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Differential Impact of CD27 and 4-1BB Costimulation on Effector and Memory CD8 T Cell Generation following Peptide Immunization

Jane E. Willoughby, Jonathan P. Kerr,1 Anne Rogel, Vadim Y. Taraban,2 Sarah L. Buchan, Peter W. M. Johnson, and Aymen Al-Shamkhani

The factors that determine differentiation of naive CD8 T cells into memory cells are not well understood. A greater understanding of how memory cells are generated will inform of ways to improve vaccination strategies. In this study, we analyzed the CD8 T cell response elicited by two experimental vaccines comprising a peptide/protein Ag and an agonist that delivers a costimulatory signal via CD27 or 4-1BB. Both agonists increased expansion of Ag-specific CD8 T cells compared with Ag alone. However, their capacity to stimulate differentiation into effector and memory cells differed. CD27 agonists promoted increased expression of perforin and the generation of short-lived memory cells, whereas stimulation with 4-1BB agonists favored generation of stable memory. The memory-promoting effects of 4-1BB were independent of CD4 T cells and were the result of programming within the first 2 d of priming. Consistent with this conclusion, CD27 and 4-1BB–stimulated CD8 T cells expressed disparate amounts of IL-2, IFN-γ, CD25, CD71, and Gp49b as early as 3 d after in vivo activation. In addition, memory CD8 T cells, generated through priming with CD27 agonists, proliferated more extensively than did 4-1BB–generated memory cells, but these cells failed to persist. These data demonstrate a previously unanticipated link between the rates of homeostatic proliferation and memory cell attrition. Our study highlights a role for these receptors in skewing CD8 T cell differentiation into effector and memory cells and provides an approach to optimize vaccines that elicit CD8 T cell responses.

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Cytotoxic T lymphocytes provide protection against intracellular pathogens and are capable of eliminating tumor cells. The interaction of naive CD8 T cells with Ag-bearing dendritic cells (DCs) instills a developmental program that drives T cell expansion, effector cell differentiation, and memory cell generation (1, 2). Following the expansion phase, the majority (90–95%) of effector T cells die by apoptosis, whereas the remaining cells persist as a memory pool that functions to protect the host upon Ag re-encounter (2). The extent of CD8 T cell expansion is strongly influenced by the affinity of the TCR/peptide MHC interaction and duration of Ag availability, such that sustained T cell expansion necessitates strong TCR signaling (3). Other factors known to promote CD8 T cell expansion include signaling by costimulatory receptors and certain cytokines, such as IL-12 and type I IFNs (4). However, proinflammatory cytokines, such as IFN-γ and IL-12, can negatively impact the generation of memory cells by promoting contraction of the T cell response and development of short-lived terminally differentiated cells (2, 4–6).

The potential of activated T cells to develop into memory cells appears to be programmed at an early stage of the T cell response. Kalia et al. (7) reported that, within 3.5 d of infection with lymphocytic choriomeningitis virus, prolonged expression of IL-2Rα (CD25) marks a population of Ag-specific CD8 T cells that is enriched in short-lived effector cells (SLECs). Compared with cells that expressed low amounts of CD25, these cells proliferated more rapidly, exhibited a more pronounced effector phenotype, and were more prone to apoptosis (7). Further support for a role for IL-2 in regulating effector cell differentiation and determining memory T cell fate was provided by studies of CD25-deficient T cells. These studies showed that the absence of CD25 led to a reduction in the function of effector T cells (8) and a lack of accumulation of SLECs, without compromising the survival of memory precursor effector cells (MPECs) (9).

