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A Novel Role of CD30/CD30 Ligand Signaling in the Generation of Long-Lived Memory CD8⁺ T Cells

Hitoshi Nishimura,* Toshiki Yajima,* Hiromi Muta,† Eckhard R. Podack,‡ Kenzaburo Tani,† and Yasunobu Yoshikai*2

Memory CD8⁺ T cells can be divided into two subsets, central memory (TCM) and effector memory (TEM) CD8⁺ T cells. We found that CD30, a member of the TNFR-associated factor (TRAF)-linked TNFR superfamily, signaling is involved in differentiation of long-lived CD8⁺ TCM cells following *Listeria monocytogenes* infection. Although CD8⁺ TEM cells transiently accumulated in the nonlymphoid tissues of CD30 ligand (CD153⁻/⁻) mice after infection, long-lived memory CD8⁺ TCM cells were poorly generated in these mice. CCR7 mRNA expression was down-regulated in CD8⁺ T cells of the spleen of CD153⁻/⁻ mice in vivo and the expression was up-regulated in CD8⁺ TEM cells by anti-CD30 mAb cross-linking in vitro. These results suggest that CD30/CD30 ligand signaling plays an important role in the generation of long-lived memory CD8⁺ T cells at least partly by triggering homing receptors for TCM cells. *The Journal of Immunology*, 2005, 175: 4627–4634.

Generation and maintenance of long-lived CD8⁺ T cell memory are important goals of vaccination because they can provide protection against reinfection of many pathogens, including intracellular bacteria (1). Immune response against the pathogens depends on the ability of lymphocytes to migrate to appropriate places within the body to find their cognate Ags (2). Memory CD8⁺ T cells have been divided into two subsets based on their anatomical location, expression of cell surface markers, and effector functions (3). Memory T cells expressing homing receptors such as CD62 ligand (CD62L)³ and CCR7, which allow efficient homing to lymph nodes (LNs), are termed central memory (TCM) cells, whereas memory T cells lacking these LN-homing receptors, which are located in nonlymphoid tissues, are termed effector memory (TEM) cells. TCM cells have been reported to produce few effector molecules but to have homeostatic proliferative capacity in response to IL-15 (4, 5). In contrast, TEM cells are thought to facilitate their entry into infected tissues expressing cognate effector molecules for these receptors (4, 5). It is suggested that TCM and TEM cells do not necessarily represent distinct subsets and that they are part of a continuum in a linear naive effector-TEM-TCM differentiation pathway (4). In contrast, there is some evidence that TCM and TEM cells might be generated differentially during primary immune response depending on the conditions of activation (5). However, very little is known about signaling that regulates the differentiation pathway of these memory T cells.

Signaling via the TNFR superfamily regulates the fate of activated T cells (6). Members of the TNFR superfamily such as Fas and TNFR type I, which contain a death domain in the cytoplasmic tail, are responsible for activation-induced T cell death (7), whereas members having no death domain such as CD40 and CD70 play a key role in effective T cell immunity (6). For example, generation of memory CD8⁺ T cells displaying an enhanced capacity for cell division and cytokine secretion requires CD40L expression by CD4⁺ T cells (8–11). CD30, a member of the TNFR superfamily, is expressed by activated or memory CD8⁺ T cells but not by resting B or T cells (12–14). A CD30 ligand (CD30L, CD153) is a 40-kDa type II membrane-associated glycoprotein belonging to the TNF family (15). CD153 is expressed on macrophages, dendritic cells, and B cells (6, 14–16). Little is known about the role of CD30/CD30L signaling in generation of memory CD8⁺ T cells, although there are several lines of evidence showing that the signaling regulates peripheral T cell response through controlling T cell survival and down-regulation of cytolytic capacity (17–22).

We previously reported that gene expression of CCR7 is up-regulated by CD30 stimulation in the human YT lymphoma cell line (23, 24). This raises the possibility that CD30/CD30L signaling plays an important role in differentiation of memory T cell subsets following Ag exposure. In the present study, we found with CD153⁻/⁻ mice that CD30/CD30L signaling is involved in differentiation of CD8⁺ TCM cells following exposure to a microbe.

