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CD4 T Cells Are Required for CD8 T Cell Survival during Both Primary and Memory Recall Responses

Patricia Novy, †Michael Quigley, †Xiaopei Huang,* and Yiping Yang²*†

The role of CD4 T cell help in primary and secondary CD8 T cell responses to infectious pathogens remains incompletely defined. The primary CD8 T response to infections was initially thought to be largely independent of CD4 T cells, but it is not clear why some primary, pathogen-specific CD8 T cell responses are CD4 T cell dependent. Furthermore, although the generation of functional memory CD8 T cells is CD4 T cell help dependent, it remains controversial when the “help” is needed. In this study, we demonstrated that CD4 T cell help was not needed for the activation and effector differentiation of CD8 T cells during the primary response to vaccinia virus infection. However, the activated CD8 T cells showed poor survival without CD4 T cell help, leading to a reduction in clonal expansion and a diminished, but stable CD8 memory pool. In addition, we observed that CD4 T cell help provided during both the primary and secondary responses was required for the survival of memory CD8 T cells during recall expansion. Our study indicates that CD4 T cells play a crucial role in multiple stages of CD8 T cell response to vaccinia virus infection and may help to design effective vaccine strategies.


Abbreviations used in this paper: DC, dendritic cell; VV, vaccinia virus; WT, wild type; HA, hemagglutinin; MFI, mean fluorescence intensity; AICD, activation-induced cell death; LCMV, lymphocytic choriomeningitis virus; LN, lymph node.

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but relatively stable CD8 memory pool was generated in the absence of CD4 T cells. Furthermore, we observed that in addition to CD4 T cell help provided during the primary response, the ‘help’ provided following a secondary challenge was also required for the survival of memory CD8 T cells during the recall expansion. These results suggest that CD4 T cell help is crucial for multiple stages of CD8 T cell response to VV infection. As VV has been used widely as vaccine vehicles for infectious diseases and cancer, our findings may have important implications for the design of effective vaccine strategies.

Materials and Methods

Mouse

B10.D2 mice were purchased from The Jackson Laboratory. CD4-deficient mice (CD4−/−) on the C57BL/6 background were purchased from The Jackson Laboratory and backcrossed onto the B10.D2 genetic background for nine generations. The clone 4 hemagglutinin (HA)-TCR-transgenic mice (CD4−/−) provided following a secondary challenge was also required for the survival of memory CD8 T cells during the recall expansion. These results suggest that CD4 T cell help is crucial for multiple stages of CD8 T cell response to VV infection. As VV has been used widely as vaccine vehicles for infectious diseases and cancer, our findings may have important implications for the design of effective vaccine strategies.

Adoptive transfer of clone 4-transgenic T cells

Naive clonotypic HA-specific CD8+ T cells (Thy1.1+) were prepared from clone 4 TCR-transgenic mice. Briefly, single-cell suspensions were prepared from spleen and lymph nodes of clone 4 TCR mice and clonotypic percentage was then determined by flow cytometry analysis of CD8+Vβ8.2+ cells as described (28, 29). The activation marker CD44 was also checked to ensure these clonotypic cells were naive. CD8 T cells were positively selected using anti-CD8 microbeads according to the manufacturer’s instructions (Miltenyi Biotec) with a purity of >98%. A total of 1×106 or 1×106 purified CD8+ T cells were adoptively transferred to naive recipients via tail vein injection in 200 μl of HBSS. In some experiments, cells were labeled with CFSE before transfer as previously described (28).

Immunizations and Ab treatment

Recombinant vaccinia virus encoding HA (rVV-HA) and rE1-deleted adenovirus encoding HA (Ad-HA) were previously described (28). rVV-HA was grown in TK-143B cells, purified by sucrose banding, and titer was determined by plaque-forming assay on TK-143B cells. Mice were infected with 5×103 or 5×104 PFU rVV-HA i.p. Ad-HA was grown in 293 cells (American Type Culture Collection), purified by two rounds of CsCl density centrifugation, and desalted by gel filtration through Sephadex G-25 column (PD-10 column; Amershams Bioscience). The titer was determined by plaque-forming assay on 293 cells. Mice were infected with 2×106 PFU i.p.

