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Complement-Dependent Enhancement of CD8\(^+\) T Cell Immunity to Lymphocytic Choriomeningitis Virus Infection in Decay-Accelerating Factor-Deficient Mice

Chongyun Fang,* Takashi Miwa,* Hao Shen,† and Wen-Chao Song\(^{2*}\)

Decay-accelerating factor (DAF, CD55) is a GPI-anchored membrane protein that regulates complement activation on autologous cells. In addition to protecting host tissues from complement attack, DAF has been shown to inhibit CD4\(^+\) T cell immunity in the setting of model Ag immunization. However, whether DAF regulates natural T cell immune response during pathogenic infection is not known. We describe in this study a striking regulatory effect of DAF on the CD8\(^+\) T cell response to lymphocytic choriomeningitis virus (LCMV) infection. Compared with wild-type mice, DAF knockout (Daf-1\(^{-/-}\)) mice had markedly increased expansion in the spleen of total and viral Ag-specific CD8\(^+\) T cells after acute or chronic LCMV infection. Splenocytes from LCMV-infected Daf-1\(^{-/-}\) mice also displayed significantly higher killing activity than cells from wild-type mice toward viral Ag-loaded target cells, and Daf-1\(^{-/-}\) mice cleared LCMV more efficiently. Importantly, deletion of the complement protein C3 or the receptor for the anaphylatoxin C5a (C5aR) from Daf-1\(^{-/-}\) mice reversed the enhanced CD8\(^+\) T cell immunity phenotype. These results demonstrate that DAF is an important regulator of CD8\(^+\) T cell immunity in viral infection and that it fulfills this role by acting as a complement inhibitor to prevent virus-triggered complement activation and C5aR signaling. This mode of action of DAF contrasts with that of CD59 in viral infection and suggests that GPI-anchored membrane complement inhibitors can regulate T cell immunity to viral infection via either a complement-dependent or -independent mechanism. *The Journal of Immunology, 2007, 179: 3178–3186.

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Materials and Methods

**Mice**

Daf-1−/− mice deficient in the Daf-1 gene, the murine homolog of human DAF, were generated by gene targeting as previously described (11). They were backcrossed onto the C57BL/6 background for 10 generations. Six time-backcrossed C57BL/6-C3−/− mice were obtained from The Jackson Laboratory, and were backcrossed in house for an additional five generations. A breeder pair of C5a receptor knockout (C5aR−/−) mice on the C57BL/6 background was provided by Dr. J. Lambris (University of Pennsylvania, Philadelphia, PA) (14). C3−/− or C5aR−/− mice were cross-bred with Daf-1−/− mice to derive Daf-1−/− or Daf-1−/−/C5aR−/− mice, respectively. Male mice ages 8–12 wk were used in this study. Gender- and age-matched WT C57BL/6 mice were obtained from The Jackson Laboratory. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

**Reagents**

Allophycocyanin-conjugated rat anti-mouse IFN-γ (clone XMG1.2), PE- or FITC-conjugated rat anti-mouse CD8α (clone 53-6.7), PerCp-conjugated rat anti-mouse CD4 (clone RM4-5), allophycocyanin-conjugated rat anti-mouse CD44 (clone IM-7), FITC-conjugated rat anti-mouse CD62 ligand (CD62L, clone MEL-14), purified rat anti-mouse Fc receptor (clone 2.4G2), GolgiStop and Cytofix/Cytoperm kit were from BD Pharmingen. DMEM, l-glutamine, HEPES, PBS, nonessential amino acids, sodium pyruvate, 2-ME, and penicillin-streptomycin were from Invitrogen Life Technologies. LCMV gp33−41 peptide was synthesized by Sigma-Aldrich. FBS was from HyClone Laboratories. [3H]Thymidine was from Amersham Biosciences. EL-4 cell line was from American Type Culture Collection.