Materials and Methods

Mice

Age- and sex-matched BALB/c mice were obtained from Charles River Breeding Laboratories. The generation and preliminary characterization of CD153⁻/⁻ mice were previously described (24). All mice were maintained under specific pathogen-free conditions and were offered food and water ad libitum. All mice were used at 6–8 wk of age.
Microorganism

*Listeria monocytogenes* strain EGD was used in all experiments. Bacterial virulence was maintained by serial passages in BALB/c mice. Mice were inoculated i.p. with various doses of viable *L. monocytogenes* in 0.2 ml of PBS on day 0. The spleen and liver were removed from each mouse and separately placed on trypto-soya agar plates, and colonies were counted after incubation for 24 h at 37°C.

Abs and reagents

FITC-conjugated anti-CD44 (4M7), anti-CD69 (H1.2F3), and anti-IFN-γ (XMG1.2) PE-conjugated anti-CD8 (53-6.7), anti-CD44 (IM7), and anti-CD62L (MEL-14); and CyChrome-conjugated anti-CD8α (53-6.7) and anti-CD4 (RM4-5) were purchased from BD Pharmingen. CyChrome and allophtococyanin-conjugated streptavidin were also obtained from BD Pharmingen. CFSE was purchased from Molecular Probes.

Generation of H2-K^I^ tetramers

MHC-peptide tetramers for staining of epitope-specific cells were generated as previously described (25, 26). Briefly, purified H chain and β2m-microglobulin were dissolved in 8 M urea and diluted in a refolding buffer containing a high concentration of synthetic peptide listeriolysin O (LLO)91–99 (27) or JAK1 self-epitope (28) to generate monomeric soluble H2-K^I^-peptide complexes. Biotinylation and tetramerization of the heterodimer were performed as described by Altman et al. (25). The monomeric complexes were tetramerized by the addition of PE-labeled streptavidin (BD Pharmingen) at a molar ratio of 4:1.

Flow cytometry analysis

The cells were incubated with saturating amounts of FITC-, PE-, CyChrome-, and biotin-conjugated mAbs for 30 min at 4°C. To detect biotin-conjugated mAbs, cells were stained with CyChrome- or allophtococyanin-conjugated streptavidin. For staining of epitope-specific CD8^T^ T cells using the tetrameric H2-K^d^-peptide complex, cells were incubated at 4°C for 20 min in unconjugated-streptavidin (0.5 mg/ml; Sigma-Aldrich) and Fn block (2.4G2), followed by triple staining with FITC-CD44, CyChrome-CD8α, and PE-conjugated tetrameric H2-K^I^-peptide complex (0.2–0.3 mg/ml) for 30 min at 4°C. The cells were analyzed using an FACS Calibur flow cytometer (BD Biosciences).

Analysis of intracellular cytokine synthesis

Spleen cells were harvested from infected mice, washed, and suspended at 10^5^ cells/ml in complete culture medium and then incubated for 4 h at 37°C in the presence of 10 mg/ml brefeldin A (Sigma-Aldrich) and 5 μg/ml LLO91–99 peptide. These cells were harvested, washed, and incubated for 30 min at 4°C with PE-conjugated anti-CD44 mAb and CyChrome-conjugated CD8 mAb. After surface staining, cells were subjected to intracellular cytokine staining using a Fast Immune Cytokine System according to the instructions of the manufacturer (BD Biosciences). The cells were washed and fixed in 100 μl of FACs lysing solution (BD Biosciences) for 10 min at room temperature and were then washed again, resuspended in 500 μl of FACs permeabilizing solution (BD Biosciences), and incubated for 10 min at room temperature. After washing, the cells were stained with FITC-conjugated IFN-γ mAb or FITC-conjugated isotype control rat IgG (BD Pharmingen) for 30 min at room temperature, and the fluorescence of the cells was analyzed using a flow cytometer.

Cell culture

Magnetic bead-separated CD8^T^ T cells (95% pure) from LN were cultured in 200 μl of complete culture medium in a 96-well flat-bottom plate (BD Biosciences) at density of 5 × 10^3^ cells/well with indicated concentrations of LLO91–99 peptide. To estimate IFN-γ production, the supernatant was collected at 48 h. The IFN-γ production in the supernatants was assessed using DuoSet ELISA development system (Genzyme). Proliferative activity of CD8^+^ T cells was assessed by incorporation of [3H]thymidine. The cells were pulsed with [3H]thyminidine 6 h before harvesting. [3H]Thymidine incorporation was then determined by liquid scintillation counting.