Because memory cell potential is imprinted during priming, it is conceivable that signaling through costimulatory receptors impacts on the ratio of SLECs/MPECs. We set out to address the effect of enforced costimulation through different receptors on the differentiation of CD8 T cells in a vaccination setting. We selected the TNFR superfamily members CD27 and 4-1BB as targets, because both are known to influence the CD8 T cell response. CD27 is expressed constitutively on naive CD8 T cells, whereas 4-1BB is induced within 24 h of T cell activation in vivo (10, 11). Studies using CD27 or 4-1BB ligand (4-1BBL)–deficient mice demonstrated a nonredundant contribution by both receptors to the accumulation of influenza virus–specific CD8 T cells during the primary response and the formation and response of memory cells (12, 13). Interestingly, the requirement for 4-1BB costimulation during the primary response to influenza virus is greater during infection with a more.
virulent strain of virus, consistent with the higher expression of 4-1BB on activated T cells from mice bearing a higher viral load (13). Although the lack of CD27 signaling results in suboptimal CD8 T cell responses (12, 14–17), deliberate triggering of CD27 by administration of soluble CD70 (18) or through transgenic expression of CD70 on DCs (19) prevents tolerance induced by injection of a peptide Ag and allows the generation of a population of effector and memory CD8 T cells. Similarly, 4-1BB triggering was shown to prevent peptide-induced CD8 T cell tolerance (20) and augment effector and memory responses following peptide or DC immunization (21–23).

The effects of CD27 and 4-1BB agonists have not been directly compared. We carried out a side-by-side evaluation of the effects of CD27 and 4-1BB triggering on the CD8 T cell response following peptide and protein immunization. Our data reveal key differences in the effects of CD27 and 4-1BB costimulation on the generation of effector and memory CD8 T cells. These data extend our understanding of the mechanisms that regulate CD8 T cell differentiation and have bearing on the use of CD27 and 4-1BB agonists as adjuvants for promoting CTL-mediated immune responses.

Materials and Methods

Mice and in vivo experiments

T cells (10^6 or 10^5) from OT-I TCR-transgenic mice were adoptively transferred into C57BL/6 recipients. Mice were challenged i.p. with OVA (Sigma; 5 mg on day 0) and either anti-CD27 mAb [clone AT124 (24); 200 µg] or anti-4-1BB mAb [clone LOB12.3 (25); 200 µg] on days 0 and 1. Alternatively, mice were primed with 30 nmol OVA peptide 257–264 (day 0) and either recombinant soluble CD70 (18) (250 µg) or 4-1BBL (25) (250 µg) given i.v. on days 0, 1, and 2. Endogenous OVA-specific CD8 T cell responses were generated in C57BL/6 mice by i.v. administration of OVA (5 mg, day 0) and either anti-CD27 or anti-4-1BB mAbs (200 µg, days 0 and 1). To detect secondary responses, primed mice were injected once with OVA peptide 257–264 (30 nmol) and anti-CD40 mAb (clone 3/23; 500 µg). Secondary responses were measured after adoptive transfer of purified (by cell sorting) memory OT-I cells (10^5) obtained 65 d post-priming. For comparison, an equivalent number of naive OT-I cells was transferred into a separate cohort. For CD4 T cell depletion, mice were administered CD4-depleting Abs (T-191.1.2 and GK 1.5; 0.5 mg each) on days −1 and 1. In some experiments, OT-I T cells isolated from the spleens of mice 4 d post-priming with OVA and either anti-CD27 or anti-4-1BB were transferred into secondary naive recipients. For these experiments, OT-I T cells were enriched using the Mouse CD8 Recovery Kit (CEDARLANE), and ∼5 million effector cells were transferred into secondary naive recipients. For IL-2 blocking, mice were injected i.p. with anti–IL-2 mAbs JES6-1A12 (50 µg) and S4B.6.1 (200 µg) for three consecutive days starting on day 0. Animal experiments were performed according to UK Home Office license guidelines and approved by the University of Southampton’s ethical committee.

Lymphocyte isolation from the colon, liver, and lungs

Isolation of colon lamina propria lymphocytes was carried out as described previously (26), except that tissue digestion was performed using 150 U/ml collagenase VIII (Sigma). To isolate lymphocytes from livers, PBS-washed organs were passed through a 100-µm cell strainer in the presence of PBS-0.5% FCS/2 mM EDTA. Lymphocytes were isolated from the 30–70% Percoll interface after centrifugation (900 × g). The same protocol was applied to lungs, except that they were first cut into small pieces.