**Infection of mice with LCMV**

LCMV-Armstrong and LCMV-clone 13 strains were propagated in vitro, and viral titers were determined by plaque assays as previously described (15). For acute LCMV infection, 2×10^6 PFU of LCMV-Armstrong were administered to mice by i.p. injection. Mice were sacrificed at day 6, 7, or 8 to collect the spleens for analysis of primary immune response or 2 mo later for memory immune response. For chronic LCMV infection, 2×10^6 PFU LCMV-clone 13 were administered to mice by i.v. injection through the tail vein. Mice were bled at day 8, 15, 30, and 45 to obtain PBMC for T cell analysis, and to obtain serum for monitoring virus clearance. Mice infected with clone 13 were sacrificed at day 50 postinfection for terminal analysis of splenocytes. Experiments were also performed to test the function of the memory immune response to LCMV infection. For these experiments, mice were first infected with 2×10^6 PFU LCMV-Armstrong (i.p.) and then challenged 5 mo later with 2×10^6 PFU LCMV-clone 13 (i.v.). Mice were sacrificed at different time points, and the titers of LCMV-clone 13 virus in their spleens were determined by plaque assays as previously described (15).

**Preparation of splenocytes and PBMC**

Spleens were first cut into small pieces and meshed with the blunt end of a plastic syringe in a petri dish on ice. Single-cell suspension was prepared by passing the spleen homogenate through a 70-μm cell strainer (Falcon) and cell pellets were collected after centrifugation at 600×g for 7 min. To determine whether DAF regulates CD8T cell expansion upon acute LCMV infection

To determine whether DAF regulates CD8 T cell immunity, we infected groups of WT and Daf-1−/− mice with LCMV-Armstrong, a virus strain that causes acute infection and a strong CD8 T cell response in the mouse (15, 18, 19). On day 6, 7, or 8 postinfection, mice were sacrificed, and the percentage and total number of CD4+ and CD8+ T cells in their spleens were determined by FACs. As shown in Fig. 1, A and B, Daf-1−/− mouse spleens were found to contain a significantly higher percentage of CD8+ T cells than WT mouse spleens at day 7 postinfection (30.40 ± 1.60% vs 20.48 ± 1.45%, n = 4 mice per group, p < 0.005). The total number of CD8+ T cells in Daf-1−/− mice was also greatly increased (32.80 ± 2.76 × 10^6 spleen vs 12.50 ± 1.64 × 10^6 spleen, p < 0.001) (Fig. 1C). Similar increases in CD8+ T cell expansion in Daf-1−/− mice were also observed on days 6 and 8 postinfection (Fig. 1D). Because there was no difference between naive (noninfected) WT and Daf-1−/− mice in their splenic CD8+ T cell frequency or total number (data not shown and Ref. 20), these results suggested that DAF deficiency either promoted Ag-specific CD8+ T cell expansion or impaired the depletion of non-specific CD8+ T cells in response to acute LCMV infection.

We observed no significant difference in the frequency of splenic
CD4<sup>+</sup> T cells between LCMV-infected WT and Daf-1<sup>−/−</sup> mice (Fig. 1A). However, in three of four independent experiments, the total number of splenic CD4<sup>+</sup> T cells was significantly greater in Daf-1<sup>−/−</sup> mice than in WT mice (data not shown).

Studies have shown that in the early phase of LCMV infection, nonspecific T cells are deleted to make space for the expansion of LCMV-specific T cells (21-23). The activated, Ag-specific T cells can be distinguished by their up-regulation of CD44 and down-regulation of CD62L (CD44<sup>hi</sup>/CD62L<sup>low</sup>) (23, 24). To determine whether the increased CD8<sup>+</sup> T cell number in Daf-1<sup>−/−</sup> mice was due to LCMV-driven expansion of Ag-specific T cells or ineffectual deletion of nonspecific T cells, we analyzed the expression of CD44 and CD62L on gated CD8<sup>+</sup> T cells. As shown in Fig. 1, E and F, the frequency of CD44<sup>hi</sup>/CD62L<sup>low</sup> cells among gated CD8<sup>+</sup> T cells was significantly higher in Daf-1<sup>−/−</sup> mice than that in WT mice (80.83 ± 0.75% vs 72.30 ± 1.33%, n = 4 mice, p < 0.0014). This implied that the much larger CD8<sup>+</sup> T cell population in Daf-1<sup>−/−</sup> mouse spleens most likely resulted from LCMV-driven expansion of Ag-specific CD8<sup>+</sup> T cells rather than ineffectual deletion of nonspecific CD8<sup>+</sup> T cells.