RT-PCR

Nylon wool-enriched spleen T cells were incubated with appropriate dilutions of FITC-conjugated anti-1-A^K^, IgM, and biotinylated anti-1-D^X^, anti-CD11c, and anti-CD4 mAbs, and washed twice in HBSS. The cells were then incubated with anti-FITC microbeads, streptavidin microbeads, and anti-CD4 mAb microbeads for 15 min at 4°C. CD8^T^ T cells were enriched to >90% by negative selection using LD-positive depletion colums (Miltenyi Biotec). T~EM~ (CD62L^−^CD44^+^CD8^+^) cells were negatively separated with anti-CD62L mAb microbeads and anti-CD4 mAb microbeads from nylon wool-enriched spleen T cells (>95% purity). Total RNA was isolated from CD8^T^ T cells and from CD62L^−^CD44^+^CD8^+^ T cells of CD153^−/−^ mice and control mice with TRIZol reagent (Invitrogen Life Technologies). The first-strand cDNA synthesized from the total RNA was amplified using 10 pmol of each primer specific for murine rRNA or chemokine receptors. cDNA encoding chemokine receptors was analyzed by real-time PCR using a TaqMan PCR kit and an ABI PRISM 7000 sequence detector thermal cycler according to the protocol recommended by the manufacturer (Applied Biosystems).

*In vivo cytotoxicity assay*

Analysis of in vivo cytolytic activity was conducted basically according to the previously reported protocol (29, 30). BALB/c splenocytes were divided into two populations and labeled with either a high concentration (3 μM) or a low concentration (0.3 μM) of CFSE. Next, CSFe^high^ cells were pulsed with 10^6^ M LLO91–99 for 1 h at 37°C in the dark, whereas CFSE^low^ cells remained unpulsed. After washing, CFSE^high^ cells were mixed with equal numbers of CFSE^low^ cells, and a suspension of 2 × 10^7^ of these cells was i.v. injected into each mouse. Spleens from recipients were obtained 15 h later for flow cytometric analysis to measure in vivo killing as indicated by the loss of the CFSE^high^ Ag-pulsed population relative to the control CFSE^low^ population. Percentage of specific lysis was calculated according to the following formula: [1 – (ratio of unprimed cells/ratio of primed cells) × 100], in which the ratio of unprimed cells is the percentage of CFSE^low^ per CFSE^high^ cells remaining in noninfected recipients, and the ratio of primed cells is the percentage of CFSE^low^ cells remaining in infected recipients.

*Statistical analysis*

Data were analyzed by Student’s t test, and Bonferroni correction was applied for multiple comparison. A value of p < 0.05 was considered statistically significant.

*Results*

**CD153 is required for generation of long-lived memory CD8^T^ T cells**

We studied the kinetics of bacterial clearance and the expansion, contraction, and stable memory of Ag-specific CD8^T^ T cells in CD153-deficient mice with a BALB/c background after i.p. inoculation with 1 × 10^6^ CFU of *L. monocytogenes* strain EGD. We found that the numbers of bacteria increased to maximal levels on day 3 in the spleen and liver and thereafter cleared completely by day 10 after inoculation in both wild-type mice and CD153^−/−^ mice. We also found that the bacteria were more rapidly eliminated in wild-type mice than in CD153^−/−^ mice (data not shown). To directly follow the fate of the *L. monocytogenes* epitope-specific CD8^+^ T cells in CD153^−/−^ mice after i.p. inoculation with *L. monocytogenes*, we used intracellular IFN-γ staining in response to LLO91–99 peptide, the immunodominant epitope recognized by H-2^K^β^−^restricted CD8^+^ T cells (Fig. 1A), and tetrameric MHC molecule folding with the LLO91–99 peptide (Fig. 1B) for staining epitope-specific CD8^+^ T cells. As shown in Fig. 1A, a significant number of CD8^+^ T cells expressing high levels of CD44 in the spleen and peritoneal cavity were stained with intracellular IFN-γ in CD153^−/−^ mice on day 7 after infection with *L. monocytogenes* at the induction stage of effector T cells, albeit at a lesser level of CD44 than that found in wild-type mice. However, the absolute number of LLO91–99-specific CD8^+^ T cells in the peritoneal cavity of CD153^−/−^ mice was 3.1 ± 0.3 × 10^6^ cells in CD153^−/−^ mice, comparable to that found in wild-type mice of 3.4 ± 0.4 × 10^6^ cells (Fig. 2). These results suggest that the generation of effector CD8^+^ T cells normally occurs in CD153^−/−^ mice following *Listeria* infection.