Flow cytometry

Abs against CD8α (53-6.7), CD62L (MEL-14), CD98 (RL388), CD71 (R17217), killer cell lectin–like receptor G1 (KLGR1; 2F1), CD127 (A7R34), CD122 (TM-b1), and perforin (eBioOMAK-D) were purchased from eBioscience. PE-labeled H-2Kb SIINFEKL tetramer was prepared in-house. Anti-CD25 mAb (7D4) was purchased from BD Pharmingen. Intracellular perforin staining was performed using the Foxp3 Staining Buffer Set (eBioscience). For intracellular cytokine staining, cells were activated with SIINFEKL (1 nM) for 4 h at 37°C in the presence of GolgiPlug (BD Pharmingen) prior to surface staining (tetramer and anti-CD8 mAb). Intracellular cytokine staining was performed using BD Cytofix/Cytoperm buffers. Cells were stained with IFN-γ–allophycocyanin (XMG1.2, BD Pharmingen) and IL-2–PE-Cy7 (JES6-5H4; eBioscience). Samples were run on a BD FACSCan II flow cytometer.

Detection of in vivo proliferation

BrDU (Sigma; 1 mg) was given i.p. daily for 3 d prior to analysis. BrdU staining was performed according to the manufacturer’s protocol (BD Pharmingen).

Quantitative real-time PCR

Total RNA extracted from in vivo–primed OT-I cells and purified by cell sorting was subjected to reverse transcription using Superscript III Reverse Transcriptase, and the resulting cDNA was analyzed by real-time PCR using TaqMan Gene Expression Assays (both from Life Technologies). Data were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Mm00446968_m1), Gpibα (Lilrb1) (Mm01614371_m1), T-bet (B201.2) (Mm00450960_m1), comoderserin (Mm01351988_m1), Blimpl1 (Pdm1) (Mm00476128_m1), and Bcl-6 (Mm00477633_m1).

Statistics

Statistical significance was determined using the two-tailed Student t test, except for the data in Fig. 2A and 2B, for which a one-tailed test was used.

Results

Both CD27 and 4-1BB agonists augment primary CD8 T cell expansion but 4-1BB is superior for establishment of memory

We compared the effects of CD27 and 4-1BB stimulation in vivo on the expansion of adoptively transferred OVA-specific TCR-transgenic OT-I T cells. Injection of OVA alone resulted in an ineffectual primary T cell response. In contrast, coinjection of agonist anti-CD27 mAb or anti-4-1BB mAb with OVA promoted strong expansion of OT-I T cells, as determined by MHC I tetramer labeling of PBMCs (Fig. 1A, Supplemental Fig. 1A) and splenocytes (Fig. 1B). Although the magnitude of OT-I T cell expansion following administration of CD27 or 4-1BB mAb was similar in this experimental setting, contraction of the T cell response was more profound following CD27 stimulation (Fig. 1A, B). As such, fewer memory OT-I cells were detected following CD27 stimulation in all of the organs examined, including non-lymphoid tissues, such as the lungs, liver, and colon (Fig. 1C, Supplemental Fig. 1B). These effects were independent of the initial number of adoptively transferred OT-I T cells. Thus, more memory cells were generated using agonist 4-1BB mAb even when the number of OT-I T cells was reduced by 10-fold (Supplemental Fig. 1C). Furthermore, evaluation of the endogenous OVA-specific CD8 T cell response revealed increased numbers of Ag-specific memory cells in mice primed with OVA and anti–4-1BB mAb compared with those that received OVA and anti-CD27 mAb (Fig. 1D). Moreover, upon rechallenge, the secondary response was significantly higher in the group of mice that were primed with OVA and agonist 4-1BB mAb (Fig. 1E). Memory T cells can be divided into central and effector memory subsets based on expression of l-selectin (CD62L) and the chemokine receptor CCR7. Because the spleen contains both memory subsets (27), we examined whether stimulation via CD27 or 4-1BB exerts differential effects on the generation of central and effector memory cells in this organ. Fifty-four days following immunization, and independent of the type of agonist used, ∼80% of OT-I T cells within the spleen displayed high expression of CD44 and CD62L, whereas the remaining ∼20% were CD44high but CD62Llow (Supplemental Fig. 1D). These data demonstrate that, although memory T cell generation after CD27 stimulation is curtailed compared with 4-1BB stimulation, the central/effector memory differentiation program remains unaffected.

