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Systemic C3 Modulates CD8⁺ T Cell Contraction after Listeria monocytogenes Infection

Yulong Tan,* Yongsheng Li,† Xiaolan Fu,* Fei Yang,* Ping Zheng,* Jue Zhang,† Bo Guo,* and Yuzhang Wu*

Ag-specific CD8⁺ T cell contraction (contraction), which occurs after the resolution of infection, is critical for homeostasis of the immune system. Although complement components regulate the primary CD8⁺ T cell response, there is insufficient evidence supporting their role in regulating contraction and memory. In this study, we show that C3-deficient (C3⁻/⁻) mice exhibited significantly less CD8⁺ T cell contraction than did wild-type mice postinfection with recombinant Listeria monocytogenes expressing OVA. Kinetic analyses also revealed decreased contraction in mice treated with cobra venom factor to deplete C3, which was consistent with the results in C3⁻/⁻ recipient mice transplanted with bone marrow cells from the same donors as wild-type recipient mice. The phenotypes of memory cells generated by C3⁻/⁻ mice were not altered compared with those of wild-type mice. Further, C5aR signaling downstream of C3 was not involved in the regulation of contraction. Moreover, the regulation of contraction by C3 may be independent of the duration of antigenic stimulation or the functional avidity of effector CD8⁺ T cells. However, reduced contraction in C3⁻/⁻ mice was accompanied by a decrease in the proportion of KLRG-1lo (killer-cell lectin-like receptor G1) CD127hi long-lived effector cells at the peak of the response and correlated with a reduction in the levels of inflammatory cytokines, such as IL-12 and IFN-γ, produced early postinfection. These results provide new insights into the role of systemic C3 in regulating contraction following intracellular bacterial infection and may help to develop vaccines that are more effective. The Journal of Immunology, 2014, 193: 3426–3435.

In response to bacterial and viral infections, the number of Ag-specific CD8⁺ T cells expands dramatically. Upon elimination of the infectious agent, the CD8⁺ T cell population decreases to homeostatic levels during a contraction phase (contraction) (1–3). In this scenario, the majority of responding CD8⁺ T cells undergoes apoptosis, whereas 5–10% of the original population survives and becomes long-lived memory cells (1–3). The contraction phase preserves the flexibility of the response to new infections while maintaining enhanced defense against previously encountered pathogens (4). Contraction is programmed early postinfection by cytokines, such as IL-12 and IFN-γ, that regulate differentiation of effector CD8⁺ T cells to KLRG-1lo (killer-cell lectin-like receptor G1) CD127hi long-lived memory precursor effector cells (MPECs) that are present at the peak of the response and preferentially survive contraction. In contrast, the CD8⁺ T cell subset KLRG-1loCD127hi is short-lived effector cells (SLECs) that may undergo apoptosis (5–8). Furthermore, cytokines, such as IL-2, IL-7, or IL-15, influence contraction through differential regulation of CD8⁺ T cell subsets (MPECs and SLECs) during the contraction phase (9–11). Nevertheless, the mechanisms that control contraction are not fully understood (12).

A large body of evidence originally established the role of the complement system in the innate immune response against pathogens through its functions of opsonization, lysis, and anaphylatoxin activity (13–15). Subsequent research revealed that the complement system is involved in the induction and regulation of B cell- and T cell-mediated responses (13, 15–18). Complement activation regulates CD8⁺ T cell priming and proliferation in naturally occurring viral infections and in models of transplantation (16, 19, 20). Delayed-type hypersensitivity, a form of a secondary cell-mediated immune response, is suppressed by complement component of C3 (21). Furthermore, a peptide-treated C3-deficient (C3⁻/⁻) mouse induces an increase in Ag-specific CD8⁺ IFN-γ⁺ cells and a higher rate of male graft rejection compared with that for wild-type (WT) mice 10 wk after grafting (22). These studies suggest that complement components may regulate contraction and the induction of memory cells.