In contrast, the numbers of LLO91–99-specific CD8^+^ T cells were significantly higher in the peritoneal cavity on days 28 and 42 after infection with *L. monocytogenes*. The numbers of LLO91–99-specific CD8^+^ T cells were determined by liquid scintillation counting.
after infection at a relatively early stage of memory T cell generation as compared with cells found in wild-type mice, whereas the number of Ag-specific CD8\(^+\) T cells in the spleen of CD153\(/^{--}\) mice did not differ from that in wild-type mice on day 28 and had decreased in CD153\(/^{--}\) mice on day 42 after infection (Fig. 1A).

Notably, the numbers of LLO\(_{01-99}\) specific CD8\(^+\) T cells were significantly decreased in the spleen, LN, and peritoneal cavity of CD153\(/^{--}\) mice on day 84 at a relatively late stage of memory T cell generation compared with those in wild-type mice (Figs. 1 and 2; \(p < 0.01\)). These results suggest that absence of CD153 in vivo does not affect generation of Ag-specific effector CD8\(^+\) T cells but hampers the generation of long-lived memory CD8\(^+\) T cells after L. monocytogenes infection.

**Impaired generation of memory CD8\(^+\) T\(_{CM}\) cells in CD153\(/^{--}\) mice after Listeria infection**

The memory T cell compartment can be divided into T\(_{CM}\) and T\(_{EM}\) cell subsets based on the expression of several cell surface molecules such as LN-homing receptors (3, 4). CD62L expression is useful in distinguishing between these two subsets because T\(_{CM}\) cells are mostly CD62L\(_{high}\), whereas T\(_{EM}\) cells are CD62L\(_{low}\) (3, 4). We first examined the expression of CD62L on LLO\(_{01-99}\) specific CD8\(^+\) T cells in spleens of wild-type and CD153\(/^{--}\) mice on day 42 after L. monocytogenes infection, at which time the number of Ag-specific CD8\(^+\) T cells in CD153\(/^{--}\) mice was much more equal to that in wild-type mice. The cells were analyzed by four-color flow cytometry for the simultaneous expression of CD8, CD44, and CD62L on LLO\(_{01-99}\) specific CD8\(^+\) T cells. The majority (87%) of LLO\(_{01-99}\) specific CD8\(^+\) T cells in CD153\(/^{--}\) mice shown gated in Fig. 3A were of the CD62L\(_{low}\) phenotype, whereas only 13% were CD62L\(_{high}\). In contrast, the memory CD8\(^+\) T cells from wild-type mice (Fig. 3A) were CD62L\(_{high}\) cells in the LLO\(_{01-99}\) specific CD8\(^+\) T cells in the spleen, LNs, and peritoneal cavity of CD153\(/^{--}\) mice were significantly lower than those in wild-type mice on day 42 after infection.

T\(_{CM}\) cells exhibit higher proliferative ability than do T\(_{EM}\) cells (4). To further confirm impairment of generation of T\(_{CM}\) cells in CD153\(/^{--}\) mice following Listeria infection, we next analyzed the functional properties of LLO\(_{01-99}\) specific CD8\(^+\) T cells from the spleens of wild-type and CD153\(/^{--}\) mice on day 42 after infection in vitro response to LLO\(_{01-99}\) peptide. The ability of CD8\(^+\) T cells from CD153\(/^{--}\) mice to produce the IFN-\(\gamma\) was comparable to that of CD8\(^+\) T cells from wild-type mice (Fig. 3B). However,
only CD8+ T cells from wild-type mice were capable of proliferating in response to LLO91–99 peptide (Fig. 3B). From these results, it is concluded that CD8+ TCM cells are poorly generated in CD153−/− mice following L. monocytogenes infection, although TEM cells accumulated in nonlymphoid tissues at a relatively early stage of memory T cell generation.