Additional experiments were conducted using soluble rCD70 and 4-1BBL as agonists instead of mAbs and the OVA 257–264 peptide instead of whole OVA. The frequency of memory cells...
The effect of CD27 and 4-1BB agonists on CD8 T cell expansion and memory formation. Kinetics of OVA-specific CD8 T cell expansion and decline in peripheral blood (A) and spleen (B), following transfer of $10^5$ OT-I cells and priming with OVA+control rat IgG, OVA+anti-CD27, or OVA+anti-4-1BB mAb. (C) The frequency of memory (day 57) OVA-specific CD8 T cells in different organs following OT-I cell transfer and priming with OVA+anti-CD27 or OVA+anti-4-1BB mAb. (D) The number of endogenous OVA-specific CD8$^+$ T cells in peripheral blood 6 wk after priming with either OVA+anti-CD27 or OVA+anti-4-1BB mAb. Each data point represents the response of an individual mouse. Mean and SE are indicated. (E) Kinetics of the primary and secondary endogenous OVA-specific CD8$^+$ T cell response. Mice were rechallenged on day 43 with OVA peptide and anti-CD40 mAb. Each data point in (A)–(C) and (E) represents the mean ± SE (data are representative of at least two independent experiments, with a minimum of three mice/group). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005.

CD27 is constitutively expressed on T cells, whereas 4-1BB is expressed transiently on CD8 T cells following engagement of the TCR by Ag and also following stimulation of memory CD8 T cells by IL-15 (13, 31). To investigate at which time point during an immune response 4-1BB influences memory differentiation, we adoptively transferred OT-I T cells into groups of mice and then immunized them with OVA and either agonist CD27 mAb or OVA and agonist 4-1BB mAb. Four days after priming, when OT-I cells began to expand, we isolated CD8 T cells using negative-selection columns that ensured that the isolated cells were devoid of CD27 or 4-1BB mAb. Equal numbers of primed OT-I T cells were transferred into secondary recipient mice, and Ag-specific memory CD8 T cells were enumerated 62 d later. In this setting, priming in the presence of higher numbers of resting memory cells

**FIGURE 1.** The effect of CD27 and 4-1BB agonists on CD8 T cell expansion and memory formation. Kinetics of OVA-specific CD8 T cell expansion and decline in peripheral blood (A) and spleen (B), following transfer of $10^5$ OT-I cells and priming with OVA+control rat IgG, OVA+anti-CD27, or OVA+anti-4-1BB mAb. (C) The frequency of memory (day 57) OVA-specific CD8 T cells in different organs following OT-I cell transfer and priming with OVA+anti-CD27 or OVA+anti-4-1BB mAb. (D) The number of endogenous OVA-specific CD8$^+$ T cells in peripheral blood 6 wk after priming with either OVA+anti-CD27 or OVA+anti-4-1BB mAb. Each data point represents the response of an individual mouse. Mean and SE are indicated. (E) Kinetics of the primary and secondary endogenous OVA-specific CD8$^+$ T cell response. Mice were rechallenged on day 43 with OVA peptide and anti-CD40 mAb. Each data point in (A)–(C) and (E) represents the mean ± SE (data are representative of at least two independent experiments, with a minimum of three mice/group). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005.

BM, bone marrow; LN, lymph node; LPL, colon lamina propria lymphocyte.