C3 plays a central role in the complement cascade (15). Hemocytes are the major source of the circulating or systemic pools of C3, although significant, but smaller, amounts are produced locally by cells of the immune system or by other cell types (23, 24). The locally produced complement components contribute to the activation of APCs and T cells and drive CD4⁺ T cell differentiation, expansion, and survival (25–27). Similarly, studies on models of transplantation show that locally produced C3 regulates the expansion of CD8⁺ T cells (20, 28). In contrast, optimal CD8⁺ T cell responses against visceral leishmaniasis depend on circulating complement activation by natural Abs (29). Moreover, C3 deletion by cobra venom factor (CVF) treatment or C3 deficiency lead to weak CD8⁺ T cell responses because fewer...
blood-borne bacteria are transferred to splenic CD8α+ dendritic cells (DCs) postinfection (30). Therefore, it is important to determine the roles of systemic and local C3 production in the regulation of the CD8α+ T cell response, because they may inform the design of more effective vaccines.

In the current study, using an informative murine model of *Listeria monocytogenes* infection (31, 32), we demonstrate that systemic C3 plays an important role in regulating CD8α+ T cell contraction and that regulation of contraction by C3 may correlate with a reduction in the fraction of SLECs at the peak of the response, as well as decreased production of inflammatory cytokines.

**Materials and Methods**

**Mice**

C57BL/6 (B6) and BALB/c mice were purchased from the Peking University Animal Center (Beijing, China). C5a receptor-deficient (C5aR−/−) mice on a BALB/c background, OT-I TCR-transgenic mice, and B6. C3−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Congenic C57BL/6 mice (Ly5.1) were a gift from Dr. Jinxiong She (Medical College of Georgia, Augusta, GA). Ly5.1-C3−/− mice were generated by crossing Ly5.1-B6 with B6. C3−/− mice were purchased from the Animal Care and Use Committee of the Third Military Medical University.

**Bacterial infection and ampicillin treatment**

Recombinant *L. monocytogenes* expressing OVA (rLmOVA) and actA-deficient rLmOVA (△actA rLmOVA) were kindly provided by Dr. John Harty (University of Iowa, Iowa City, IA). Age-matched mice were injected i.v. with a sublethal dose of 4 × 10⁶ CFU △actA rLmOVA or 3 × 10⁶ CFU rLmOVA in 300 μl saline. For secondary infections, mice were injected i.v. with 1 × 10⁶ CFU △actA rLmOVA. Mice were provided 2 mg/ml ampicillin (Dingguo Bio, Beijing, China) in drinking water for 1 wk, starting on day 4 postinfection. The number of bacteria present in each spleen was determined by plating diluted spleen homogenates on brain–heart infusion plates containing erythromycin.

**Depletion of C3**

To deplete C3, mice were injected i.p. with 10 μg CVF (Quidel, San Diego, CA) in sterile PBS (300 μl) on day −1 before infection with △actA rLmOVA and then on days 1, 2, 3, 5, 7, 9, 11, and 13 postinfection. To determine the effects of complement during the early priming/expansion phase or the later contraction phase, mice were treated with CVF on days −1, +1, +2, +4, and +6 or were treated with CVF on days +7, +8, +10, and +12 or were treated with sterile PBS (control group) on the same day as CVF treatment.

**ELISA**

Murine C3 was measured as described (33). Capture and detection Abs were from Cappel (ICN Pharmaceuticals, Costa Mesa, CA). Serum levels of systemic C3 plays an important role in regulating CD8α+ T cell contraction and that regulation of contraction by C3 may correlate with a reduction in the fraction of SLECs at the peak of the response, as well as decreased production of inflammatory cytokines.

**Abs and flow cytometry**

- Anti-CD8α (clone 53-6.7), anti-Ly5.1 (clone A20), anti-Ly5.2 (clone 104), anti-CD127 (IL-7Rα, clone A7R34), anti–KLRL1 (clone 2F1), anti–CD44 (clone IM7), anti–IFN-γ (clone XMG1.2), rat IgG2a and IgG2b isotype controls and mouse IgG1 isotype controls, anti–IFN-γ (clone XMG1.2), and anti–IL-2 (clone JES6-5H4) were purchased from eBio-science.
- anti–CD8α (clone 53-6.7) and anti–CD26L (clone MEL-14) were purchased from BioLegend; and anti–TCR Vβ4 (clone KT4), anti–TCR Vβ5.1, 5.2 (clone MR9-4), anti–TCR Vβ6 (clone RR4-7), anti–TCR Vβ7 (clone TR310), and anti–TCR Vβ8.1, 8.2 (clone MR5-2) were purchased from BD Biosciences. Splenocytes or PBMCs were prepared and stained with mAbs specific for proteins expressed on the cell surface. To detect intracellular cytokines, cells were cultured at 37°C for 4–5 h in complete medium supplemented with 10% calf bovine serum and 1 μg/ml brefeldin A (eBioscience), in the absence or presence of OVA257–264 peptide (SIINFEKL; 1.0 μg/ml). Listeriolysin O (91–99) (LLD9a-b) peptide (GYKDGNEYI; 1.0 μg/ml) was used to detect the CD8α+ T cell response of mice with a BALB/c background. These peptides were synthesized by Chinese Peptide (Hangzhou, Zhejiang, China). After culture, cells were harvested and stained using a Foxp3 Staining Buffer Set (eBioscience), following the manufacturer’s protocol. OVA-specific CD8α+ T cell responses were also determined by staining with H2-K1/OVA257–264 pentamers (Proimmune, Oxford, U.K.), according to the manufacturer’s instructions. All FACS data were acquired using a BD FACSCan II and analyzed using FlowJo software (TreeStar).

