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CD27 costimulation contributes substantially to the expansion of functional memory CD8$^+$ T cells after peptide immunization

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Naive T cells require signals from multiple costimulatory receptors to acquire full effector function and differentiate to long-lived memory cells. The costimulatory receptor, CD27, is essential for optimal T-cell priming and memory differentiation in a variety of settings, although whether CD27 is similarly required during memory CD8$^+$ T-cell reactivation remains controversial. We have used OVA and anti-CD40 to establish a memory CD8$^+$ T-cell population and report here that their secondary expansion, driven by peptide and anti-CD40, polyI:C, or LPS, requires CD27. Furthermore, antigenic peptide and a soluble form of the CD27 ligand, CD70 (soluble recombinant CD70 (sCD70)), is sufficient for secondary memory CD8$^+$ T-cell accumulation at multiple anatomical sites, dependent on CD80/86. Prior to boost, resting effector- and central-memory CD8$^+$ T cells both expressed CD27 with greater expression on central memory cells. Nonetheless, both populations upregulated CD27 after TCR engagement and accumulated in proportion after boosting with Ag and sCD70. Mechanistically, sCD70 increased the frequency of divided and cytolytic memory T cells, conferred resistance to apoptosis and enabled retardation of tumor growth in vivo. These data demonstrate the central role played by CD27/70 during secondary CD8$^+$ T-cell activation to a peptide Ag, and identify sCD70 as an immunotherapeutic adjuvant for antitumor immunity.

Keywords: CD27 · CD70 · Memory · T cell · TLR · TNFRSF

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Introduction

T lymphocytes mediate immunity to infection and can protect against tumor growth. For full activation, T cells require TCR engagement and costimulatory signals provided predominantly through members of the Ig superfamily (e.g. CD28 and ICOS) and TNF receptor superfamily (TNFRSF; e.g. CD27 and 4-1BB). TNFRSF receptors signal via the NF-κB, MAPK, and PI3K pathways to facilitate cell cycle progression, upregulation of antiapoptotic Bcl-2 family members, secretion of cytokines, and expression of

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cytokine receptors leading to improved T-cell survival and proliferation. The existence of multiple TNFRSF members allows for tight temporal and spatial control of T-cell activation, regulated in part by the expression patterns of the receptors and their ligands [1].

Unlike many TNFRSF receptors, CD27 is highly expressed on naive $CD4^+$ and $CD8^+$ T cells and is further upregulated after TCR engagement [2]. The only known ligand for CD27/CD70 exhibits limited expression at rest but is upregulated on DC and B cells within 24 h of incubation with anti-CD40 or TLR agonists [3–5]. CD27/CD70 is therefore well poised to contribute to early T-cell activation events. Indeed, CD27 signals are essential for maximal T-cell priming by protein Ag, cellular vaccines, or infectious agents [3, 5–9], and for the establishment of T-cell memory after influenza infection [6]. Furthermore, DC-dependent expansion of $CD8^+$ T cells and tumor protection triggered by Ag and anti-CD40 both require CD70, and anti-CD40 can be replaced with agonist anti-CD27 mAb to recapitulate the antitumor response [4, 5, 10, 11]. Together these data suggest that the signal delivered from a CD40-licensed DC to a naive $CD8^+$ T-cell requires T-cell-expressed CD27, a finding recently confirmed by others [12].

Memory T cells provide rapid protection against antigenic rechallenge and have been delineated into two major subsets: effector-memory $CD8^+$ T (T_{EM}) cells reside primarily in nonlymphoid tissues and constitutively express perforin and granzyme B, whereas central-memory $CD8^+$ T (T_{CM}) cells express CD62L and CCR7 and are present in secondary lymphoid organs [13]. Both subsets express CD27 and the absence of CD27 during priming equally impairs the differentiation of both memory T-cell populations [14, 15]. Once established, high expression of CD27 is a feature of cells with a T_{CM}-like phenotype and greater capacity for mounting recall responses compared with $CD27^{lo}$ cells [14, 16]. In contrast, $CD27^{lo}$ cells are associated with a terminally differentiated/senescent memory T-cell phenotype in both humans and mice [17, 18].