**4-1BB promotes memory precursor cell generation early during the primary response**

CD27 is constitutively expressed on T cells, whereas 4-1BB is expressed transiently on CD8 T cells following engagement of the TCR by Ag and also following stimulation of memory CD8 T cells by IL-15 (13, 31). To investigate at which time point during an immune response 4-1BB influences memory differentiation, we adoptively transferred OT-I T cells into groups of mice and then immunized them with OVA and either agonist CD27 or 4-1BB mAb. Four days after priming, when OT-I cells began to expand, we isolated CD8 T cells using negative-selection columns that ensured that the isolated cells were devoid of CD27 or 4-1BB mAb. Equal numbers of primed OT-I T cells were transferred into secondary recipient mice, and Ag-specific memory CD8 T cells were enumerated 62 d later. In this setting, priming in the presence
of 4-1BB stimulation was again more effective than stimulation via CD27 in generating memory T cells (Fig. 3A). These data demonstrate that 4-1BB triggering by anti–4-1BB mAb beyond the peak of the primary response is not essential for its CD8 T cell memory–promoting effects. To further investigate the time frame during which 4-1BB exerts its programing effect on the generation of memory CD8 T cells, we first analyzed the kinetics of 4-1BB expression on OT-I T cells. 4-1BB was undetectable on naive cells, peaked 1 d after OVA administration, and declined by day 2 (Supplemental Fig. 2A, 2B). In contrast, CD27 was present on naive OT-I T cells, but its expression increased 1 d after priming (Supplemental Fig. 2C, 2D). Fig. 3B shows that the memory-promoting effects of agonist anti–4-1BB mAb correlated with the kinetics of 4-1BB expression on OT-I T cells. Thus, delaying administration of 4-1BB mAb by 2 d resulted in an accelerated T cell contraction phase that impacted negatively on the generation of the Ag-specific memory pool (Fig. 3B). These data demonstrate that early 4-1BB signaling is required for optimal generation of memory precursor cells. Next, we examined whether coadministration of anti–4-1BB mAb and anti-CD27 mAb rescues the progressive decline in memory CD8 T cells observed with anti-CD27 mAb alone. These experiments showed that, when the two agonists were combined, the magnitude and kinetics of the OT-I cell response resembled that seen with anti-CD27 mAb alone (Fig. 3C). Thus, the CD27-differentiation pathway dominates over the 4-1BB activation pathway, possibly reflecting the constitutive and inducible expression pattern of CD27 and 4-1BB, respectively. Interestingly, agonist CD27 also mediated its costimulatory effects prior to day 2 (Supplemental Fig. 2E). Together, these data show that CD27 and 4-1BB influence the T cell–differentiation program relatively early during T cell activation.

The impact of CD27 and 4-1BB triggering on effector CD8 T cells

CD27 and 4-1BB engagement induced similar proliferation of OT-I T cells when measured on days 2 and 3 postpriming by CFSE dilution or on day 6 by Ki67 staining (Supplemental Fig. 3, data not shown). We then examined whether effector cell generation was differentially regulated by CD27 and 4-1BB signaling. We isolated splenocytes from mice following adoptive transfer of OT-I cells and priming with OVA+anti-CD27 mAb or OVA+anti–4-1BB mAb. Effector OVA-specific CD8 T cells were isolated from mice, and equal numbers were transferred into secondary recipients. The frequency of OVA-specific CD8 T cells was determined in peripheral blood on day 62. (B) Delaying 4-1BB triggering until day 2 negatively impacts on the generation of memory cells. Following adoptive transfer of OT-I cells and priming with OVA (day 0) or OVA+anti–4-1BB mAb (200 μg) on day 0 or 2, the frequency of OVA-specific CD8 T cells was monitored in blood. (C) The addition of 4-1BB agonist does not rescue the decline in memory OVA-specific CD8 T cells generated through priming with OVA+anti-CD27 mAb. Following adoptive transfer of OT-I cells, the frequency of OVA-specific CD8 T cells was monitored in the blood of mice primed with OVA+anti-CD27 mAb with control rat IgG or anti–4-1BB mAb (200 μg; day 0). Each data point represents the mean ± SE (data are representative of at least two independent experiments with three mice/group). *p ≤ 0.05, ***p ≤ 0.005.
cells and examined perforin expression by intracellular staining and flow cytometry. The frequency of perforin-expressing cells within the OVA-specific CD8 T cell population was higher in mice primed with OVA and anti-CD27 mAb compared with those that received OVA and anti–4-1BB mAb (Fig. 4A, Supplemental Fig. 4A). These findings were verified by additional experiments involving lower numbers of adoptively transferred OT-I T cells (Supplemental Fig. 4B). IL-2 is known to promote CTL effector function (8). Therefore, we examined whether IL-2 is preferentially produced after CD27 costimulation. We found significantly more IL-2–producing OVA-specific CD8 T cells in mice primed with OVA and anti-CD27 mAb compared with those primed with OVA and anti–4-1BB mAb (Fig. 4B, Supplemental Fig. 4C). Furthermore, the frequency of IFN-γ–producing cells was slightly higher on day 3 in mice given agonist CD27 compared with those receiving agonist 4-1BB (Fig. 4C). We also assessed expression of the transcription factors T-bet, comesodomin, Blmp-1, and Bcl-6, which regulate various facets of the effector and memory CD8 T cell–differentiation programs (2), but none displayed differential expression following CD27 or 4-1BB triggering (Supplemental Fig. 4D).