**Adoptive transfer**

CD8α+ T cells were enriched (∼90%) from splenocytes by immunodepletion of CD8α+ cells using a CD8α+ T Cell Isolation Kit II (Miltenyi Biotec), according to the manufacturer’s instructions. To further purify CD8α+ T cell subpopulations, magnetic bead–purified CD8α+ T cells were stained with appropriate mAbs on ice in PBS containing 1% FCS and were further sorted using a FACSAria (Becton Dickinson). The purity of FACS–sorted samples was ∼98%. Groups of congenic recipient mice were injected i.v. with equivalent numbers of the respective CD8α+ T cell subpopulations.

**Chimeric mice**

B6 WT and C3−/− mice were irradiated (1100 cGy) and injected i.v. with 10 × 10⁶ bone marrow (BM) cells from congenic B6 or C3−/− mice, respectively. After 2 mo, the animals were bled, and CD8α+ T cells were analyzed by surface staining to confirm chimerism. The mice were immunized with △actA rLmOVA, and analyzed 7, 14, and 21 d after immunization.

**Quantitative analysis of C3 expression by CD8α+ T cells**

Total RNA was extracted using TRIzol reagent (Invitrogen) from the FACS–sorted populations of CD8α+ T cells, according to the manufacturer’s instructions. RNA purity and concentration were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Total RNA (1 μg) was reverse transcribed using the PrimeScript RT Reagent Kit with gDNA Eraser (TAKARA BIO, Otsu, Japan), according to the manufacturer’s instructions. PCR conditions were as follows: denaturation at 95°C for 30 s, followed by 45 cycles of amplification at 95°C for 5 s, 60°C for 30 s, and 72°C for 30 s. Quantitative PCR was performed using a Stratagene Mx3000P system (Agilent Technologies, Santa Clara, CA) and a SYBR Premix Ex Taq (Perfect Real Time) Kit (TaKaRa), according to the manufacturer’s instructions. Each reaction mixture, consisting of 7.0 μl water, 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), and 10 μl× Master mix, was added to 1.0 μl cDNA. Data are expressed relative to those of the controls. The average threshold-cycle differences among the samples were normalized to the β-actin (Actb) (control) in the corresponding cDNA preparation. Primers were used: 5′-ACC TTA CCT CGG CAA GTT CCT-3′ and 5′-TTG TAG GCC TGG TCA GGG GCA G-3′. The housekeeping gene β-actin (Actb) (control) in the corresponding cDNA preparation. Primers were used: 5′-ACC TTA CCT CGG CAA GTT CCT-3′ and 5′-TTG TAG GCC TGG TCA GGG GCA G-3′.

**Statistical analysis**

Statistical analysis was performed using an unpaired two-tailed Student t test by Prism GraphPad Software (La Jolla, CA). The p values < 0.05 were considered statistically significant.
Results

Decreased CD8+ T cell contraction in C3−/− mice postinfection with L. monocytogenes

C3−/− mice were immunized with ΔactA rLmOVA, and changes in the numbers of OVA-specific CD8+ T cells were determined by staining for intracellular IFN-γ from days 7 to 50. On day 7 following immunization, effector CD8+ T cell expansion was approximately two-thirds lower in C3−/− mice compared with WT mice (Fig. 1A, 1B), which is consistent with the results of another study showing that CD8+ T cell responses to L. monocytogenes infection were downregulated in the absence of C3 (35). Interestingly, compared with control mice, the contraction (days 7–16) in C3−/− mice was much slower, whereas the numbers of OVA-specific effector CD8+ T cells in C3−/− mice were greater (~2–3-fold) on day 50 postinfection (Fig. 1B). Kb/OVA pentamer analysis also revealed decreased contraction in C3−/− mice (Fig. 1C, 1D). In addition, we used rLmOVA to immunize mice and found slower contraction in C3−/− mice than in WT mice (Fig. 1E). These results suggest that C3 is required for CD8+ T cell contraction after L. monocytogenes infection.