To further characterize the highly expanded CD8<sup>+</sup> T cells in Daf-1<sup>−/−</sup> mice, we analyzed the number of splenic CD8<sup>+</sup> T cells that were specific for the LCMV antigenic gp33–41 peptide by staining total splenocytes with the gp<sub>33–41</sub>/Db tetramer-positive (16). Fig. 2, A–C, shows that the percentage and total number of gp<sub>33–41</sub>/Db tetramer-positive CD8<sup>+</sup> T cells in Daf-1<sup>−/−</sup> mouse spleens were significantly higher than that in the WT mouse spleens (percentage: 2.02 ± 0.15% vs 1.30 ± 0.10%; total number: 21.84 ± 2.39 × 10<sup>4</sup>/spleen vs 7.76 ± 0.43 × 10<sup>4</sup>/spleen; n = 4 mice per group, p < 0.01 for both measurements). Separately, we stimulated splenocytes from LCMV-infected WT and Daf-1<sup>−/−</sup> mice with gp<sub>33–41</sub> and analyzed the number of IFN-γ-secreting CD8<sup>+</sup> T cells after intracellular staining of IFN-γ. Fig. 2, D–G, shows that Daf-1<sup>−/−</sup> mouse spleens were again found to contain a significantly higher percentage and total number of IFN-γ-producing CD8<sup>+</sup> T cells than WT mouse spleens (percentage: 4.44 ± 0.41% vs 2.50 ± 0.29%; total number: 48.23 ± 6.18 × 10<sup>4</sup>/spleen vs 14.99 ± 1.57 × 10<sup>4</sup>/spleen; n = 4 mice, p < 0.01 for both measurements). These data indicated that Daf-1<sup>−/−</sup> mice had increased Ag-specific CD8<sup>+</sup> T cell expansion upon acute LCMV infection. Notably, the relative percentage of gp<sub>33–41</sub>/Db tetramer-positive (Fig. 2D) or IFN-γ-producing cells (Fig. 2F) among gated CD8<sup>+</sup> T cells showed a trend of increase in Daf-1<sup>−/−</sup> mice but did not differ significantly from that of WT mice, suggesting that DAF deficiency caused expansion of gp<sub>33–41</sub>-specific as well as other epitope-specific CD8<sup>+</sup> T cells.

Daf-1<sup>−/−</sup> mouse splenocytes exhibited higher total CTL activity and contained lower titers of LCMV

To evaluate the functional significance of enhanced Ag-specific CD8<sup>+</sup> T cell immunity in Daf-1<sup>−/−</sup> mice, we compared the total CTL activities of WT and Daf-1<sup>−/−</sup> mouse splenocytes at day 7 after LCMV-Armstrong infection. For this experiment, EL-4 cells pulsed with the viral antigenic peptide gp<sub>33–41</sub> were used as target cells (25). Fig. 3A shows that the total CTL activity of Daf-1<sup>−/−</sup> mouse splenocytes was significantly higher than that of WT mouse splenocytes at three different E:T ratios. This most likely reflected an increased number of gp<sub>33–41</sub>-specific CD8<sup>+</sup> T cells in Daf-1<sup>−/−</sup> mice rather than higher CTL activity of individual Daf-1<sup>−/−</sup> CD8<sup>+</sup> T cells. Indeed, in a separate assay wherein we normalized the effector cell number in splenocytes based on the frequency of

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**FIGURE 1.** Increased CD8<sup>+</sup> T cell expansion after acute LCMV infection in Daf-1<sup>−/−</sup> mice. WT and Daf-1<sup>−/−</sup> mice (n = 4 for each group) were infected with LCMV-Armstrong. At indicated time points postinfection, the percentage and total number of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in their spleens were determined by FACS analysis. **A,** Representative FACS analysis showing increased frequency (percentage of cells shown) of CD8<sup>+</sup> but not CD4<sup>+</sup> T cells in the spleens of Daf-1<sup>−/−</sup> mice. **B,** Scatter plot of splenic CD8<sup>+</sup> T cell frequencies in WT and Daf-1<sup>−/−</sup> mice (n = 4). **C,** Scatter plot of splenic CD8<sup>+</sup> T cell numbers in WT and Daf-1<sup>−/−</sup> mice (n = 4). **D,** Time course analysis of CD8<sup>+</sup> T cell frequency in the spleens of WT and Daf-1<sup>−/−</sup> mice (day 6, 7, and 8). **E,** Representative FACS analysis of gated splenic CD8<sup>+</sup> T cells showing a higher percentage (each quadrant) of CD8<sup>+</sup> T cells that were specific for the LCMV antigenic gp33–41 peptide by CD44 and CD62L on gated CD8<sup>+</sup> T cells. **F,** Representative FACS analysis of gated splenic CD8<sup>+</sup> T cells having an activated phenotype (CD44 high/CD62Llow) in Daf-1<sup>−/−</sup> mice. **G,** Scatter plot of the percentage of CD44<sup>hi</sup>/CD62L<sup>low</sup> cells among gated splenic CD8<sup>+</sup> T cells in WT and Daf-1<sup>−/−</sup> mice (n = 4). Except in **D,** all data are from day 7 postinfection. Data are representative of four independent experiments.
gp33–41/Db tetramer-positive CD8 T cells, we observed no difference in total CTL activity between WT and Daf-1−/− mice (data not shown). In a parallel experiment, we investigated the antiviral activity of CD8 T cells in vivo by determining the LCMV titers in WT and Daf-1−/− mouse spleens at day 3, 5, and 7 post infection with LCMV-Armstrong. As expected, we observed that the virus titers in both groups of mice decreased exponentially between days 3 and 7, indicating that the virus was being cleared by the host (Fig. 3B). Notably, we found that the viral titer in the spleen of Daf-1−/− mice was significantly lower than that in the spleen of WT mice at days 5 and 7, suggesting that Daf-1−/− mice eliminated LCMV more efficiently than WT mice. These data correlated well with the increased CD8 T cell number in Daf-1−/− mice as described and suggested that the more vigorously expanded CD8 T cells in the mutant mice were functionally relevant.