Our data show that, compared with 4-1BB, CD27 engagement substantially enhances IL-2 production by CD8 T cells, thus promoting strong autocrine signaling via the IL-2 receptor and skewing differentiation of CTLs toward terminal effectors. Consistent with this notion, priming with OVA and agonist anti-CD27 resulted in higher and more prolonged expression of CD25 (Fig. 5A; day 3), a known target of IL-2 signaling and a component of the IL-2R complex (32). This finding was verified by additional experiments using lower numbers of OT-I T cells (Supplemental Fig. 4E). CD27 triggering also preferentially induced expression of the transferrin receptor (CD71) (Fig. 5B) and transcription of Gpl49b (Lilrb4) (Fig. 5C), both known to be expressed following IL-2–mediated generation of CTLs in vitro (33, 34). To address the role of IL-2 in CTL differentiation after CD27 triggering, we neutralized IL-2 in vivo during priming with OVA and agonist anti-CD27. Neutralization of IL-2 had no effect on accumulation of OT-I cells at the peak of the response on day 6, demonstrating that CD27–mediated expansion of OT-I cells is independent of IL-2 production (Fig. 5D). In contrast, expression of perforin was markedly reduced after IL-2 neutralization (Fig. 5E). Additionally, IL-2 blockade during priming accentuated the death of T cells during the contraction phase, reducing memory T cell numbers (Fig. 5F).

Overall, our data demonstrate contrasting roles for CD27 and 4-1BB costimulation in CD8 effector T cell differentiation, as well as memory generation.

A higher rate of homeostatic proliferation impaired by CD27 signaling is incompatible with long-term maintenance of memory cells

Following Ag clearance, memory CD8 T cells are maintained largely through the action of IL-7 and IL-15 (2). Furthermore, in some models of infection, memory precursor cells can be identified at the peak of the response by increased expression of IL-7Rα and decreased expression of KLRG1 (2). Therefore, we determined whether CD27 and 4-1BB triggering differentially regulate T cell expression of IL-7Rα, the IL-15Rβ subunit (CD122), and KLRG1. Our data reveal that, at early time points and compared with 4-1BB, the downregulation of IL-7Rα on OT-I cells is delayed following CD27 triggering (Fig. 6A). However, when IL-7Rα expression was re-established on memory cells, no obvious differences were detected between the two groups (Fig. 6B). Interestingly, expression of KLRG1 on OT-I T cells initially was induced more efficiently by 4-1BB triggering (Fig. 6A), although at later time points both CD27– and 4-1BB–stimulated cells expressed similar levels of KLRG1 (Fig. 6B). Thus, increased memory formation by 4-1BB costimulation did not correlate with the presence of cells that expressed high IL-7Rα and decreased levels of KLRG1. Moreover, no differences were detected in the expression of CD122 between the CD27 and 4-1BB groups (Fig. 6B).

To address possible differences in homeostatic proliferation between CD27– and 4-1BB–generated memory T cells, we examined the proportion of cycling memory OT-I cells by measurement of BrdU incorporation. To our surprise, we found that
the proportion of OT-I cells that incorporated BrdU was consistently higher in the CD27 group (Fig. 6C). These data suggest that the waning of CD27-generated memory CD8 T cells is a consequence of a defect in cell survival.