Compared with primary T cells, memory $CD8^+$ T cells show an increased cell frequency, longevity, improved effector function, and increased responsiveness to lower Ag concentration [19–21]. Nonetheless, memory T cells remain dependent on DC for reactivation, reflecting an ongoing requirement for costimulation [22, 23]. Several studies have reported CD28 as a critical mediator of T-cell recall responses [24–27] and some evidence suggests that CD40 also contributes to secondary memory differentiation after restimulation [28]. In contrast, the absence of 4–1BBL or OX40L on APC during reexposure to influenza has no effect on T-cell secondary expansion [29]. With regard to CD27, data are conflicting with evidence both for and against a role for this receptor during secondary T-cell expansion [12, 15, 30–33].

In this study, we investigated the role of CD27 costimulation during a secondary $CD8^+$ T-cell response. Specifically, we show that maximal reactivation of memory $CD8^+$ T cells by peptide and either anti-CD40 or the TLR agonists, polyI:C and LPS, requires CD70. Furthermore, boosting with Ag plus soluble recombinant CD70 (sCD70) was sufficient to drive significant accumulation of T_{EM} and T_{CM} $CD8^+$ T cells in different organs and to expand secondary $CD8^+$ T cells with cytotoxic function capable of protecting against tumor growth in vivo. Our findings have implications for the use of sCD70 in prime-boost vaccination protocols and provide insight into the role of CD27–CD70 signals during recall responses.

Results

CD40-driven secondary expansion of memory $CD8^+$ T cells requires CD27 and CD28

To analyze the role of costimulation during the recall response of endogenous memory $CD8^+$ T cells, we generated memory OVA-specific $CD8^+$ T cells by immunizing mice with OVA and agonistic anti-CD40 mAb, as described [4, 10]. This protocol primed a strong $CD8^+$ T-cell response and established a stable memory population, representing 0.2–0.5% of total lymphocytes as reported previously ([10] and data not shown). Memory cells had typical memory $CD8^+$ T-cell markers, including high expression of CD44 and a bimodal distribution of CD62L, characteristic of T_{CM} ($CD62L^{hi}$) and T_{EM} ($CD62L^{lo}$) cells (Fig. 1A). Additionally, the majority of memory $CD8^+$ T cells expressed surface CD27 at heterogeneous levels (Fig. 1A). Upon rechallenge with the immunodominant OVA peptide ($OVA_{257–264}$) and anti-CD40, OVA-specific $CD8^+$ T cells increased in number to comprise 12% (±4.0%) of total lymphocytes by day 7 (Fig. 1B). Expansion was dependent on coinjection of anti-CD40 since no significant increase was observed after administration of Ag alone (Fig. 1B). Importantly, injection of $OVA_{257–264}$ and anti-CD40 to naive mice did not result in a detectable OVA-specific $CD8^+$ T-cell response (Fig. 1B), confirming that the increased T-cell frequency after rechallenge is a bona fide recall response of OVA-specific memory $CD8^+$ T cells.

Anti-CD40 leads to upregulation of costimulatory ligands on DCs, including CD80, CD86, and CD70, which interact with CD28 and CD27 on T cells to mediate priming of naïve $CD8^+$ T cells [4, 34, 35]. We therefore addressed the role of CD27 or CD28 during the recall response. Blockade of either CD70 or CD80/86 reduced the anti-CD40-stimulated expansion of OVA-specific memory $CD8^+$ T cells by approximately 75 to 3.1% (±0.5%) and 2.9% (±0.8%) of total lymphocytes, respectively, at day 7 (Fig. 1B). Secondary $CD8^+$ T-cell expansion driven by peptide in combination with LPS or polyI:C was also largely dependent on CD70 (Supporting Information Fig. 1) showing that TLR agonists cannot compensate for a lack of CD70 during boost.

Soluble CD70 promotes the recall response of memory $CD8^+$ T cells

We next investigated whether Ag and CD27 costimulation is sufficient to boost a secondary response. Mice were primed as before and boosted with $OVA_{257–264}$ and soluble recombinant murine CD70 (sCD70) [2]. $OVA_{257–264}$ and sCD70 caused a 19-fold expansion of the OVA-specific memory $CD8^+$ T-cell pool with an