Lack of CD153 causes a progressive loss in protective immunity against reinfection with Listeria

As described, the numbers of long-lasting Ag-specific memory CD8+ T cells in both nonlymphoid and lymphoid tissues were greatly decreased on day 84 after Listeria infection. We examined the functional relevance of this decrease by analyzing Ag-specific CTL activity in vivo in CD153−/− mice on day 84 after Listeria infection. This analysis was done by monitoring the specific eradication of an adoptive transferred target population of LLO91–99-pulsed splenocytes that had been differentially labeled with CFSEhigh so as to be distinguishable from a cotransferred reference population (CFSElow). Fig. 4A shows that strong and specific cytotoxic effectors were detectable in the spleens of infected wild-type mice. In contrast, the numbers of CTLs in CD153−/− mice were greatly decreased on day 84 after infection with L. monocytogenes (Fig. 4A). The loss of functional CTL activity in CD153−/− mice might be results of quantitative, but not qualitative, decrease by the Ag-specific CD8+ T cells because the number of tetrameric H2-Kd/LLO91–99-positive cells was significantly decreased in the CD153−/− mice on day 84 after infection compared with the number of cells in wild-type mice (Fig. 2).

To evaluate protective immunity against L. monocytogenes in wild-type and CD153−/− mice inoculated with Listeria 84 days previously, we tested the ability to control a lethal challenge of L. monocytogenes (1 × 10⁶ CFU). As expected, LLO91–99-specific CD8+ T cells poorly expanded in CD153−/− mice compared with those in wild-type mice on day 5 after a secondary challenge (Fig. 4B). There was no difference in the number of bacteria in the spleen and liver of between unimmunized CD153−/− and wild-type mice (Fig. 4C). However, consistent with the results of levels of memory CD8 T cells, there was a marked deficiency in the protection of CD153−/− mice against lethal challenge with L. monocytogenes (Fig. 4C). Collectively, CD153−/− mice poorly generated long-lived memory CD8+ T cells, which were not able to lyse peptide-coated target cells and were not able to confer protective immunity against reinfection. Although TEM cells had accumulated in the CD153−/− mice on day 42 after infection, the mice poorly generated long-lived memory CD8+ T cells at a later stage of infection. This result suggests that TEM cells may be important for generation of long-lived memory CD8+ T cells following Ag exposure.
We found that long-lasting CD8+ TCM cells are poorly generated in CD153−/− mice, whereas CD8+ TEM cells accumulate in nonlymphoid tissues at a relatively early stage of memory T cell generation after L. monocytogenes infection. Furthermore, we previously found by DNA chip analysis that CD30 signaling up-regulates CCR7 mRNA (23, 24). These findings give rise to the possibility that CD30 signaling makes TEM cells by inducing expression of LN-homing receptors, including CCR7. To address this issue, we first examined changes in expression of LN-homing receptors of purified CD62L−CD44+CD8+ TEM after CD30 stimulation. The purified CD62L−CD44+CD8+ TEM cells were stimulated with plate-bound CD30 mAb for 24 h. We first examined the expression of CD62L on the surfaces of TEM cells after anti-CD30 mAb cross-linking, but TEM cells did not express CD62L molecules after stimulation. We next examined gene expression of chemokine receptors, including CCR5, CCR6, and CCR7, in the TEM cells stimulated with anti-CD30 mAb. As shown in Fig. 5 after anti-CD30 mAb cross-linking, the gene expression of CCR5s was greatly up-regulated and the gene expression of CCR7s was down-regulated in TEM cells. These results suggest that CD30/CD30L signaling may play an important role in the generation of long-lived memory CD8 T cells after Ag exposure by triggering the expression of CCR7 in TEM cells to make them migrate and differentiate into TCM cells in nonlymphoid organs.

These results suggest that CD30/CD30L signaling plays an important role in the differentiation of TCM cells through induction of CCR7 mRNA expression.