Discussion
In this study, we demonstrated that CD27 and 4-1BB promote generation of effector and memory cells to different extents. Both receptors increase T cell accumulation during the primary response (Fig. 1A, 1B, 1E, Supplemental Fig. 1E); however, engagement of CD27 favors the generation of effector T cells, exemplified by perforin expression (Fig. 4A, Supplemental Fig. 4A, 4B). In addition, the memory T cell pool generated by CD27 triggering is short-lived and gradually wanes with time, which is in sharp contrast with the situation observed following priming with Ag and 4-1BB agonists (Fig. 1E, Supplemental Fig. 1E). Consequently, mice primed with Ag and 4-1BB agonists generated a significantly higher secondary response than those given Ag and CD27 agonists (Figs. 1E, 2A, 2B, Supplemental Fig. 1E). It is noteworthy that the inclusion of a CD27 agonist during immunization enhanced the magnitude of the primary and secondary responses compared with peptide alone (Supplemental Fig. 1E), consistent with our previous observation (18). However, by examining memory responses at later time points and conducting a side-by-side comparison with 4-1BB agonists, we discovered that CD27-generated memory CD8 T cells are relatively short-lived compared with those generated by 4-1BB agonists. Thus, targeting 4-1BB during peptide/protein vaccination should provide a more optimal method for generating long-term protective immunity. Whether CD27/4-1BB triggering exerts similar effects on CD8 T cell differentiation by guest on April 16, 2017 http://www.jimmunol.org/ Downloaded from
when using vaccines that induce a higher degree of inflammation remains to be established. The outcome of disrupting the CD27–CD70 interaction on the generation of memory cells has been variable; some, but not all, studies showed a reduction in the number of memory CD8 T cells (12, 35, 36). It was suggested that CD27 signaling promotes the accumulation of MPECs only under conditions in which IL-12 is strongly induced (36), which may explain the conflicting data regarding the effect of CD27 signaling on the generation of memory cells.

Our data demonstrate that continuous triggering of CD27 and 4-1BB during the memory phase is not required for the differences that we observed in the longevity of memory cells generated by CD27 or 4-1BB agonists (Fig. 3, Supplemental Fig. 2). These data suggest that the memory cell outcome is predetermined during the priming phase. We provide evidence for this notion by demonstrating differences in CD25 expression on CD8 T cells as early as 3 d after priming with CD27 and 4-1BB agonists (Fig. 5A, Supplemental Fig. 4E). Prolonged expression of CD25 on a subset of activated CD8 T cells during acute viral infection was shown to mark a population of cells with enhanced effector function and inability to mature into functional long-lived memory cells (7). Therefore, our data identify CD27 as a key driver of sustained CD25 expression on activated CD8 T cells. In addition to CD25, the transferrin receptor (CD71) and Gp49b (34, 37) were preferentially induced in CD27-stimulated CD8 T cells (Fig. 5B, 5C).

These molecules, together with perforin, are known to be upregulated by IL-2 (8, 32–34). Because IL-2 production by CD8 T cells is induced more strongly by CD27 triggering compared with 4-1BB (Fig. 4B), our data suggest a scenario in which differentiation toward cytotoxic effectors is regulated in a CD8 T cell–autonomous manner through CD27 and IL-2 signaling (Fig. 5E). However, not all of the costimulatory effects of CD27 require IL-2; early expansion and accumulation of OT-I T cells at the peak of the primary response occurred independently of IL-2 (Fig. 5D). Whether the skewed differentiation of CD27-stimulated CD8 T cells into short-lived memory cells is mediated by the increased production of IL-2 could not be discerned. IL-2 neutralization did not restore stable memory but instead accentuated the contraction of the T cell response (Fig. 5F), suggesting that IL-2 maintains the survival of short-term memory cells (9). Our data are consistent with the notion that CD27 triggering skews the CD8 T cell–differentiation program toward terminal differentiation.