Depletion of C3 by CVF treatment reduces contraction independent of C5aR signaling

CVF treatment leads to complement depletion by activating the alternative pathway of C3 activation (36). To verify the role of C3 in regulating contraction, we depleted circulating C3 in WT mice by ~90% by continuous administration of CVF from days −1 to +13 (Fig. 2A). The primary response of the CVF-treated mice was ~50% of that of the controls on day 7 postinfection. Contraction was significantly slower, whereas the percentage of OVA-specific CD8+ T cells on day 50 was higher in CVF-treated mice than in the controls (Fig. 2B, 2C).

To further determine the effect of systemic C3 during the priming/expansion or contraction phases, we treated mice with CVF from days −1 to +6 or from days +7 to +12. Only the mice treated with CVF from days −1 to +6 showed decreased contraction postinfection (Fig. 2D), indicating that systemic C3 was involved in the regulation of contraction during the early priming/expansion phase.

C3 activation generates downstream pathway components, including anaphylatoxin C5a (15, 37). Because C5a/C5aR signaling enhances the CD8+ T cell response (38, 39), we hypothesized that C3 regulated CD8+ T cell contraction through C5a. Therefore, we used a peptide antagonist (C5aR-A) specific for C5aR to test this possibility. Contraction in mice treated with C5aR-A did not differ significantly compared with controls (Supplemental Fig. 1). We also assessed the LLO91–99-specific CD8+ T cell response in C5aR−/− mice on a BALB/c background and found that CD8+ T cell contraction was not significantly different from that in WT mice (Fig. 2E). These results suggest that the regulation of CD8+ T cell contraction under conditions of C3 deficiency is independent of C5aR signaling.

Systemic C3 regulates CD8+ T cell contraction after BM transplantation

To further address the role of systemic C3 on contraction, we generated chimeras by infusing WT BM cells into WT or C3-deficient recipients. Thus, in the presence or absence of serum...
C3, BM cells produced local C3. C3⁻/⁻ BM cells also were infused into WT or C3-deficient recipients. Thus, BM cells do not produce local C3, regardless of whether serum C3 is present or absent. The presence of chimerism after 2 mo was determined using flow cytometry (Fig. 3A). The chimeric animals were immunized with OVA-expressing L. monocytogenes (ΔactA rLmOVA, and the changes in CD8⁺ T cell responses were analyzed using PBLs on days 7, 14, and 21 postinfection. We found that the C3⁻/⁻ recipients exhibited slower contraction compared with WT recipients when engrafted with cells from the same donor BM. In contrast, CD8⁺ T cell contraction in the same recipient mice, when engrafted with BM from C3⁻/⁻ or WT donors, respectively, showed no significant difference (Fig. 3B, 3C). These findings support the conclusion that systemic C3 regulates CD8⁺ T cell contraction.

**Memory CD8⁺ T cells function normally in C3⁻/⁻ mice**

Because the unique phenotype of memory CD8⁺ T cells contributes to their protective effect (5, 8, 40), we tested whether the phenotype of OVA-specific CD8⁺ T memory cells from C3⁻/⁻ mice was similar to those from WT mice (5, 8, 40). The expression of CD62L, KLRG-1, CD127, and CD44 of OVA-specific CD8⁺ T cells, as well as the production of IL-2, was not significantly different between WT and C3⁻/⁻ mice (Fig. 4).