In vivo CD4+ T cell depletion in B10.D2 mice was performed by i.p. injection of the anti-CD4 mAb GK1.5 (150 μg) for 3 days beginning 10 days before rVV-HA infection and every third day thereafter until completion of the experiment as described (19).

Isolation of lymphocytes from nonlymphoid tissues

Lymphocytes were isolated nonlymphoid tissues as described (30). Briefly, liver or lung issue was homogenized and passed through a 70-μm cell strainer. The single-cell suspension was resuspended in 35 ml of HBSS and centrifuged on a 15 ml of Ficoll gradient (Amersham). Cells were harvested from the Ficoll gradient and washed twice with HBSS before analysis.

Abs and flow cytometry

mAbs (all from BD Biosciences unless indicated) used for staining were PE-Cy5-conjugated anti-CD8; FITC-conjugated anti-Thy1.1, -CD44, -CD62L, -CD69, -IFN-γ, -CD122, -TNF-α, and -granzyme B (eBioscience); PE-conjugated anti-Thy1.1, annexin V, and anti-Bcl-xL (Santa Cruz Biotechnology); biotin-conjugated anti-CD127. Collection of flow cytometry data was conducted using a FACSscan or FACS calibur (BD

FIGURE 1. Defective clonal expansion of CD8 T cells in the absence of CD4 T cell help during a primary response to VV infection. A total of 104 purified naive clone 4 CD8 T cells (Thy1.1+) were adoptively transferred into congenic WT, CD4-deficient (CD4−/−) B10.D2 mice (Thy1.2+), or WT mice treated with the depleting CD4 mAb GK1.5 (+GK1.5), which were subsequently infected with rVV-HA. Seven days later, spleen and other lymphoid and nonlymphoid organs were harvested for analysis of transferred cells. A. Expansion and function of clonotypic cells. Splenocytes were stained with anti-CD8, anti-TCR, and anti-IFN-γ intracellularly. Percentage of total (left panels) and IFN-γ-producing (right panels) clonotypic cells among total lymphocytes is indicated with the numbers in parentheses showing the MFI (×104) of IFN-γ-producing clonotypic cells. B. The mean absolute numbers of clonotypic T cells per spleen, combined six peripheral LNs, combined six Peyer’s patches, whole liver, or whole lung are indicated with SDs. Data shown are representative of three independent experiments.
Intracellular staining

To measure intracellular levels of Bcl-xL, splenocytes were stained with anti-CD8 and -Thy1.1 Abs. Cells were then permeabilized using the Cytofix/Cytoperm kit according to the manufacturer’s instructions (BD Biosciences) and subsequently stained intracellularly with anti-Bcl-xL Ab. To assess production of effector molecules, splenocytes were cultured in 200 μl of CTL medium (RPMI 1640 supplemented with 10% FBS, 2 mM l-glutamine, 100 IU/ml penicillin, 100 IU/ml streptomycin, and 50 μM 2-ME) at a concentration of 10^7 cells/ml in the presence of 2 μM of the K^d HA518-526 peptide and 5 μg/ml brefeldin A containing Golgi-Plug (BD Biosciences) for 6 h at 37°C. After incubation, cells were washed and stained with anti-CD8 and -Thy1.1. Cells were then permeabilized using the same protocol as for Bcl-xL and subsequently stained intracellularly with anti-IFN-γ, -TNF-α, or -granzyme B.

Real-time quantitative PCR

Total RNA was isolated from purified cells using TRIzol reagent (Invitrogen Life Technologies) and cDNA was generated using a reverse transcription kit (Promega). Real-time PCR was performed using an iCycler (Bio-Rad) to measure SYBR green incorporation. The following primer sets were used: Bcl-xL, 5'-TGGTGTCGACCTTCTCTCC-3', 5'-CTCCA TCCGAAAGAGTTCA-3'; TRAIL, 5'-TCACCAACGAGATGAAGC AG-3', 5'-GGCCTAAGGTCTTTCCATCC-3'. Amounts of mRNA were normalized to hypoxanthine phosphoribosyltransferase RNA levels within each sample.