**FIGURE 4.** Lack of CD153 causes a progressive loss in protective immunity against reinfection with Listeria. A, Cytotoxic responses of CD8 T cells in CD153−/− mice and wild-type mice at 84 days after L. monocytogenes infection. In vivo killing of LLO91-99 peptide-pulsed target cells labeled with CFSE in uninfected mice, wild-type mice and CD153−/− mice at 84 days after infection was determined by monitoring the specific eradication of an adoptive transferred target population of LLO91-99-pulsed splenocytes that had been differentially labeled with CFSE so as to be distinguishable from a cotransferred reference population (CFSElow). The number shown represents the percentage of specific killing to that of control CFSE−/− labeled cells (mean ± SD; n = 5). B, Expansion of LLO91-99-specific CD8 T cells in CD153−/− mice and wild-type mice on day 5 after secondary challenge. Tetrameric H2-Kk peptide complexes or intracellular cytokine staining of LLO91-99-specific CD8 T cells in CD153−/− mice and wild-type mice were examined by flow cytometry and analyzed by gating on CD8+ T cells. C, Protection against lethal challenges with L. monocytogenes in CD153−/− mice and wild-type mice infected with L. monocytogenes 84 days previously. CD153−/− mice and wild-type mice that had each been infected with 1 × 105 CFU L. monocytogenes 84 days previously were each challenged with a lethal dose of L. monocytogenes (1 × 106 CFU), the numbers of bacteria in spleens and livers were counted 2 days later. Each column and vertical bar represent mean ± SD of five mice in each group. *, p < 0.05, significant difference between the values for wild-type CD8 to BALB/c and the values for immune CD153−/− CD8 T cells to BALB/c.

**CD30L signaling induces CCR7 expression in TEM**

Up-regulation of CCR7 expression in TEM cells in response to CD30 cross-linking. Purified CD62L−CD44+CD8+ TEM cells were stimulated with plate-bound anti-CD30 mAb (clone CD30.1) for 24 h. Gene expression of the chemokine receptors CCR5, CCR6, and CCR7 in TEM cells stimulated with anti-CD30 mAb was examined by quantitative RT-PCR. First-strand cDNA was synthesized with random primers. cDNA encoding chemokine receptors were analyzed by real-time PCR using a TaqMan PCR kit and an ABI PRISM 7000 sequence detector thermal cycler according to the recommended protocol of the manufacturer (Applied Biosystems). Data of gene expression were normalized on the basis of rRNA mRNA levels. Representative results from three separate experiments are shown.

**FIGURE 5.** CD30L signaling induces CCR7 expression in TEM cells. Up-regulation of CCR7 expression in TEM cells in response to CD30 cross-linking. Purified CD62L−CD44+CD8+ TEM cells were stimulated with plate-bound anti-CD30 mAb (clone CD30.1) for 24 h. Gene expression of the chemokine receptors CCR5, CCR6, and CCR7 in TEM cells stimulated with anti-CD30 mAb was examined by quantitative RT-PCR. First-strand cDNA was synthesized with random primers. cDNA encoding chemokine receptors were analyzed by real-time PCR using a TaqMan PCR kit and an ABI PRISM 7000 sequence detector thermal cycler according to the recommended protocol of the manufacturer (Applied Biosystems). Data of gene expression were normalized on the basis of rRNA mRNA levels. Representative results from three separate experiments are shown.
Upon encounter with a pathogenic microbe, Ag-specific T cells proliferate and differentiate into activated effector T cells. Most of the activated T cells die by apoptosis (31), but the few that survive become memory cells and persist for a long period of time, sometimes throughout the life of an animal (32–34). Memory is dependent on the amount of surviving T cells after primary TCR-medi- ated activation and presumably on escape from activation-induced cell death or starvation cell death by apoptosis (35). It has been recently reported that naive CD8+ T cells receiving prolonged or strong stimulation of T cell receptors can differentiate into effector cells and survive as memory T cells by enhancing IL-15/IL-7 re- sponsiveness (36). TNF/TNFR superfamily is known to play im- portant roles in generation of effector T cells as accessory mole- cules during an immune response (6). CD153 has been detected on activated mouse DCs (16). Therefore, the CD153−CD30 pair may contribute to T cell priming at dendritic cell-T cell interaction like 4-1BB and OX40 (37–39). In addition, CD153 is expressed on activated T cells and can support T cell expansion during T-T cell interaction (37). There are several lines of evidence that CD4 help is important for generation of memory CD8 T cells following micro- biological infection (40). Therefore, it is possible that defective den- dritic cell and T cell help may account for the altered CD8 T cell responses in CD153−/− mice. However, the results of the present study indicate that the generation of effector CD8+ T cells seems to occur normally in CD153−/− mice at induction phase of effector T cells after Listeria infection. Furthermore, the number of TEM cells increased, albeit transiently, after Listeria infection in CD30/ CD30L−/− mice. CD30/CD30L signaling might not be essential at least for the generation of effector CD8+ T cells and CD8 TEM cells after exposure to a microbe.

