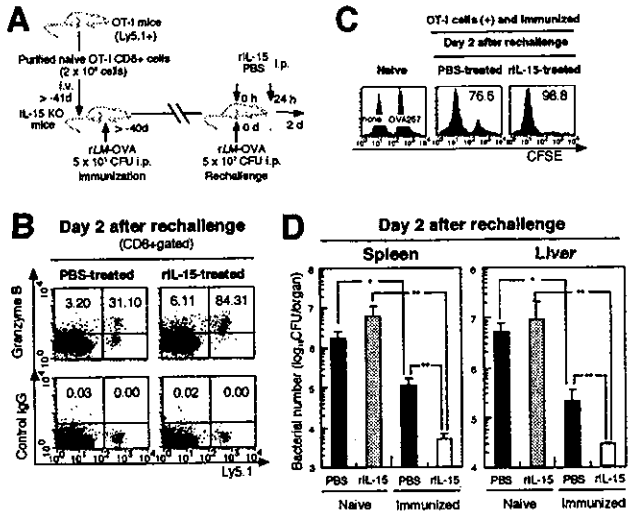

FIGURE 4. In vivo administration of exogenous IL-15 directly induced the expression of cytotoxic effector molecules in memory $CD8^+$ T cells after secondary *infection. A,* Purified naive OT-I cells were adoptively transferred into naive IL-15 KO hosts that were immunized with rLM-OVA 24 h later. At 40 or more days after immunization, IL-15 KO mice harboring memory OT-I cells were rechallenged with a lethal dose of rLM-OVA. rIL-15 (2 μg) or PBS for control was injected i.p. at 0 and 24 h after rechallenge with rLM-OVA. *B,* On day 2 after rechallenge with rLM-OVA, splenocytes from rIL-15-treated and PBS-treated mice harboring memory OT-I cells were prepared and intracellular granzyme B staining was performed. Dot plots are gated on $CD8^+$ cells, and the number indicated is the percentage of donor cells ($Ly5.1^+$) or recipient cells ($Ly5.1^-$) stained positive for granzyme B or the isotype control. Data are representative of three independent experiments using pooled cells from three mice and are shown as typical two-color profiles. C, Spleen cells from naive mice ($Ly5.1^+Ly5.2^+$) were pulsed with OVA peptides or left unpulsed and then injected i.v. into rIL-15-treated or PBS-treated *IL-15* KO mice rechallenged with rLM-OVA 2 days previously, and then in vivo CTL activity was examined at 5 h after adoptive transfer in target cells. Histograms are gated on $Ly5.1^+Ly5.2^+$ cells in the spleen from infected mice. The values in the *right corner* of each panel represent the percentage of specific killing compared with nonpulsed cells. *D,* The numbers of bacteria in the spleens and livers from rIL-15-treated or PBS-treated IL-15 KO mice harboring memory OT-I cells were determined on day 2 after secondary infection. Data were obtained from three separate experiments, and each value shown is the mean +SD for five mice. *, $p < 0.05$; **, $p < 0.01$.

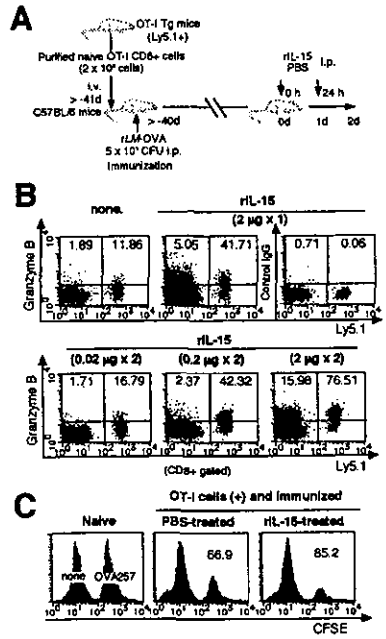

FIGURE 5. In vivo administration of exogenous IL-15 alone can induce the expression of cytotoxic effector molecules and in vivo CTL activities in memory $CD8^+$ T cells. *A,* Purified naive OT-I cells were adoptively transferred into naive C57BL/6 hosts that were immunized with rLM-OVA 24 h later. At 40 or more days after immunization, C57BL/6 mice harboring memory OT-I cells were injected i.p. with various doses of rIL-15 or PBS for control. *B,* At 24 h after administration of various doses of rIL-15 once or twice, mice were sacrificed and expression of intracellular granzyme B was analyzed. Dot plots are gated on $CD8^+$ cells, and the number indicated is the percentage of donor cells ($Ly5.1^+$) or recipient cells ($Ly5.1^-$) stained positive for granzyme B or the isotype control IgG. *C,* C57BL/6 mice harboring memory OT-I cells were injected i.p. with 2 μg rIL-15 or PBS for control. At 24 h after a single injection of rIL-15, spleen cells from naive mice ($Ly5.1^+Ly5.2^+$) were pulsed with OVA peptides or left unpulsed and then injected i.v. into each mouse. Then in vivo killer activity at 5 h after adoptive transfer in targets cells was examined. Histograms are gated on $Ly5.1^+Ly5.2^+$ cells in the spleen. The values in the *right corner* of each panel represent the percentage of specific killing compared with nonpulsed cells.