Memory T cell isolation and secondary transfer

Purified clone 4 CD8 T cells were adoptively transferred into naive mice as described above. Forty-five days post-rVV-HA infection, mice were sacrificed and spleen, superficial lymph nodes, and mesenteric lymph nodes were pooled. Cells were stained with PE-conjugated anti-Thy1.1 and FITC-conjugated anti-CD8. Thy1.1^+ T cells were positively selected using anti-PE beads according to the manufacturer’s instructions (Miltenyi Biotec). Enriched Thy1.1^+ cells were then subjected to cell sorting gated on Thy1.1^+CD8^+ with a high speed cell sorter FACSVantage (BD Biosciences). The purity of FACS-sorted populations of cells was 95%.

Ovary VV titer assay

Viral load in the ovaries was measured by plaque-forming assay as previously described (31). A total of 10^4 purified clone 4 CD8 T cells were transferred into female mice that were subsequently infected with 5 × 10^6 PFU rVV-HA. Mice were sacrificed 3 or 28 days postinfection and ovaries were harvested and stored at −80°C. Ovaries from individual mice were
homogenized and freeze-thawed three times. Serial dilutions were performed and the viral titers were determined by plaque assay on confluent TK-143B cells.

Statistical analysis
Results were expressed as mean ± SD. Differences between groups were examined for statistical significance using the Student t test.

Results
CD4 T cell help is needed for clonal expansion of CD8 T cells during the primary response to VV infection
To better understand the role of CD4 T cells in primary and secondary CD8 T cell responses to infection, we used a model of influenza HA-specific CD8 T cell response to rVV-HA in vivo. A total of 10⁴ naive clone 4 CD8 T cells were transferred to WT, GK1.5-treated WT (+GK1.5), or CD4⁻/⁻ mice that were subsequently infected with rVV-HA. Some WT mice were left uninfected (Naive). Seven days later, splenocytes were harvested for subsequent analysis. A, Annexin V staining. The percentage of Annexin V⁺ cells among clonotypic cells is indicated. B and C, Clonotypic cells were purified by cell sorting and subjected to real-time quantitative PCR to measure the expression of Bcl-xL (B) and TRAIL (C). Data are presented as normalized mRNA abundance to hypoxanthine phosphoribosyltransferase. D, Cells from different recipients were stained with Bcl-xL intracellularly or an isotype control Ab (Isotype). The MFI of clonotypic cells is indicated. Plots are gated on CD8⁺Thy1.1⁺ cells. Data shown are representative of three independent experiments.

CD4 T cells promote the survival of activated CD8 T cells during priming in vivo. A total of 10⁴ purified naive clone 4 CD8 T cells were transferred to WT, GK1.5-treated WT (+GK1.5), or CD4⁻/⁻ mice that were subsequently infected with rVV-HA. Some WT mice were left uninfected (Naive). Seven days later, splenocytes were harvested for analysis. As in CD4⁻/⁻ mice, clone 4 CD8 T cells in the CD4-depleted mice had a significant (p < 0.001) reduction in clonal expansion compared with the untreated WT mice (Fig. 1). Collectively, these results indicate that CD4 T cell help is critical for clonal expansion during the primary response to VV infection in vivo.

CD4 T cells in CD8 T cell responses

FIGURE 3. CD4 T cells promote the survival of activated CD8 T cells during priming in vivo. A total of 10⁴ purified naive clone 4 CD8 T cells were transferred to WT, GK1.5-treated WT (+GK1.5), or CD4⁻/⁻ mice that were subsequently infected with rVV-HA. Some WT mice were left uninfected (Naive). Seven days later, splenocytes were harvested for analysis. As in CD4⁻/⁻ mice, clone 4 CD8 T cells in the CD4-depleted mice had a significant (p < 0.001) reduction in clonal expansion compared with the untreated WT mice (Fig. 1). Collectively, these results indicate that CD4 T cell help is critical for clonal expansion during the primary response to VV infection in vivo.

FIGURE 4. Diminished, but relatively stable, CD8 memory pool can develop in the absence of CD4 T cells. A total of 10⁴ purified naive clone 4 CD8 T cells were transferred to WT, GK1.5-treated WT (+GK1.5), or CD4⁻/⁻ mice that were subsequently infected with rVV-HA. A, Seven, 15, 28, and 55 days after infection, splenocytes were stained with anti-CD8 and -Thy1.1. The mean absolute numbers with SDs of clonotypic cells is indicated. B–E, Fifty-five days after infection, the mean absolute numbers with SDs of clonotypic cells per spleen (B), combined six peripheral LNs (C), combined six Peyer’s patches (D), and whole liver (E), are indicated. Data are representative of two independent experiments.
CD8 T cell activation and effector differentiation in response to VV infection is not affected by a lack of CD4 T cell help