Because both TCM and TEM cells can be generated during the same immune response to L. monocytogenes, a key question is how these subpopulations are related to each other. Recent studies have provided new insights into the lineage relationship between TCM and TEM cells (1–3). There is evidence suggesting that TEM cells can convert into TCM cells under appropriate in vivo conditions (4). The numbers of TEM and TCM cells changed substantially over time and did so in a reciprocal manner, with the number of TCM cells increasing and the number of TEM cells decreasing. Wherry et al. (4) investigated this possibility directly by transferring purified populations of TEM cells or TCM cells into secondary recipients and examining the fate of the transferred cells. These researchers found that TCM cells retained their phenotype for at least 30 days after transfer, whereas half of the TEM cells acquired the phenotype of TCM cells during this period. They therefore proposed a linear differentiation model of a memory CD8+ T cell subset. In the present study, the generation of TCM cells was im- paired in the absence of CD30L/CD30 signaling following Listeria infection in vivo, and an LN-homing receptor, CCR7, was strongly up-regulated in TEM cells after anti-CD30 mAb cross-linking in vitro. On the basis of the linear differentiation theory, our results suggest that CD30L/CD30 signaling plays an important role in the generation of long-lived memory CD8+ T cells after exposure to an Ag by triggering differentiation into TCM cells via induction of the expression of CCR7 on TEM cells in nonlymphoid organs. In contrast, in the case of CD8+ T cells, there is some evidence that TCM and TEM cells might be generated differentially during an immune response depending on the conditions of activation (5, 41). On the basis of the different lineage theory that CD8+ TCM and TEM cells are largely independent subpopulations, the possibility that fewer CD8+ TCM cells are generated in the absence of CD30L/CD30 signaling at the time of immune response after primary infection with L. monocytogenes cannot be excluded.

It is notable that CD8+ TEM cells transiently accumulated in nonlymphoid tissues in CD153−/− mice at a relatively early stage of the memory phase. L. monocytogenes was eliminated both in CD153−/− mice and wild-type mice in <10 days after infection, but preferential accumulation of TEM cells in CD153−/− mice was still observed on day 42 after infection with L. monocytogenes. It is possible that TEM cells can survive in nonlymphoid tissues for several weeks in the absence of a relevant Ag or CD30L. According to the linear differentiation theory, CD30L/CD30L signaling is essential for conversion of TEM cells into TCM cells at least partly via induction of CCR7 expression. Memory T cells in nonlym- phoid tissues may express CCR7 by CD30L/CD30 signaling and then migrate to lymphoid tissues with abundant expression of li- gands for CCR7 such as secondary lymphoid-tissue chemokine. Because of the lack of CCR7 expression by CD30L/CD30L signaling, TEM cells may remain in nonlymphoid tissues due to the lack of CCR7 expression, resulting in accumulation of TEM cells in nonlymphoid tissues of CD153−/− mice after infection.

CD30 has been reported to be preferentially expressed by Th2 and T cytotoxic type-2 (Tc2) cells (5, 14, 42–44), and blocking of CD30L/CD30 signaling has been reported to suppress the develop- ment of Th2 cells and to enhance the development of Th1 cells in vitro (45). These results suggest that CD30L/CD30L signaling plays a role in the proliferation, cytokine secretion, and survival of Th2 cells, although controversy results have been reported (46). Tc1 and Tc2 cells are known to mutually regulate in their differen- tiation, and Th1 cells have been reported to be subjected to apoptosis weaker than Tc2 cells (7, 31). Therefore, it is alterna- tively possible that Tc2 cells producing IL-4 are selectively sup- pressed after infection in CD153−/− mice, resulting in preferential accumulation of TEM cells in nonlymphoid tissues. Alternatively, according to a different line- age theory, TEM cells may be preferentially generated in lym- phoid tissues in the absence of CD30L and migrate to nonlym- phoid tissues due to the lack of CCR7 expression, resulting in accumulation of TEM cells in nonlymphoid tissues of CD153−/− mice after infection.