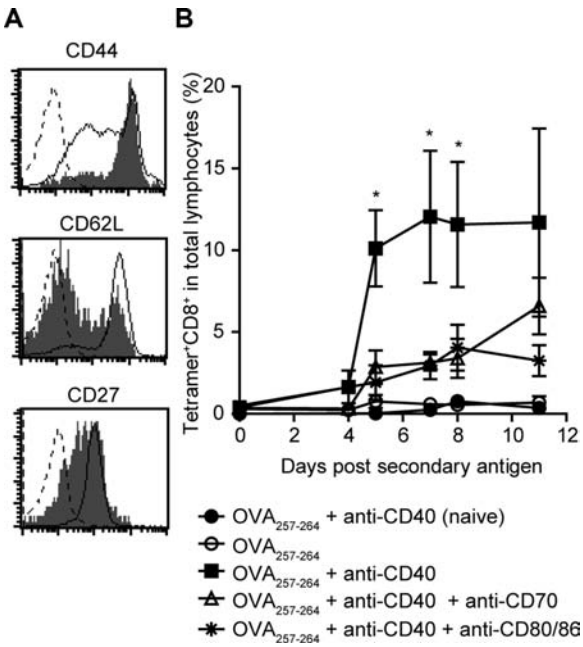

Figure 1. CD70 and CD80/86 are required for maximal memory CD8$^+$ T-cell reactivation driven by anti-CD40. Mice were immunized with OVA plus anti-CD40. (A) Twenty days later the phenotype of splenic OVA-specific memory CD8$^+$ T cells was analyzed by staining with H-2K^b/OVA$_{257–264}$ tetramers and anti-CD8 with antibodies/soluble ligands for CD44, CD62L, and CD27. Expression by tetramer$^+$CD8$^+$ (filled histograms) and tetramer$^-$CD8$^+$ (open histograms) T cells from the same mouse is shown; dotted lines represent isotype control staining on tetramer$^+$CD8$^+$ T cells. Data are representative of two independent experiments of three mice each. (B) On day 23, primed (or naive control) mice received OVA$_{257–264}$ alone or with agonistic anti-CD40 in the presence of blocking antibodies anti-CD70, anti-CD80/86, or control Ig (Mc106A5). The frequencies of primed and reactivated memory CD8$^+$ T cells in peripheral blood were determined by staining with H-2K^b/OVA$_{257–264}$ tetramer and anti-CD8. The mean percentages of tetramer$^+$CD8$^+$ T cells in the lymphocyte population (±SEM) on the days indicated are shown, pooled from three experiments with three mice per group (i.e. $n = 9$) except groups OVA$_{257–264}$ + anti-CD40 (naive) and OVA$_{257–264}$ + anti-CD40 + anti-CD80/86, which were included in one experiment ($n = 3$). $^*p < 0.05$ comparing OVA$_{257–264}$ + anti-CD40 with OVA$_{257–264}$ + anti-CD40 + anti-CD70 groups, Student's *t*-test.