We next investigated what contributed to the defect in CD8 T cell expansion during the primary response to VV infection in the absence of CD4 T cells. One possibility is that CD8 T cells are not fully activated without CD4 T cell help. To address this, we transferred 10^6 naive clone 4 CD8 T cells into WT, GK1.5-treated, or CD4^-/- mice and subsequently infected the hosts with 5 x 10^5 PFU rVV-HA. Higher clone 4 T cell numbers (10^6) were used due to the fact that 10^4 transferred cells were below the limit of detection at early time points. Twenty-four hours after infection, clone 4 CD8 T cells in WT, GK1.5-treated, and CD4^-/- mice displayed a similarly activated phenotype of CD44 high and CD69 low (Fig. 2A). Three days after infection, CFSE-labeled clone 4 CD8 T cells in WT, GK1.5-treated, and CD4^-/- mice underwent several rounds of division similarly by CFSE dilution (Fig. 2B), suggesting CD8 T cell proliferation was also not affected by a lack of CD4 T cell help. Furthermore, despite a reduced clonal size, the effector differentiation of clone 4 CD8 T cells in both GK1.5-treated and CD4^-/- mice appeared to be intact at day 7 after infection as the production of IFN-γ on a per cell basis (as measured by mean fluorescence intensity (MFI)) was similar to that in WT mice (Fig. 1A). Similarly, the production of other effector molecules such as TNF-α and granzyme B appeared to be normal in GK1.5-treated and CD4^-/- mice compared with that in WT mice (Fig. 2C). Additionally, the phenotype of effector CD8 T cells as measured by CD62L down-regulation, CD122 up-regulation, and CD127 re-up-regulation was not affected by the lack of CD4 T cells in both the GK1.5-treated and the CD4^-/- hosts as compared with WT mice (Fig. 2D). These data suggest that CD8 T cell activation and effector differentiation in response to VV infection in vivo is not affected by a lack of CD4 T cell help.

The survival of activated CD8 T cells during priming is dependent on CD4 T cell help

Because CD8 T cell activation, proliferation, and effector differentiation do not appear to be altered due to the lack of CD4 help, we then asked whether the difference in clonal expansion could be due to decreased survival of the activated CD8 T cells in the absence of CD4 T cells. We used annexin V staining to assess CD8 T cells undergoing apoptosis. A total of 10^4 naive clone 4 CD8 T cells were transferred into WT, GK1.5-treated, or CD4^-/- mice, followed by infection with 5 x 10^5 PFU rVV-HA. Seven days after infection, mice were harvested for analysis. Indeed, activated clone 4 CD8 T cells in both GK1.5-treated and CD4^-/- mice displayed a significant (p < 0.001) increase in annexin V positivity (51.1 and 55.7%, respectively) compared with WT mice (20.3%, Fig. 3A). This increased apoptosis of activated CD8 T cells in the absence of CD4 T cell help correlated with a significant (p < 0.001) reduction in the expression of the prosurvival molecule, Bcl-xL, at both the message RNA and protein levels (Fig. 3, B and D). TRAIL expression has been implicated in regulating secondary expansion of the “helpless” memory CD8 T cells (33). Here, we showed that TRAIL expression was also significantly (p < 0.001) up-regulated in the activated CD8 T cells during primary response to VV infection in the absence of CD4 T cells (Fig. 3C). Taken together, these results suggest that the diminished

![Figure 5](http://www.jimmunol.org/) Phenotypic and functional analyses of the “helpless” memory CD8 T cells. A total of 10^6 purified naive clone 4 CD8 T cells were transferred to WT, GK1.5-treated WT (+GK1.5), or CD4^-/- mice that were subsequently infected with rVV-HA. Fifty-five days later, ovaries were harvested and lysate was used to determine viral titer by plaque assay.
clonal expansion of CD8 T cells in response to VV infection in the absence of CD4 T cells is not caused by a reduction in T cell activation, but by poor survival of activated CD8 T cells.