Memory CD8⁺ T cells undergo a vigorous expansion to protect against a challenge infection (1, 41). Therefore, we next investigated whether memory T cells harvested from C3⁻/⁻ mice functioned normally. Because complement is involved in phagocytosis and killing of C3-bound L. monocytogenes by activated macrophages (42) and is required for the efficient generation of CD8⁺ T cell responses (35), it is reasonable to hypothesize that C3⁻/⁻ mice acquire greater bacterial loads after L. monocytogenes infections. Surprisingly, the bacterial load was significantly lower in the spleen of C3⁻/⁻ mice than in WT mice postinfection because of reduced blood-borne bacterial shuttling to splenic CD8⁺⁺ DCs (30). We speculated that bacterial loads might not correlate with the protective function of effector CD8⁺ T cells in the model of L. monocytogenes infection. Therefore, we performed an in vivo killing assay on day 34 and found that approximately twice as many OVA-specific target cells were killed in C3⁻/⁻ mice compared with WT mice (Fig. 5A, 5B). Because the amount of memory T cells in C3⁻/⁻ mice was about twice that in WT mice (Fig. 1), these results indicate that the cytotoxic potential of memory cells in C3⁻/⁻ mice was comparable to that in WT mice.

We next determined whether the expansion abilities of the memory cells of C3⁻/⁻ and WT mice were the same. After rechallenge at 31 d after primary infection, higher numbers of CD8⁺ T cells were detected in C3-depleted mice, and the slopes of the CD8⁺ T cell response curves for C3-depleted and control mice were not significantly different (Fig. 5C). This result suggests that memory cells in C3-depleted and control mice underwent equivalent response after challenge. Unexpectedly, the population of OVA-specific CD8⁺ T cells in WT mice expanded faster and to a greater extent than did that in C3⁻/⁻ mice after rechallenge, although more memory CD8⁺ T cells were detected in C3⁻/⁻ mice than in WT mice (Supplemental Fig. 2).

The recall response of memory CD8⁺ T cells of C3⁻/⁻ mice differs from that of C3-depleted mice. We hypothesized that the difference may be attributed to the direct effect of C3 on CD8⁺ T cell proliferation (35), as well as on contraction and memory induction. Therefore, we asked whether CD8⁺ memory T cells generated in C3⁻/⁻ mice mounted a full response in mice with normal levels of C3. For this purpose, CD8⁺ memory T cells from

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**FIGURE 2.** Depletion of serum C3 decreased contraction and increased the population of memory cells. B6 mice were divided into two groups and treated with CVF or PBS from days −1 to +13. Both groups of mice were immunized with ΔactA rLmOVA. The number of OVA-specific CD8⁺ T cells in the PBMC population on days 7 and 50 postinfection was determined by staining of intracellular IFN-γ. (A) The sera of C3⁻/⁻ mice treated with CVF were assayed using an ELISA at the indicated times. The C3 level of each mouse is shown as the percentage of baseline C3 before treatment (100%). (B) Dot plots show the percentage of IFN-γ-producing splenic CD8⁺ T cells. (C) Graphs represent the frequencies of IFN-γ⁺ CD8⁺ T cells on days 7 and 50 (mean ± SEM). (D) B6 mice were divided into three groups: two groups were treated again with CVF from days −1 to +6 or from days +7 to +12, and the control group was treated with PBS. The line graph (left panel) and bar graph (right panel) show the numbers of OVA-specific CD8⁺ T cells in PBMCs at the indicated days postinfection (mean ± SEM). Data represent at least two independent experiments. (E) BALB/c WT and C5aR⁻/⁻ mice were immunized with ΔactA rLmOVA, and the number of LLO91–99-specific CD8⁺ T cells in the spleen (mean ± SEM) was determined on days 7 and 33 postinfection. Three or four mice were sacrificed per group/treatment. The data represent at least three independent experiments. *p < 0.05.
and WT mice were transferred into WT naive mice 40 d postinfection (Fig. 5D). One day later, recipient mice were infected with OactA rLmOVA. The fold increase in memory CD8+ T cells in the recall response was not significantly different between C3/2 and WT donors (Fig. 5E, 5F), suggesting that the recall capability of memory CD8+ T cells in C3-deficient and C3-depleted mice were similar compared with WT mice. Taken together, these data indicate that the phenotypes of memory cells generated in C3-deficient and C3-depleted mice were comparable to those in WT mice and that the memory cells protected against infection.

