/CR2^{-/-} mice. As shown in Fig. 5, the cytotoxic activity in the spleens of CR1/CR2^{-/-} mice was similar to that of +/+ B6 mice. We also examined the activation and expansion of LCMV-specific CD8 T cells using MHC class I tetramers. As illustrated in Fig. 6a, the relative proportions of CD8 T cells specific to the immunodominant epitopes NP396–404 and gp33–41 were comparable between +/+ B6 and CR1/CR2^{-/-} mice. Further, the absolute numbers of NP396–404- and gp33–41-specific CD8 T cells in the spleens of CR1/CR2^{-/-} mice were similar to those of +/+ B6 mice (Fig. 6b). Intracellular cytokine staining was performed to assess the effect of CR1/CR2 deficiency on the expansion of CD8 T cells specific to multiple LCMV CTL epitopes. CR1/CR2 deficiency had minimal effects (statistically insignificant) on the activation and expansion of CD8 T cells specific to both dominant (NP396, gp33, and gp34) and subdominant (gp276, gp118, and NP205) epitopes (data not shown). Taken together, these data suggest that generation of LCMV-specific CD8 T cell responses is independent of CR1/CR2. Consistent with these findings, in +/+ mice, NP396–404-specific CD8 T cells did not express detectable levels of CR1/2 on their surface. However, a subpopulation of LCMV-specific CD8 T cells expressed cell surface CR3 (Mac-1) and CR5aR (data not shown).

Discussion

In this manuscript, we have documented the role of complement component C3 in regulating the activation and expansion of Ag-specific CD8 T cells during an acute systemic viral infection in mice. The main point is that C3, a component of innate immunity regulates epitope selection and is essential for optimal expansion of CD8 T cells during an acute infection with LCMV. Studies in CR1/CR2^{-/-} mice indicated that C3-mediated regulation of the CD8 T cell response was largely independent of complement receptors 1 and 2.

It is becoming increasingly evident that components of innate immunity modulate the specific immune response. Although the importance of complement proteins in regulating humoral immunity is well documented, the role of C3 in orchestrating T cell

FIGURE 6. Expansion of LCMV-specific CD8 T cells in CR1/CR2-deficient mice. Eight days after infection with LCMV-Arm, the number of LCMV-specific CD8 T cells in the spleens of +/+ B6 and CR1/CR2^{-/-} mice was determined by staining with anti-CD8 Abs and MHC class I (D^b) tetramers specific to the LCMV CTL epitopes NP396–404 and gp33–41. The flow cytometry plots are gated on total splenocytes based on forward and side scatter and the numbers denote percentages of LCMV-specific CD8 T cells among splenocytes. The data in b are the mean of five mice per group.



responses has been unclear (13, 20). However, recent studies have ascribed a role for complement in the elicitation of T cell responses in mice. Depletion of C3 protected mice against T cell-dependent autoimmune myocarditis (16). In C3^{-/-} mice, the development of T cell-dependent autoimmune allergic encephalitis was attenuated (32). Upon infection with influenza virus, C3-deficient mice exhibited poor viral clearance on account of suboptimal activation and trafficking of CD4 and CD8 T cells to the lung (17). In kidney and skin transplant models, local synthesis of C3 was shown to be essential for T cell priming and rejection of allografts (18, 30). These studies showed that C3 plays an important role in T cell activation, in organ-specific autoimmunity, in transplant rejection, and in a localized infection. Our studies confirm and extend these findings. In our studies, we investigated the role of C3 in the generation of Ag-specific T cell responses to multiple epitopes during a systemic viral infection of mice with LCMV. We show that C3 activity affects priming and expansion of LCMV-specific CD8 T cells in an epitope-dependent fashion. In 129/B6 mice, loss of C3 results in a selective decrease in the expansion of CD8 T cells specific to the epitope, NP396–404 (Fig. 2, *a* and *b*). In B6 mice, CD8 T cell responses to the gp-derived epitopes were markedly reduced by C3 deficiency, as compared with NP epitopes (Figs. 2, *c* and *d*). Discussion of the mechanism(s) underlying the differential effect of C3 deficiency on the epitope selection (NP396 vs gp33–41) in 129/B6 and B6 mice is purely speculative. Differences in the genetic background of the mice may influence the efficiency of processing and presentation of the NP396–404 and gp33–41 peptides. As a result, the C3 dependency of peptide processing and presentation may vary from one strain to the other. Why are CD8 T cell responses to some epitopes more affected by C3 deficiency within the 129/B6 or B6 mice? It is worth noting that the same class I MHC molecule (D^b) presents both NP396–404 and gp33–41 epitopes in 129/B6 (H-2^b) and B6 (H-2^b) mice. Further, our studies in LCMV-infected +/+ B6 and C3^{-/-} B6 mice showed that C3 deficiency did not affect the cell surface expression of D^b molecules on macrophages and B cells (data not shown). Therefore, at least in the B6 mice, the C3 deficiency-induced epitope-specific inhibition of CD8 T cell responses cannot be attributed to the differences in levels of D^b expression on APCs. Previous studies have shown that lack of costimulatory interactions (CD28/B7 or CD40L/CD40) or CD4 deficiency did not affect the epitope hierarchy of the LCMV-specific CD8 T cell response (33–35). Therefore, it is less likely that costimulation or CD4 T cell help are factors in C3-dependent epitope selection in LCMV-infected mice. It was recently shown that epitope selection can be influenced by IFN- γ during an infection of mice with *Listeria monocytogenes* (36). However, the role of IFN- γ in regulating epitope selection during an acute LCMV infection is not known. The mechanism(s) regulating the epitope hierarchy of the anti-LCMV CD8 T cell responses are not well understood. The role of C3 in epitope selection during an acute LCMV infection warrants further investigation. Nonetheless, our studies suggest that the complement system, an important element of the innate immune response can interfere with T cell epitope selection. The level of C3, a changing parameter in the course of systemic inflammation, might interfere with immunodominance.