**Diminished, but relatively stable, CD8 memory pool can develop in absence of CD4 T cells**

We next determined the ability of effector CD8 T cells to develop into stable memory cells in the absence of CD4 T cell help. After the peak of clonal expansion at day 7, splenic clone 4 effector CD8 T cells in the WT recipients underwent marked contraction between days 7 and 14, and those that survived developed into stable memory CD8 T cells (Fig. 4, A and B). This is consistent with previous observations in other models of bacterial or viral infections (1, 2, 6). Similarly, after contraction, clone 4 effector CD8 T cells generated in both the GK1.5-treated and CD4−/− hosts were also capable of differentiating into memory cells, but with a significant (p < 0.001) reduction in memory size that was proportional to the size of effectors (Fig. 4, A and B). This reduction was not a result of differential homing of memory cells in the absence of CD4 T help as a similar degree of decrease was observed in other lymphoid and nonlymphoid organs such as peripheral lymph nodes (Fig. 4C), Peyer’s patch (Fig. 4D), and liver (Fig. 4E), in both the GK1.5-treated and CD4−/− mice. Neither was this decrease in the memory size due to a persistent viral infection, as viral titers performed on day 28 after infection showed that the virus was cleared in the GK1.5-treated and CD4−/− hosts as efficiently as WT mice (Table I). Despite a reduction in their size, the memory CD8 T cells generated in the GK1.5-treated and CD4−/− hosts appeared relatively stable at least up to day 55 after infection (Fig. 4A). Furthermore, the production of the effector molecules IFN-γ, TNF-α, and granzyme B, as well as the expression of the surface markers CD62L, CD122, and CD127 appeared to be similar in the WT, GK1.5-treated, and CD4−/− mice (Fig. 5). Thus, a diminished, but relatively stable memory CD8 pool can develop following VV infection in the absence of CD4 T cell help.

**CD4 T cell help is also required for CD8 memory recall expansion following secondary challenge**

One hallmark of memory cells is a rapid and more efficacious response upon secondary encounter with a pathogen. It is not entirely clear what controls this rapid recall potential of memory cells. Previous studies have suggested that CD4 T cell help during the primary response is needed for the generation of fully functional memory cells that can respond to secondary challenge rapidly (15, 23, 24). However, it is less clear whether CD4 T cells are needed following rechallenge for recall expansion. If so, what is the relative contribution of CD4 T cell help provided during the primary response vs following rechallenge to the recall expansion? To address these questions, clone 4 memory CD8 T cells were purified by FACS sorting from WT, GK1.5-treated, or CD4−/− mice 45 days after infection with rVV-HA. Equal numbers (3.5 × 10^5) of purified memory cells were then transferred into naive WT, GK1.5-treated, or CD4−/− mice that were subsequently challenged with 5 × 10^5 PFU rVV-HA i.p. Seven days after rechallenge, splenocytes were analyzed for the recall expansion and effector function of transferred memory cells. Vigorous recall expansion of clone 4 CD8 T cells was detected in WT recipients that received memory cells from WT donors, whereas the extent of recall expansion was significantly (p < 0.05) reduced in WT recipients transferred with memory cells from either GK1.5-treated or CD4−/− donors (Fig. 6). This is consistent with the notion that CD4 T cell help during the primary response provides the necessary “instructive” signals for the generation of fully functional memory cells (15, 23, 24). To our surprise, a much greater reduction in recall expansion (p < 0.001) was observed when memory cells from WT, GK1.5-treated, or CD4−/− donors were transferred into either CD4-depleted or CD4−/− recipients (Fig. 6). Similar results were obtained when mice were challenged with recombinant adenovirus-expressing HA (data not shown). These results indicate that CD4 T cell help provided following secondary challenge is also critical for recall expansion of memory CD8 T cells, in addition to that provided during the primary response.