The principal attribution of memory T cells is their ability to undergo cell division called “homeostatic proliferation” to maintain their number (1, 47). Homeostatic proliferation is thought to be required for the long-term maintenance of Ag- driven memory CD8+ T cells in vivo. A TEM cell population that is CCR7+ CD62L+ has little homeostatic proliferative po- tential, and this subset therefore does not seem to be a perma- nent memory population (4). In contrast, a TEM cell subset that is CCR7+ CD62Lhigh is capable of efficient homeostatic prolifer- ation and may survive for a long time in lymphoid tissues (4). In the present study, memory CD8+ T cells in both lymphoid and nonlymphoid tissues of CD153−/− mice decreased dramat- ically at the late stage of infection. Most of the memory TEM cells in nonlymphoid tissues of CD153−/− mice might die by apoptosis due to the lack of their responsiveness to survival signals or survival signals themselves. Recent studies have sug- gested that cytokines such as IL-15/IL-7 are involved in the proliferation and survival of Ag-driven memory CD8+ T cells in the absence of Ag (48–53).
The TNFR-associated factor (TRAF)-linked TNFR family members CD40, OX40, 4-1BB, and CD27 by virtue of their anti-apoptotic effects, would be prime candidates to shape T cell memory (6). These molecules might be responsible for maintenance of T_{CM} cells in the lymphoid tissues. It has recently been reported that CD4^{+}CD3^{−} accessory cells have low levels of CD80 or CD86 expression but express high levels of the two TNF ligands OX40 ligand and CD153 in the B cell area of lymphoid tissues (16). OX40 has been found to up-regulate expression of the CXC chemokine receptor (CXCR) 5 and allows T cell migration into B cell areas of peripheral lymphoid organs (54). We found that CD30 stimulation also induced CXCR5 expression on T_{EM} cells (our unpublished observation). Therefore, it is possible that CD30 signaling up-regulates the expression of CCR7 and CXCR5 on memory T cells, enabling these T cells to migrate to B cell areas of lymphoid tissues. Survival signaling from CD4^{+}CD3{−} accessory cells in lymphoid tissues may play an important role in the maintenance of T_{CM} cells for a long period. It is also possible that CD30 signaling provides direct survival signal or the responsiveness to memory CD8^{+} T cells in addition to up-regulation of the expression of homing receptors. CD30 uses TRAF molecules, particularly TRAF2 and TRAF5, to induce downstream signals (55–57). TRAF2 is thought to be responsible for NF-κB activation and for the anti-apoptotic effect mediated by CD30 (55–57). Although lymphocyte homeostasis does not seem to be affected in TRAF5 mice (36). We previously showed by gene microarray analysis strong expression of CCR7 in large granular lymphocyte lymphoma by CD30 is expressed by activated and memory CD8^{+} T cells (19). However, it should be noted that triggering through the TCR has been shown to result in both down-regulation of CCR7 expression on CD8^{+}T_{EM} cells and up-regulation of CCR7 expression in CD8^{+}T_{CM} cells (36). We previously showed by gene microarray analysis strong up-regulation of CCR7 in large granular lymphocyte lymphoma by CD30 (23, 24). We found that CD30, but not other TRAF2 involving molecules containing CD40 and IL-15reduced CCR7 expression in the CD62L^{+}CD4^{+}CD8^{+}T_{EM} cells (our unpublished observations) (Fig. 5A). These results suggest that other signaling such as by the MAPK pathway may be involved in the up-regulation of CCR7 on memory CD8^{+}T cells. Further analysis is needed to elucidate the effects of CD30 signaling on memory CD8^{+} T cells. It is also interesting whether CCR7 expression level in naive CD8^{+} T cells is altered in CD153^{−/−} mice. However, homing of naive CD8^{+} T cells in CD153-deficient mice was not affected. CD153 is expressed by activated dendritic cells, and CD30 is expressed by activated and memory CD8^{+} T cells but not by naive CD8^{+} T cells (6, 14–16). Therefore, CD30L/CD30 signaling for up-regulation of CCR7 may only serve to function on activated/memory CD8^{+} T cells.

In conclusion, we have shown that CD30/CD30L signaling is involved in the expression of CCR7 in T_{EM} cells following Listeria infection. Ag-specific T_{EM} cells accumulated preferentially in CD153^{−/−} mice at the early stage of L. monocytogenes infection. In contrast, long-lived memory CD8^{+} T cells, which function in protective immunity, were not generated in large numbers in CD153^{−/−} mice at the later stage of infection. These results suggest that CD30/CD30L signaling plays an important role in the generation of long-lived memory CD8^{+} T cells after exposure to an Ag by triggering differentiation into T_{CM} cells via induction of expression of CCR7 on T_{EM} cells in nonlymphoid organs.

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Disclosures
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