Enhanced CD8 T cell response to chronic LCMV infection in Daf-1−/− mice

The kinetics of viral clearance and immune response to chronic LCMV infection is significantly different from that of acute LCMV infection (26). Chronic LCMV infection is characterized by a high virus load in multiple tissues for 2–3 mo (15). Although the infection is eventually under control in most tissues, the virus is not completely eliminated from the host (15). The induction of CD8 T cell response in chronic LCMV infection also differs in that, although CD4 T cells are not necessary for an acute CD8 T cell response, they are indispensable for CD8 T cell responses in...
were assayed for killing activity toward gp33–41-pulsed EL-4 cells (targets).

A

Daf-1

deficient mice had higher killing activity and lower virus titers. WT and Daf-1/−/− mice (n = 4 per group) were infected with LCMV-Armstrong. A, On day 7 postinfection, splenocytes (effectors) were assayed for killing activity toward gP33–41-pulsed EL-4 cells (targets). Daf-1/−/− mouse splenocytes showed higher killing activities at all three E:T ratios. Each curve represents splenocytes from a single mouse. B, In separate experiments, virus titers in the mouse spleens were determined by plaque-assays on days 3, 5, and 7 postinfection. Results are representative of two independent experiments each. **, p < 0.01 between WT and Daf-1/−/− groups.

B

Daf-1

Daf-1/−/− mice developed more Ag-specific memory CD8+ T cells

In the case of acute LCMV infection, most of the activated T cells are eliminated by activation-induced cell death after resolution of virus infection (16). Only ~5% of the these cells will survive and develop into memory T cells, which can give rise to a quicker and more vigorous recall response upon reencounter with the same Ag. To determine whether DAF deficiency also influences the development of memory CD8+ T cells, groups of WT and Daf-1/−/− mice were infected with LCMV-Armstrong and examined 2 mo later. Mice were sacrificed and splenocytes were analyzed for the

chronic LCMV infection (27, 28). To examine the CD8+ T cell response of Daf-1/−/− mice during chronic LCMV infection, we infected groups of WT and Daf-1/−/− mice with LCMV clone 13, a more virulent LCMV strain that causes chronic infection in mice (15). We then monitored their CD8+ T cell response by analyzing PBMC (day 8, 15, 30, and 45) or splenocytes (day 50, terminal) for the frequency of CD8+ or gp33–41/Db tetramer-positive T cells. As shown in Fig. 4, A and B, we found that the percentage of CD8+ or gp33–41/Db tetramer-positive T cells in PBMC of Daf-1/−/− mice was significantly higher than the percentage found in WT mice at all time points examined. Notably, increased total and gp33–41-specific CD8+ T cells in Daf-1/−/− mice correlated with lower viral titers in these mice at latter but not early time points after infection (Fig. 4C). When splenocytes were restimulated at day 50 with gp33–41, a higher percentage (Fig. 4, D and E) and total number (Fig. 4F) of Daf-1/−/− mouse splenocytes secreted IFN-γ as assessed by intracellular staining (percentage: 0.32 ± 0.01% vs 0.13 ± 0.01%; total number: 2.3 ± 1.26 × 10^5/spleen vs 0.80 ± 0.28 × 10^5/spleen, n = 4 mice per group, p < 0.001 for both measurements). These data demonstrated that Daf-1/−/− mice could mount a persistently stronger CD8+ T cell response to chronic LCMV infection.