**CD4 T cell help promotes the survival of CD8 T cells during recall expansion**

Despite the compromised recall expansion in the absence of CD4 T cell help either during the primary response or following rechallenge, the effector function of clone 4 CD8 T cells after recall expansion decreased to a similar level in the WT and CD4−/− hosts as expected. Therefore, while CD4 T cell help is needed for CD8 memory pool stability and rapid recall potential upon rechallenge, the primary response is not essential for the survival of memory CD8 T cells in the absence of CD4 T cell help.
expansion appeared to be intact as their ability to produce IFN-γ on a per cell basis (as measured by MFI) was similar to that of “helped” WT control (Fig. 6B). This result suggested that the dependency of CD8 memory recall expansion on CD4 T cell help was likely mediated by promoting their survival, similar to our observations during the primary response. To address this question, clone 4 memory CD8 T cells (3.5 × 10^5) were purified from the WT, GK1.5-treated, or CD4^{-/-} donors 45 days after infection with rVV-HA and transferred into naive WT, GK1.5-treated (+GK1.5) WT, or CD4^{-/-} recipients that were subsequently infected with VV-HA. Seven days postchallenge, spleens were harvested and stained with anti-CD8, anti-Thy1.1, and annexin V. The percentage of Annexin V^+ cells among clonotypic cells is indicated. Plots are gated on CD8^+Thy1.1^+ cells. Data shown are representative of three independent experiments.

**FIGURE 7.** The survival of memory CD8 T cells during a recall expansion is dependent on CD4 T cells. A total of 3.5 × 10^5 memory cells were purified from day 45 rVV-HA-infected WT, GK1.5-treated (+GK1.5) WT, or CD4^{-/-} mice and adoptively transferred into naive WT, GK1.5-treated (+GK1.5) WT, or CD4^{-/-} recipients that were subsequently infected with VV-HA. Seven days postchallenge, spleens were harvested and stained with anti-CD8, anti-Thy1.1, and annexin V. The percentage of Annexin V^+ cells among clonotypic cells is indicated. Plots are gated on CD8^+Thy1.1^+ cells. Data shown are representative of three independent experiments.

Discussion

In this study, we have shown that CD4 T cell help plays a critical role in both primary and memory CD8 T cell responses to VV infection. We demonstrate that although CD4 T cell help is not needed for activation and effector differentiation of Ag-specific CD8 T cells during a primary CD8 T cell response to VV infection, the survival of activated CD8 T cells is dependent on CD4 T cell help, leading to a reduction in clonal expansion and a diminished, but stable, CD8 memory pool. Furthermore, we also demonstrate that the “help” provided by CD4 T cells both during the primary response and following secondary challenge is required for recall expansion of memory CD8 T cells by promoting their survival.

It has been well-documented that CD4 T cell help is important for the induction of primary CD8 T cell response to non-inflammatory Ags such as minor histocompatibility Ags, tumor Ags, or protein Ag in vivo (7-11). This is achieved by activating or “licensing” the DCs through CD40-CD40L interactions between DCs and CD4 T cells (12-14) or via direct CD40-CD40L interactions between CD8 and CD4 T cells (15). On the contrary, it had been initially thought that primary CD8 T cell response to infectious pathogens is largely independent of CD4 T cell help as pathogens can provide the inflammatory signals to promote full activation of DCs (16-18). However, some primary CD8 T cell responses to pathogens such as adenovirus (19), influenza virus (20), HSV-1 (21), and L. monocytogenes (22), are CD4 T cell help dependent. Because direct CD40-CD40L interaction between CD8 and CD4 T cells is not involved in these infections (34, 35), it has been unclear why CD4 T cell help is needed in these settings. Our results presented here demonstrate that CD4 T cell help is also required for primary CD8 T cell response to VV infection in vivo. Consistent with the notion that pathogens can activate DCs directly for efficient T cell priming and thus bypass the need for CD4 T cell help (18), the activation and effector differentiation of CD8 T cells during the primary response to VV infection is independent of CD4 T cells. However, the survival of activated CD8 T cells is critically dependent on CD4 T cell help and as a result, the clonal expansion of Ag-specific CD8 T cells is diminished without CD4 T cell help.

How does CD4 T cell help promote the survival of activated, Ag-specific CD8 T cells during the primary response in vivo? It is possible that CD4 T cells could either directly provide survival signals to activated CD8 T cells or indirectly act on an intermediate cell that provides CD8 T cells with such signals. A recent report in vitro has implicated CD4 T cells in protecting activated CD8 T cells from activation-induced cell death (AICD) through a direct cell-to-cell contact mechanism (36). Although AICD of CD4 T cells has been considered to be mediated by Fas-FasL interaction (37, 38), it remains controversial which death receptors are involved in AICD of CD8 T cells. Regulation of TRAIL expression by CD4 T cell help has been implicated in protecting memory CD8 T cells from AICD during a recall expansion (33). However, a recent study has suggested that CD4 T cell help consists of both TRAIL-dependent and -independent mechanisms (39). In line with these observations, we provided evidence that in the absence of CD4 T cell help, TRAIL expression is up-regulated in the activated CD8 T cells during the primary response to VV infection. In addition, there is a significant reduction in the expression of the pro-survival molecule, Bcl-xL, in the “helpless” CD8 T cells, suggesting that the intrinsic apoptotic pathway (40) may also be involved in CD4 T cell-mediated protection of activated CD8 T cells from AICD in vivo. Thus, future studies will be needed to elucidate the protective signals that CD4 T cells provide, and the signaling pathways involved in promoting the survival of activated CD8 T cells during the primary response in vivo.