those in the case of PBS administration. These results suggest that IL-15 plays an important role in the induction of effector *functions* in Ag-specific memory $CD8^+$ CTL following re-exposure to microbes.

IL-15 has been reported to directly up-regulate expression of cytotoxic molecules such as granzyme B and perforin that are closely correlated with cytotoxicity effector function of human $CD8^+$ memory cells in vitro (25). Therefore, we next investigated whether in vivo administration of rIL-15 alone can induce cytotoxic activity of memory OT-I cells. C57BL/6 mice harboring memory OT-I cells were injected i.p. with various dose of rIL-15 (Fig. 5*A*), and the expression levels of intracellular granzyme B and the cytolytic activity levels of splenic memory OT-I cells at 24 h after administration of various doses of *rIL-15 once* or twice were examined. As shown in Fig. 5*B*, *upper panel*, memory OT-I cells contained low levels of granzyme B before rIL-15 treatment, but high intracellular levels of granzyme B in memory OT-I cells had been induced at 24 h after a single administration of 2 μg rIL-15. Furthermore, injection of 2 μg rIL-15 twice induced ~80% of expression levels of intracellular granzyme B in memory OT-I cells in the absence of TCR triggering, and this up-regulation occurred in a dose-dependent manner (Fig. 5*B*, *lower panel*). Intracellular expression levels of granzyme B in endogenous $CD8^+$ T cells were also significantly increased after administration of rIL-15. In correlation with the expression of *intracellular* granzyme B in memory OT-I cells, in vivo CTL activity was significantly increased in rIL-15-treated mice compared with that in PBS-treated control mice at 24 h after a single administration of 2 μg rIL-15 (Fig. 5*C*). These results suggest that the ability to induce granzyme B in response to IL-15 is independent of prior Ag challenge.

Discussion

In the present study, we examined the roles of IL-15 in expansion and activation of Ag-specific naive and memory $CD8^+$ T cells by direct comparison of naive and memory $CD8^+$ T cells that exhibit the same Ag specificity for $OVA_{257-264}/K^b$ *in* experiments on adoptive transfer into IL-15 Tg mice and IL-15 KO mice after infection with rLM-OVA. The absolute numbers of and the frequencies of division of naive $OVA_{257-264}/K^b$-specific $CD8^+$ T cells in IL-15 Tg mice and IL-15 KO mice were almost the same as those in control C57BL/6 mice after primary infection with

rLM-OVA, confirming that IL-15 is not essential in priming naive CD8$^+$ T cells for expansion and differentiation into effector CTL following microbial infection. In contrast, in vivo CTL activity levels of memory $OVA_{257-264}/K^b$-specific CD8$^+$ T cells were significantly higher in IL-15 Tg mice but lower in IL-15 KO mice at the early stage of secondary immune response, well before the division of memory CD8$^+$ T cells occurred. Moreover, in vivo administration of exogenous IL-15 confers robust protection against reinfection via induction of a cytotoxic molecule in memory CD8$^+$ T cells. These results suggest that IL-15 plays an important role in early activation of Ag-specific memory CD8$^+$ T cells following secondary infection with microbes.

It is notable finding that in vivo CTL activity levels of memory OT-I cells were significantly higher in IL-15 Tg mice but lower in IL-15 KO mice at the early stage of reinfection, well before the division of memory CD8$^+$ T cells occurred. Perforin/granzyme-mediated cytolysis is the major pathway involved in lysis of target cells infected with intracellular pathogens. It has been reported that perforin-mediated cytolysis is an essential effector function in CD8$^+$ T cell-mediated secondary resistance to *L. monocytogenes* (32, 33). We demonstrated that in correlation with in vivo CTL activity levels, the expression levels of granzyme B in memory OT-I CD8$^+$ T cells were significantly higher in IL-15 Tg mice but lower in IL-15 KO mice at the early stage after secondary infection. IL-15 has been reported to directly up-regulate expression of cytotoxic molecules such as granzyme B and perforin that are closely correlated with cytotoxicity effector function of CD8$^+$ memory cells in vitro (25). We showed in the present study that in vivo administration of exogenous IL-15 alone could induce up-regulation of intracellular granzyme B in memory CD8$^+$ T cells in C57BL/6 mice. There have been several lines of evidence for IL-15 production by nonlymphoid cells after infection with various microbes (34–42). A sufficiently high concentration of IL-15 produced by macrophages and epithelial cells might induce up-regulation of cytotoxic molecules in Ag-specific memory CD8$^+$ T cells at the early stage after secondary infection with microbes and contribute to rapid elimination of reinvading microbes.