Daf-1/−/− mice infected with LCMV-clone 13 and their PBMC or splenocytes were examined at different time points. A, Percentage of CD8+ T cells in PBMCs at various time points after infection. B, Percentage of gp33–41/Db tetramer-positive CD8+ T cells in PBMCs. C, Blood LCMV titers at various time points after infection. D, Representative FACS analysis showing that, after restimulation in vitro with gp33–41, splenocytes from LCMV-clone 13-infected Daf-1/−/− mice (day 50) had more IFN-γ-secreting CD8+ T cells than that of WT mice. Scattering plots of the frequency (D) and number (E) of IFN-γ-secreting CD8+ T cells in the spleens of WT and Daf-1/−/− mice. Results are representative of two independent experiments. *, p < 0.05; **, p < 0.01.
frequency of gp33–41/Db tetramer-positive cells directly or of IFN-γ-producing CD8+ T cells after gp 33– 41 restimulation. Although there was no difference in the spleen size or total number of splenocytes between the two groups of mice, Daf-1+/− mice were found to contain significantly more gp33–41/Db tetramer-positive CD8+ T cells in their spleens than WT mouse spleens (percentage: 0.95 ± 0.08% vs 0.52 ± 0.03%; total number: 4.58 ± 10^5 vs 2.75 ± 10^5; n = 5 mice per group, p < 0.005 for both measurements) (Fig. 5, A–C). Similarly, in response to gp 33– 41 peptide restimulation, Daf-1+/− mouse spleens contained significantly more IFN-γ-secreting CD8+ T cells than WT mouse spleens (percentage: 1.09 ± 0.05% vs 0.70 ± 0.03%; total number: 5.18 ± 10^5 vs 2.51 ± 10^5; n = 5 mice, p < 0.001 for both measurements) (Fig. 5, D–F). These data indicated that more memory CD8+ T cells had developed in Daf-1+/− mouse spleens after acute LCMV infection.

Memory T cells can be divided into two subpopulations according to the level of CD62L expression: CD62L low effector memory T cells and CD62L high central memory T cells (29, 30). It has been thought that CD62L high central memory T cells are derived from CD62L low effector memory T cells and that the former are more potent in controlling secondary viral infection (30, 31). To further characterize the memory CD8+ T cell phenotype in Daf-1+/− mice, we analyzed CD62L expression on LCMV-specific memory CD8+ T cells at 3 or 5 mo postinfection. A, Representative FACS analysis of splenocytes at 3 mo postinfection showing that a higher percentage (shown in each quadrant) of CD62Llow cells were detected in Daf-1+/− mice than in WT mice among gated gp33–41/Db tetramer-positive CD8+ T cells. B, Scatter plot of the ratio of effector memory CD8+ T cells (CD62Llow) to central memory CD8+ T cells (CD62Lhigh) in WT and Daf-1+/− mice (n = 5). C, Scatter plot showing that the average titer of LCMV-clone 13 virus in the spleens of Daf-1+/− mice was lower than that in WT mice (n = 5). Mice were re-infected with LCMV-clone 13 five months after LCMV-Armstrong infection and spleens were examined for LCMV-clone 13 virus load at day 5 after re-infection.
sequences of C3 or C5aR gene deficiency in Daf-1/H11002
CD8
Daf-1 expansions upon acute LCMV-Armstrong infection in WT, Daf-1−/−, Daf-1−/−/C3−/−, and Daf-1−/−/C5aR−/− mice (n = 4 for each group) were infected with LCMV-Armstrong. On day 7 postinfection, splenocytes were collected and were either analyzed by FACS directly for CD8+ and gp33/Db tetramer-positive T cells or were stimulated with gp33/Db and then analyzed for IFN-γ-secreting CD8+ T cells. A. Scatter plot of CD8+ T cell frequencies in splenocytes of the four groups of mice. B. Scatter plot of the frequencies of gp33/Db tetramer-positive CD8+ T cells in splenocytes of the four groups of mice. C. Scatter plot of the frequencies of IFN-γ-secreting CD8+ T cells in splenocytes of the four groups of mice. In each case, there was significant difference between WT and Daf-1−/− mice but no difference between WT and Daf-1−/−/C3−/− or Daf-1−/−/C5aR−/− mice. NS, p > 0.05; **, p < 0.01.