Several phenomena have been described that could explain how C3 promotes expansion of LCMV-specific CD8 T cells. It has been previously shown that complement factors can directly interact with T cells and can modulate the function of Ag presenting T cells, which are crucial for T cell expansion (15, 16, 37–40). CD46, a receptor that interacts with complement products C3b and C4b, is expressed on all nucleated cells, including T cells (41). Depending on the isoform of CD46, C3b and C4b can either aug-

ment or suppress CD8 T cell responses (42). When both isoforms of CD46 are coexpressed on a T cell, stimulatory effects seem to dominate over suppressive effects (42). Therefore, in the LCMV infection in C3^{-/-} mice, abrogation of CD46/C3b interactions might have reduced expansion of virus-specific CD8 T cells.

It has been shown that Ag-bound C3b enhances the efficiency of Ag presentation to T cells (43, 44). Although the underlying mechanisms are still unclear, it has been reported that C3 deposition on APCs augments T cell proliferation (40, 45). Taken together, these reports suggest that lack of C3 activity can also affect the efficiency of Ag presentation, thereby limiting the expansion of LCMV-specific CD8 T cells in vivo. Moreover, complement activation products iC3b/C3dg bind to Ag/natural Ab complexes and promote Ag uptake and expression of costimulatory molecules by APCs (46). Although CD4-deficient and B cell-deficient mice develop normal CD8 T cell responses to an acute LCMV infection, the role of Abs needs further investigation (35, 47).

Products of complement activation can modulate cellular responses via complement receptors CR1 and CR2, which are predominantly expressed on APCs including B cells (9). CR1/CR2 signaling in B cells regulates activation threshold, Ag uptake, processing and presentation, isotype switching, and generation of memory B cells (9). Moreover, expression of CR1/CR2 on T cells has been reported, and may promote binding between the APC and T cells via C3 (16, 40, 45). Therefore, C3 can regulate T cell responses by interacting directly with T cells or indirectly via APCs. Our studies in CR1/CR2^{-/-} mice clearly show that CR1/CR2 signaling is not obligatory for optimal activation and expansion of LCMV-specific CD8 T cells (Fig. 6). Similar to our findings, generation of CD8 T cell responses to influenza infection in mice is dependent upon C3, but independent of CR1/CR2 signaling (17).

C3 might regulate CD8 T cell responses by its influence on cell trafficking. The chemotactic and proinflammatory activities of C3a and C5a, which are the cleavage products of C3 and C5, respectively, are well documented. Activated T lymphocytes can express functional receptors for C3a (C3aR) and C5a (C5aR) (48, 49), that influence trafficking in inflammatory disorders (48, 49). Further, C5a may affect the trafficking of dendritic cells and initiation of immune responses (50). Hence, it is plausible that during LCMV infection, lack of C3 activity (and subsequent C5 activity) might hinder recruitment, trafficking, and optimal priming of CD8 T cells. It is worth emphasizing that all the mechanisms listed above by which C3 may regulate the generation of LCMV-specific CD8 T cell responses are not mutually exclusive. Further mechanistic studies are warranted to examine the above-listed hypotheses. Nevertheless, the data presented in this paper provide compelling evidence for an important role for C3 in epitope selection and expansion of virus-specific CD8 T cells during a systemic infection. These findings have implications in devising immunotherapeutic modalities in the treatment of autoimmune diseases and prevention of transplant rejection.

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