Diminished CD8+ T cell contraction is not due to a shortened duration of infection in C3/2 mice

Next, we sought to identify factors that participated during the priming/expansion phase. Curtailing prolonged stimulation of CD8+ T cells toward the end (days 6–7) of an acute infection decreases the differentiation potential of terminal effectors (7, 8). To test the possibility that curtailed bacterial persistence in C3/2 mice inhibited CD8+ T cell contraction, C3/2 and WT mice were treated with ampicillin from day 4 postinfection to eliminate persistent bacteria; on day 6 postinfection, the bacteria were eliminated from C3/2 and WT mice (Fig. 6A). Moreover, the numbers of OVA-specific CD8+ T cells in C3/2 mice were less than half of those in WT mice on day 6, whereas this population contracted more slowly, which resulted in ~2-fold more OVA-specific CD8+ T cells on day 50 (Fig. 6B, 6C). These results indicate that the reduced CD8+ T cell contraction is not due to the duration of Ag persistence.

Decreased CD8+ T cell contraction is independent of functional avidity

Functional avidity of effector CD8+ T cells may contribute to their initial fate decision and to the generation of SLECs (43). The functional avidity of effector CD8+ T cells is determined by their sensitivity to peptide Ag or their distribution of TCR Vb (44).

We attempted to determine whether the functional avidity of effector CD8+ T cells in C3/2 mice differed from that in WT mice and correlated with reduced CD8+ T cell contraction. We found that the distribution of TCR Vb expression among OVA-specific

FIGURE 3. Systemic C3 regulates CD8+ T cell contraction in mice with chimeric BM. BM cells from WT mice (Ly5.1+) and C3/2 mice (Ly5.2+) were prepared and transferred separately into recipient WT mice and C3/2 mice (Ly5.2+ or Ly5.1+) after sublethal irradiation. Two months later, the chimeric mice were identified using flow cytometry with CD8a and Ly5.1 mAbs before immunization. (A) Graphs show the percentage of the Ly5.1+ subset among CD8+ T cells in chimeric mice. The chimeric mice were immunized with ΔactA rLmOVA and OVA-specific CD8+ T cells and analyzed for expression of intracellular IFN-γ on days 7, 14, and 21 postinfection. (B) Dot plots show the percentage of IFN-γ+ CD8+ T cells in PBMCs from WT→WT, WT→C3/2, C3/2→WT, or C3/2→C3 BM chimeric mice at the indicated days postinfection. (C) Kinetics of appearance of OVA-specific CD8+ T cells in PBMCs of chimeric mice are shown in the line graph (left panel) and bar graph (right panel) (mean ± SEM). Data represent at least five mice/group and two independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
CD8+ T cells in C3−/− mice was not significantly different from that in WT mice on day 7 postinfection (Fig. 7A). To determine whether the sensitivity to peptide of effector CD8+ T cells differed between C3−/− and WT mice, splenocytes were isolated on day 7 postinfection and stimulated with different concentrations of OVA peptide. There was no significant difference in the peptide sensitivity between C3−/− and WT mice (Fig. 7B). Together, these data suggest that altered contraction of effector CD8+ T cells is not associated with changes in the functional avidity of effector CD8+ T cells.

**Reduced CD8+ T cell contraction correlates with decreased generation of SLECs**

Postinfection or postimmunization, early effector CD8+ T cells were proposed to differentiate into at least two major subsets that can be distinguished by their expression of cell surface markers, such as CD127 and KLRG-1 (5, 7, 8). KLRG-1hiCD127lo SLECs are more likely to die after the peak response, whereas KLRG-1loCD127hi MPECs preferentially survive contraction and slowly develop into memory cells (7, 8). Therefore, CD8+ T cell contraction correlates with the proportions of these two effector CD8+ T cell subsets after differentiation (7).

We hypothesized that decreased contraction in C3−/− mice may be due to diminished differentiation of SLECs. Indeed, there were fewer SLECs in C3−/− mice than in WT mice (p < 0.01; Fig. 8A, 8B). In contrast, more MPECs were generated in C3−/− mice than in WT mice (p < 0.01; Fig. 8A, 8B). This result indicates the generation of more MPECs during the effector T cell differentiation phase and is consistent with our findings that more memory cells.
only KLRG-1hiCD127lo SLECs subpopulations were transferred. A sufficient number of cells to perform the transfer experiments; represented a very small proportion, we were unable to obtain Inflammatory cytokines in C3 infected with O 2

C3 (Fig. 8A, 8B), we asked whether cytokines secreted by DCs in

infection-matched m ice. Because KLRG-1loCD127hi MPECs contributed to reduced contraction. WT or controls on day 7 postinfection (Fig. 8C, 8D). These results suggest that C3 facilitates early CD8+ T cell differentiation toward SLECs, which promotes CD8+ T cell contraction. Their contraction was not affected by C3 during the later contraction phase (Supplemental Fig. 3), which was consistent with the result of the CVF-treatment experiment (Fig. 2D). These results suggest that C3 facilitates early CD8+ T cell differentiation toward SLECs, which promotes CD8+ T cell contraction. 