FIGURE 7. Enhanced CD8+ T cell responses in Daf-1−/− mice were complement- and C5aR-dependent. WT, Daf-1−/−, Daf-1−/−/C3−/−, and Daf-1−/−/C5aR−/− mice were infected with LCMV-Armstrong. On day 7 postinfection, splenocytes were collected and were either analyzed by FACS directly for CD8+ and gp33/Db tetramer-positive T cells or were stimulated with gp33/Db and then analyzed for IFN-γ-secreting CD8+ T cells. A. Scatter plot of CD8+ T cell frequencies in splenocytes of the four groups of mice. B. Scatter plot of the frequencies of gp33/Db tetramer-positive CD8+ T cells in splenocytes of the four groups of mice. C. Scatter plot of the frequencies of IFN-γ-secreting CD8+ T cells in splenocytes of the four groups of mice. In each case, there was significant difference between WT and Daf-1−/− mice but no difference between WT and Daf-1−/−/C3−/− or Daf-1−/−/C5aR−/− mice. NS, p > 0.05; **, p < 0.01.

gp33−/−/Db tetramer-positive CD8+ T cells in Daf-1−/− and WT mice 3 mo after LCMV-Armstrong infection. Interestingly, we found that the percentage of CD62Llow cells among gated gp33−/−/Db tetramer-positive CD8+ T cells was significantly higher in Daf-1−/− mice than the percentage found in WT mice (Fig. 6, A and B). This result suggested that the increased memory CD8+ T cells in Daf-1−/− mice were biased toward an effector memory CD8+ T cell phenotype.

To evaluate the functional significance of increased memory CD8+ T cells in Daf-1−/− mice in vivo, we infected groups of Daf-1−/− and WT mice with LCMV-Armstrong, and after 5 mo, rechallenged these groups with the more virulent LCMV-clone 13 strain. Five days after LCMV-clone 13 infection, mice were sacrificed and their spleens were collected to determine the virus titer by plaque assay. Fig. 6C shows that the virus titer in Daf-1−/− mouse spleens was significantly lower than that in WT mouse spleens, suggesting that Daf-1−/− mice mounted a more effective anti-LCMV recall response.

Enhanced CD8+ T cell response to LCMV infection in Daf-1−/− mice is C3- and C5aR-dependent

To explore the mechanism of enhanced CD8+ T cell immunity to LCMV infection in Daf-1−/− mice, we investigated the consequences of C3 or C5aR gene deficiency in Daf-1−/− mice. Daf-1−/− mice were crossed with C3−/− and C5aR−/− mice, respectively, to generate Daf-1−/−/C3−/− and Daf-1−/−/C5aR−/− mice. Comparison of the total and Ag-specific CD8+ T cell expansions upon acute LCMV-Armstrong infection in WT, Daf-1−/−, Daf-1−/−/C3−/−, and Daf-1−/−/C5aR−/− mice revealed that the enhanced CD8+ T cell immunity phenotype in Daf-1−/− mice was completely rescued by either C3 or C5aR deficiency (Fig. 7). This result indicated that enhanced CD8+ T cell immunity to LCMV infection in Daf-1−/− mice was dependent on complement activation and C5aR signaling.

Discussion

We have demonstrated in this study that mice deficient in the GPI-anchored membrane complement regulator DAF mounted a markedly more vigorous CD8+ T cell response to LCMV infection. This finding extends our previous observation in Daf-1−/− mice of enhanced CD4+ T cell recall response to model Ag immunization (13) and shows that DAF could regulate both CD4+ and CD8+ T cell immunity. It also shows that such a regulatory effect of DAF on T cell immunity could be manifested during a natural immune response to viral infection as well as in the setting of adjuvant-based single Ag immunization (13). We further demonstrated that the enhanced CD8+ T cell response in Daf-1−/− mice had functional significance in that these mice had lower splenic virus titers at days 5 and 7 after LCMV-Armstrong infection and that their splenocytes exhibited higher total CTL activities. Because there was no difference between WT and Daf-1−/− mice in their virus titers at day 3 postinfection (Fig. 3B), the lower virus load in Daf-1−/− mice at the latter time points must have resulted from accelerated clearance of the virus rather than altered susceptibility to LCMV infection. Similarly, in the chronic LCMV infection model increased CD8+ T cell expansion in Daf-1−/− mice correlated with significantly diminished virus titer at latter time points (Fig. 4C). The higher CTL activity demonstrated by Daf-1−/− mouse splenocytes most likely reflected the increased number of virus-specific CD8+ T cells and not enhanced CTL activity of individual Daf-1−/− CD8+ T cells.