Two subsets of memory CD8$^+$ T cells based on their anatomical location, expression of cell surface markers, and effector functions have been described (30, 31). Memory CD8$^+$ T cells expressing homing receptors such as CD62L and CCR7, which allow efficient homing to LN, are termed T_{CM}, whereas memory T cells lacking these LN homing receptors, which are located in nonlymphoid tissues, are termed T_{EM}. T_{CM} have been reported to produce few effector molecules but to have a high proliferative capacity in response to IL-2/IL-15 in autocrine and/or paracrine manners (43). In contrast, T_{EM} cells, which have greater cytolytic effector functions, facilitate their entry into infected tissues and play a role as the first line of host defense against re-exposure to microbes (30). However, the T_{EM} population has little homeostatic proliferative potential, and this subset therefore does not seem to be a permanent memory population (43). Although we did not separate CD8$^+$ T_{CM} and T_{EM} from memory OT-I cells in the spleen, IL-15 may affect mainly the function of CD8$^+$ T_{EM} because intracellular granzyme B was up-regulated in memory CD8$^+$ T cells well before cell division occurred at the early stage after secondary infection. CD8$^+$ T_{EM}, which reside mainly in nonlymphoid tissues, serve as the fist line of host defense against microbial invasion.

It has been reported that memory CD8$^+$ T cells expressing a Tg $\alpha\beta$ TCR specific for the male Ag expanded more than did their naive counterparts and that they accumulated much faster in recombination activating gene-2-deficient female mice (44). In contrast, a recent study has suggested that there was no significant difference between naive and memory CD8$^+$ T cells in their proliferative capacities after LCMV infection in naive normal hosts using a system of adoptive transfer of CD8$^+$ T cells from P14 Tg mice (specific for the GP-33 LCMV epitope) (11). We also found no difference between kinetics of the division of naive and memory OT-I cells transferred into naive hosts after rLM-OVA infection (Figs. 1*B* and 2*B*). Thus, there may not be a marked difference between naive and memory CD8$^+$ T cells in their proliferative capacities in vivo after Ag re-exposure in naive hosts. However, in physiological conditions of secondary immune response, the help of memory CD4$^+$ T cells in expansion of memory CD8$^+$ T cells must be considered. Tanchot and Rocha (45) reported that CD4$^+$ T cells are required for expansion of memory CD8$^+$ T cells but that they are no longer needed for their function. Consistent with this finding, we found that in vivo depletion of CD4$^+$ T cells completely inhibited the early expansion of memory OT-I cells in immunized hosts after rLM-OVA reinfection (our unpublished data). These results suggest that memory CD4$^+$ T cells are indispensable for early expansion of memory CD8$^+$ T cells after secondary infection and that memory CD8$^+$ T cells may not expand in an autocrine manner during secondary infection. In is most likely that IL-2 derived from CD4$^+$ T cells is important for expansion of memory CD8$^+$ T cells during secondary immune responses. However, Tuma et al. (46) reported that CD40L/CD40 signaling is required for long-lasting protective immunity by transferred memory CD8$^+$ T cells against *Listeria* infection. Therefore, it is possible that both IL-2 and CD40L provided by activated CD4$^+$ T cells may be required for rapid expansion of memory CD8$^+$ T cells during secondary immune responses. Additional experiments are needed to clarify these possibilities.

In conclusion, IL-15 plays important roles not only in maintenance of memory CD8$^+$ T cells by homeostatic proliferation in the absence of Ag but also in the early activation of memory CD8$^+$ T cells as secondary effector cells when microbes invade again. In vivo administration of rIL-15 to enhance cytotoxic activities of Ag-specific memory CD8$^+$ T cells may be used for controlling microbial infection in vaccinated hosts and treating patients with chronic viral and bacterial infection or malignancy.

Acknowledgments

We thank Yoko Kobayashi and Kazue Kaneda for their excellent technical assistance.

Disclosures

The authors have no financial conflict of interest.

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