Inflammatory cytokines in C3−/− mice are decreased during the early phase

Inflammatory cytokines regulate CD8+ T cell contraction through their influence on early priming and differentiation (6, 7). For example, IL-12 promotes the preferential differentiation of CD8+ T cells toward the SLEC phenotype (7). Because Ag-specific CD8+ T cells differentiated into fewer SLECs in C3−/− mice, their influence on early priming and differentiation (6, 7) was undertaken to determine the role of C3 in regulating CD8+ T cell contraction and memory induction. Our findings demonstrate that CD8+ T cell contraction was less in C3−/− mice than in WT mice, as well as upon C3 depletion induced by CVF treatment. The contraction also was diminished in C3−/− recipient mice to a greater extent than in WT recipient mice when each was engrafted with the same BM cells. Moreover, our findings suggested that decreased CD8+ T cell contraction in C3−/− mice may correlate with the generation of fewer SLECs and reduced levels of serum inflammatory cytokines. Marked increases in primary viral Ag–specific CD8+ T cell expansion and numbers of memory CD8+ T cells occur in decay-accelerating factor (CD55/Daf1)-knockout mice after acute infection.

The levels of IL-12, IL-23, and IFN-γ in the sera of C3−/− mice were significantly lower compared with those of WT mice (Fig. 9). These data suggest that the decreased inflammatory cytokines in C3−/− mice may correlate with the inhibition of differentiation to SLECs and decreased contraction.

Discussion

During an immune response against acute infection, CD8+ T cell contraction is a critical homeostatic process that occurs following the peak of Ag-specific T cell responses. Accumulating evidence shows that the complement system plays a very important role in the regulation of T cell immunity (16, 17, 46), particularly in the regulation of primary CD8+ T cell responses in certain models of bacterial and viral infection (19, 29, 35, 39). The present study was undertaken to determine the role of C3 in regulating CD8+ T cell contraction and memory induction. Our findings demonstrate that CD8+ T cell contraction was less in C3−/− mice than in WT mice, as well as upon C3 depletion induced by CVF treatment. The contraction also was diminished in C3−/− recipient mice to a greater extent than in WT recipient mice when each was engrafted with the same BM cells. Moreover, our findings suggested that decreased CD8+ T cell contraction in C3−/− mice may correlate with the generation of fewer SLECs and reduced levels of serum inflammatory cytokines.

Marked increases in primary viral Ag–specific CD8+ T cell expansion and numbers of memory CD8+ T cells occur in decay-accelerating factor (CD55/Daf1)-knockout mice after acute infection.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** OVA-specific CD8+ T cells also contracted more slowly in C3−/− mice after antibiotic treatment. WT and C3−/− mice were immunized with ΔactA rLmOVA and treated with 2 mg/ml ampicillin in drinking water from days 4 to 11. OVA-specific CD8+ T cells were determined in the spleen of each mouse by intracellular IFN-γ staining. (A) Bacterial clearance was determined in the spleen 3 and 6 d after immunization. The graphs show the CFU (mean ± SEM for three mice/group) in the spleen. (B) Frequencies of OVA-specific CD8+ T cells from representative mice at the indicated days after immunization. (C) Kinetics of OVA-specific CD8+ T cells in the spleen (mean ± SEM). Data represent three mice/time point and three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001. LOD, limit of detection.

![FIGURE 7](http://www.jimmunol.org/)

**FIGURE 7.** Similar functional avidities of OVA-specific CD8+ T cells in WT and C3−/− mice. WT and C3−/− mice were infected with ΔactA rLmOVA, and splenocytes from each group were harvested on day 7 postinfection. (A) Cells were stained with anti-CD8, anti-IFN-γ, and anti-VB Abs. The data represent the fraction of OVA-specific CD8+ T cells expressing the indicated VB-chains. (B) Peptide dose-response curves normalized to the level of maximum numbers of IFN-γ+ CD8+ T cells. Data represent the mean ± SEM of three mice/group and two independent experiments.
with lymphocytic choriomeningitis virus compared with those in WT mice (39). Deletion of C3 or C5aR in Cd55−/− mice reverses the enhanced CD8+ T cell response but does not influence contraction (39). In this study, we show reduced contraction in C3-deficient or C3-depleted mice compared with the controls. Moreover, CD8+ T cell contraction was not influenced in C5aR−/− mice or by treatment with C5aR-A. Therefore, the effect of C3 activation on the regulation of CD8+ T cell responses may be complex and variable, depending on the pathogen.