It is notable that the phenotype of increased T cell immunity in LCMV-infected Daf-1−/− mice was more pronounced with CD8+ T cells than with CD4+ T cells. This difference may be related to the nature of LCMV-elicited T cell response as the virus is known to drive primarily a CD8+ T cell response in the mouse (32–35). Nevertheless, this result contrasted with the specific inhibition of CD4+ T cell immunity by CD59, another GPI-anchored membrane complement regulator, in mice infected with recombinant
vaccinia virus (36). A further important difference between the inhibitory functions of DAF and CD59 in T cell immunity is that the activity of CD59 was shown to be complement-independent (36), whereas we have demonstrated that enhanced CD8+ T cell immunity in LCMV-infected Daf-1−/− mice was dependent on C3 and C5aR. Thus, GPI-anchored membrane complement regulators could influence T cell immunity to viral infection via at least two different mechanisms.

The complement-dependent nature of the Daf-1−/− mouse phenotype in LCMV infection is consistent with our previous finding that C3 and C5 were necessary for Daf-1−/− mice to develop a CD4+ T cell recall hyperresponse after OVA or MOG immunization (13). It also agrees with previous studies showing that C3 was required for optimal T cell expansion in response to viral infection in WT mice (37, 38). Suresh et al. (37) have shown earlier that C3 but not CR1/CR2 deficiency caused epitope-specific impairment in CD8+ T cell expansion after LCMV infection in mice of two genetic backgrounds. On the 129/B6 background, NP396–404-specific CD8+ T cells infected. In the case of LPS-treated Daf-1−/−, T cell immunity was amplified, presumably by increased C5a generation.

Several questions arise from our hypothesis and remain to be addressed experimentally. First, does the LCMV cause more complement activation in Daf-1−/− mice than in WT mice and, if so, does it occur in the fluid phase (plasma) or on specific cell types? Second, how and on what cells does the C5a-C5aR interaction promote T cell immunity? Although we have not attempted to measure and compare complement activation in LCMV-infected WT and Daf-1−/− mice, complement is known to be activated by pathogens (14). Passage through infected cells and CD8+ CTLs, and severely impaired infection of virus-specific T cells into the lungs. Consistent with our demonstration of an essential role for C5aR in the Daf-1−/− mouse phenotype, blocking the interaction of C5a-C5aR by a peptide antagonist effectively inhibited the expansion of CD8+ T cells specific for the immunodominant NP366–374 peptide in Daf-1−/− mice, whereas on the C57BL/6 background gp33–41-specific CD8+ T cells were twice as many in WT mice than in C3-deficient mice (37). In a model of influenza virus infection, Kopf et al. (38) demonstrated that C3 deficiency resulted in delayed viral clearance, reduced priming of CD4+ Th cells and CD8+ CTLs, and severely impaired infection of virus-specific T cells into the lungs. Consistent with our demonstration of an essential role for C5aR in the Daf-1−/− mouse phenotype, blocking the interaction of C5a-C5aR by a peptide antagonist effectively inhibited the expansion of CD8+ T cells specific for the immunodominant NP366-374 peptide of influenza virus (39). Taken together, these data suggested that complement augmented antiviral T cell immunity via a C5aR-dependent, CR1/CR2-independent mechanism and that in the absence of DAF, this adjuvant effect of complement on T cell immunity was amplified, presumably by increased C5a generation.

In summary, we have shown a marked C5aR-dependent enhancement in anti-LCMV CD8+ T cell immunity in Daf-1−/− mice. Further work by others has shown that LCMV-driven innate and CD8+ T cell immunity in mice was dependent on TLR2 and the obligatory TLR signaling adaptor molecule MyD88 (44).

References


In Fig. 1F the data for WT and Daf-1−/− were reversed. The corrected figure is shown below.


In the title, “Nitrogen” is incorrect. The corrected title is shown below.

Cutting Edge: A Critical Role of Nitric Oxide in Preventing Inflammation upon Apoptotic Cell Clearance


In Footnotes, the current address for the first author is incorrect. The corrected footnote is shown below.

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