Despite the poor survival of activated CD8 T cells without CD4 T cell help during priming, which leads to a reduction in clonal expansion, these “helpless” effector CD8 T cells can develop into relatively stable memory cells albeit with a diminished memory size that is proportional to the size of effector T cells. This suggests...
that after contraction phase, the maintenance of memory CD8 T cells after VV infection is independent of CD4 T cells. This is in contrast to the previous observation that the maintenance of memory CD8 T cells after an acute infection with lymphocytic choriomeningitis virus (LCMV) is compromised in MHC class II-deficient mice that lack CD4 T cells (26). The reasons for the discrepancy are not clear, but could be related to the pathogens used for the experiments. Indeed, recent studies have shown that the requirement for CD4 T cell help in memory CD8 T cell maintenance might be pathogen specific (20, 22). We have further observed that the “helpless” memory CD8 T cells are similar to the “helped” ones phenotypically as measured by the expression of CD62L, CD122, and CD127, as well as functionally in terms of the production of the effector molecules such as IFN-γ, TNF-α, and granzyme B. This is in contrast to a previous report with LCMV that the “helpless” memory CD8 T cells showed a CD62Llow CD122low phenotype, suggesting a defect in the formation of CD62Lhigh central memory cells (41). Again, it is not clear what contributes to the differences, but might be pathogen related.

The requirement for CD4 T cells in promoting fully functional memory CD8 T cells that can respond rapidly upon secondary challenge has been well-studied (15, 23–25). However, the majorities of studies have focused on the CD4 T cell help provided during initial priming phase, which delivers the necessary “instructive” signals for the generation of fully functional memory CD8 T cells. It is less clear whether CD4 T cells are also needed following secondary challenge for the rapid recall expansion. Consistent with the previous observations, we have shown in this study that indeed CD4 T cell help is required during the primary response to VV infection for the generation of rapid recall response. We have also demonstrated that the presence of CD4 T cells following a secondary challenge is also crucial to the recall expansion of memory CD8 T cells. Our results are in contrast to the observations by Shedlock and Shen (24). In their study, only CD4 T cell help provided during initial priming with the VV-encoding gp33–41 epitope from LCMV was important for recall expansion following a secondary challenge. However, LCMV was used for the secondary challenge instead of VV. Because VV has been used extensively as vaccine vehicles for infectious diseases and cancer, our results may be more relevant to the design of effective vaccine strategies.

We have also provided evidence that defective recall expansion in the absence of CD4 T cell help either during the primary response or following rechallenge is due to poor survival. Similar to the requirement of CD4 T cells for the survival of CD8 T cells during the primary response, the mechanism(s) underlying the dependency of memory CD8 T cell survival on CD4 T cell help during recall expansion remains to be defined. Although TRAIL expression has been implicated in regulating the memory CD8 T cells that lack CD4 T cell help during initial priming from AICD during a recall expansion (33), it is not clear whether the same mechanism applies to the CD4 T cell help provided following secondary challenge. Future studies are needed to delineate the exact mechanism(s) by which CD4 T cells promote the survival of memory CD8 T cells during a recall expansion in vivo.

In summary, we have demonstrated that CD4 T cells are crucial to both primary and memory CD8 T cell responses to VV infection. This is achieved by promoting the survival of Ag-specific CD8 T cells during the initial priming and the recall expansion following rechallenge. As one major goal of vaccination is to maximize the magnitude of CD8 T cell response and to generate fully functional memory CD8 T cells, our results may have important implications for the design of effective strategies for treating infectious diseases and cancer.

Disclosures

The authors have no financial conflict of interest.

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