Although in this study we used enforced costimulation by administering agonists, a setting pertinent to vaccination, other investigators showed that the lack of CD27 can compromise IL-2 production by influenza virus–specific CD8 T cells (38). This suggests that the effects of CD27 agonists on IL-2 production reported in this article resemble the effects of the endogenous CD27–CD70 interaction.

IFN-γ is a potential factor that contributes to the decline in memory after priming with CD27 agonists because it is elevated in CD27-stimulated T cells both after acute stimulation (Fig. 4C) and in transgenic mice that constitutively express CD70 (39). Thus, IFN-γ–deficient mice have normal expansion of Ag-specific CD8 T cells postinfection with attenuated Listeria monocytogenes but reduced contraction, culminating in the persistence of elevated numbers of Ag-specific CD8 T cells (5, 40).

Although in certain infection models memory precursor cells can be identified by increased expression of the IL-7Rα subunit and decreased expression of KLRG-1 (2), this was not the case in our vaccination protocol (Fig. 6A, 6B). In contrast, prolonged expression of CD25 was a more informative predictor of cells that differentiate into short-term memory cells, thus corroborating previous findings obtained in the acute lymphocytic choriomeningitis virus infection model (7). Additionally, our data highlight increased expression of CD71 and Gp49b as potentially useful in identifying short-term memory precursor cells. The general applicability of these markers in distinguishing memory precursor subsets during infection requires further attention.

The differential effects elicited by CD27 and 4-1BB agonists during priming on homeostatic memory cell proliferation are intriguing and indicate that the relative lack of memory in CD27-primed mice is due to a higher rate of cell death. CD8 T cell death during the contraction phase is controlled by the Bcl-2–regulated “intrinsic” death pathway, as well as the “extrinsic” death pathway that is dependent on CD95 (41). Using a previously established protocol (42), we administered a neutralizing anti-CD95 ligand mAb to address whether CD95 signaling contributed to T cell death in our model. Administration of anti-CD95 ligand mAb did not affect OT-I T cell death following priming with OVA and agonist anti-CD27 (data not shown), consistent with the lack of CD95 involvement reported in other studies of acute CD8 T cell responses (41). In contrast, transgenic overexpression of Bcl-2 in OT-I T cells [yav-bcl-2 × OT-I mice (43)] markedly enhanced T cell survival at the peak of the response and during the contraction phase, independent of the type of agonist used for priming. Nevertheless, 4-1BB–generated memory cells still exhibited better long-term survival than did memory cells generated by priming with an agonist anti-CD27 (data not shown).

The inability of Bcl-2 to restore CD27-generated memory cell frequencies to levels similar to those produced after priming with the 4-1BB agonist could be due to insufficient sequestration of proapoptotic Bcl-2 family proteins. Alternatively, it may be that expression of the Bcl-2 transgene is sufficient to neutralize proapoptotic Bcl-2 family proteins, but cell death ensues through a nonapoptotic programmed cell death pathway (44). Moreover, it is possible that CD27-generated memory cells, which proliferate more extensively than do memory cells generated with a 4-1BB agonist (Fig. 6C), die because they cannot sustain their bioenergetic needs. Because memory T cells generally rely on oxidative phosphorylation for their bioenergetic requirements compared with effector cells, which primarily use glycolysis (45, 46), a reduced mitochondrial mass and, thus, lower mitochondrial spare respiratory capacity in CD27-generated memory cells, could lead to their rapid demise.

In summary, we showed that an early and narrow window of CD27 or 4-1BB triggering during priming skew the differentiation program of Ag-stimulated CD8 T cells, such that effector function and memory cell numbers are differentially affected. Both receptors recruit TNFR-associated factors through short peptide motifs as a way of activating a largely shared set of signaling pathways (11). However, differences may exist in the magnitude and duration of signaling as a result of subtle changes in the kinetics of binding of TNFR-associated factors to specific receptors that could translate into functional differences. Taken together, our study identifies a novel approach for regulating effector and memory cell generation and, thus, a strategy to optimize vaccines that elicit CD8 T cells.

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Disclosures

The authors have no financial conflicts of interest.
References


8. CD8 T CELL PROGRAMING BY CD27 AND 4-1BB