Although understanding how the complement regulates T cell responses is incomplete and varies in different models of infection, recent data generated by transplantation and infection models suggest that local production of C3 determines the responses of CD8+ T cells (28, 35). For example, in a model of L. monocytogenes infection, C3 directly promotes CD8+ T cell expansion in vitro (35), which suggests that locally produced C3 acts on CD8+ T cells. We detected increased C3 mRNA expression in activated CD8+ T cells from WT mice (Supplemental Fig. 4). However, there was no significant difference in contraction in the WT recipient mice when respectively engrafted with BM from WT or C3−/− donors; also the contraction was not significantly different in the C3−/− recipient mice with BM adoptive transfer from WT or C3−/− donors, suggesting that local C3 might not play an important role in regulating CD8+ T cell contraction.

How does systemic C3 regulate CD8+ T cell contraction? We show in this study that C3 exerted an effect early postinfection but

![FIGURE 8.](http://www.jimmunol.org/) Fewer SLECs are generated in C3−/− mice. (A and B) WT and C3−/− mice were immunized with ΔactA rLmOVA, and splenocytes were analyzed by staining of intracellular IFN-γ on day 7 postinfection. (A) Expression of KLRG-1 and CD127 in IFN-γ+ CD8+ T cells. (B) Percentages of KLRG-1hiCD127lo, KLRG-1loCD127hi, and KLRG-1hiCD127hi cells among OVA-specific CD8+ T cells. Data represent the mean of six mice/group. One of three independent experiments is shown. (C and D) Splenocytes from CVF-treated and control mice were harvested and analyzed by staining intracellular IFN-γ on day 7 postinfection. (C) Expression of KLRG-1 in IFN-γ+ CD8+ T cells. (D) Percentages of KLRG-1hi cells among OVA-specific CD8+ T cells. The data represent three independent experiments.

![FIGURE 9.](http://www.jimmunol.org/) Cytokine levels are altered in C3−/− mice early after rLmOVA infection. C3−/− and WT mice were infected with ΔactA rLmOVA, and serum levels of IL-12, IL-23, IFN-γ, and TNF-α were determined using ELISA at 24 h postinfection. The results represent the mean ± SEM of three mice/group and two independent experiments.
not during later contraction. DCs are essential for the induction of antilisterial CD8+ T cell immunity early postinfection (47, 48), and multiple signals integrated (e.g., inflammatory cytokines) by DCs program the CD8+ T cell response (2, 49). Therefore, we hypothesize that systemic C3 depletion or C3 deficiency may alter the characteristics of DCs to regulate contraction. Evidence indicates that the strength or duration of an antigenic signal influences contraction through altering SLEC differentiation (8, 43). Nevertheless, we found that CD8+ T cell contraction was independent of prolonged duration of infection or functional avidity of effector CD8+ T cells. The present results suggest that antigenic signals were not involved in regulating contraction, consistent with findings that the dose and duration of infection do not influence contraction (4, 6). Moreover CD8+ T cells do not undergo cell division in response to residual Ag (50), and C3 deficiency does not affect the ability of DCs to present Ag in vitro or during the maturation of DCs in vivo after L. monocytogenes infection (35).

In contrast, we found that the levels of critical cytokines, such as IL-12 and IFN-γ, were reduced 24 h postinfection. Because IL-12 and IFN-γ program contraction (6, 7), it is reasonable to conclude that these cytokines may link C3 to CD8+ T cell contraction.

In summary, our results reveal an important role for systemic C3 in influencing CD8+ T cell contraction following an infection with intracellular bacteria and provide insights into the mechanisms by which complement regulates the CD8+ T cell response. Furthermore, because depletion of C3 by CVF treatment reduced CD8+ T cell contraction and increased the generation of CD8+ memory T cells with enhanced protective function, our findings suggest that strategies that alter the functions of the complement pathway may enhance vaccine efficacy.

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Disclosures

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References