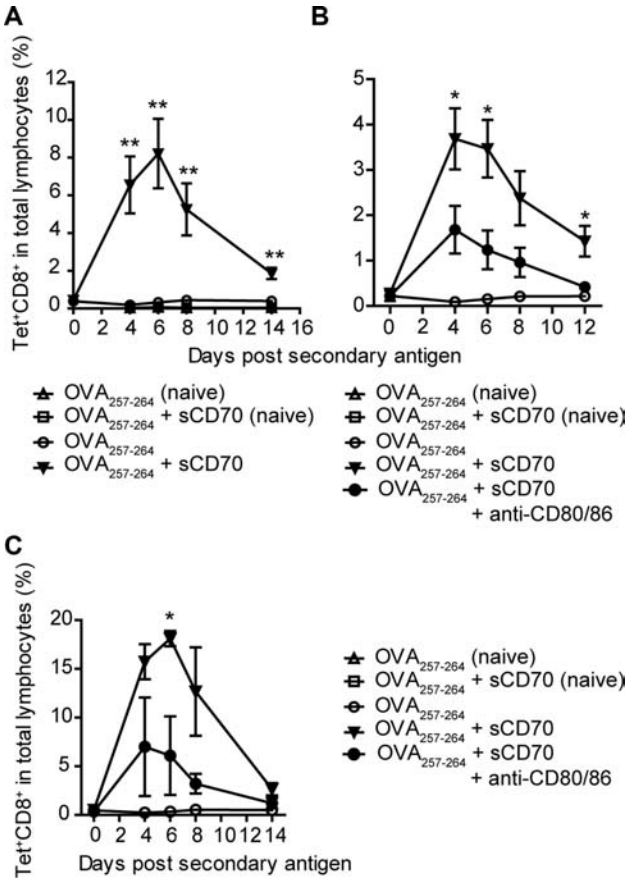

Figure 2. Soluble CD70 and peptide Ag drive robust memory CD8$^+$ T-cell expansion. B6 mice were immunized with OVA plus anti-CD40. Fifteen to 48 days later immunized mice or naïve controls were restimulated with OVA$_{257–264}$ alone or with sCD70 ± blocking anti-CD80/86. Primary and memory CD8$^+$ T-cell responses were tracked over time in peripheral blood by staining with H-2K^b/OVA$_{257–264}$ tetramer and anti-CD8. Data points represent the mean percentage of tetramer$^+$CD8$^+$ T cells in the lymphocyte population ± SEM and are pooled from four experiments (A) or show replicate experiments (B, C). $^{**}p < 0.005$, Student's *t*-test comparing OVA$_{257–264}$ with OVA$_{257–264}$ + sCD70 groups; $^*p < 0.05$, Student's *t*-test comparing OVA$_{257–264}$ + sCD70 with OVA$_{257–264}$ + sCD70 + anti-CD80/86 groups.

average of 8% (±1.8%) of the peripheral blood lymphocytes being OVA-specific CD8$^+$ T cells on day 6 (Fig. 2A). As before, we could detect no expansion of memory cells in mice boosted with OVA$_{257–264}$ alone. Naive mice that received OVA$_{257–264}$ and sCD70 in parallel did not show an increase in the frequency of OVA-specific CD8$^+$ T cells (Fig. 2A) confirming that memory, and not primary, CD8$^+$ T cells are activated by OVA$_{257–264}$ and sCD70. In common with the anti-CD40-driven secondary response (Fig. 1B), increased accumulation of CD8$^+$ T cells triggered by sCD70 was largely dependent on CD80/86 (Fig. 2B and C).

To exclude the possibility that OVA-specific memory CD8$^+$ T cells aid the priming of naïve T cells as was previously reported [36, 37], we transferred endogenous memory CD8$^+$ T cells into congenic naïve recipients in order to track primary and secondary responses within the same animal. Adoptive transfer of 1×10^7 CD90.2$^+$ purified CD8$^+$ T cells (containing endogenous OVA-specific memory CD8$^+$ T cells) to CD90.1$^+$ congenic mice resulted in 6–10% of their total CD8$^+$ T cells being CD90.2$^+$ (data not shown). Subsequent stimulation of these mice with OVA$_{257–264}$ and sCD70 expanded CD90.2$^+$ OVA-specific memory CD8$^+$ T cells as expected, but had little effect on the naive CD90.1$^+$ CD8$^+$ subset (Supporting Information Fig. 2). Finally, to ensure that the sensitivity of memory CD8$^+$ T cells to sCD70 is not a peculiarity of priming with anti-CD40, similar experiments were performed in which OVA-specific memory CD8$^+$ T cells were established by priming with OVA and the glycolipid α-galactosylceramide. We have previously reported that α-galactosylceramide activates invariant NKT cells and drives CD40-, DC-, and CD70-dependent primary T-cell responses and the establishment of resting T-cell memory ([38] and data not shown). On secondary restimulation with OVA$_{257–264}$ plus sCD70, memory CD8$^+$ T cells primed by OVA and α-galactosylceramide underwent significant expansion (increasing approximately 35-fold by day 4), whereas no

secondary response was detected in mice restimulated with $OVA_{257–264}$ alone (Supporting Information Fig. 3). Together these data show that the CD27/CD70 axis is necessary for expansion of memory $CD8^+$ T cells and that sCD70 is a potent driver of secondary $CD8^+$ T-cell activation.

Boosting with $OVA_{257–264}$ and sCD70 increases the frequency of $CD8^+$ T_{EM} and T_{CM} cells

To assess whether CD27 costimulation causes an overall expansion in memory $CD8^+$ T-cell number or merely alters their recirculation pattern, we assessed OVA-specific memory $CD8^+$ T-cell frequency in secondary lymphoid organs and peripheral tissues. Following restimulation with $OVA_{257–264}$ and sCD70, the frequency of OVA-specific $CD8^+$ T cells increased at multiple sites (13-fold in the LN, 14-fold in the spleen, and 10-fold in the liver; Fig. 3A) by day 6, the peak of the secondary response in the blood (Fig. 2). In contrast, $OVA_{257–264}$ alone resulted in only two-, 1.4-, and threefold expansion in the LN, spleen, and liver, respectively. By day 8, no significant increase in OVA-specific $CD8^+$ T cells over that seen in nonstimulated mice was observed in the $OVA_{257–264}$ alone group, whereas animals that received $OVA_{257–264}$ and sCD70 retained significantly increased frequencies (Fig. 3A) and absolute numbers (Fig. 3B) of secondary $CD8^+$ T cells in all the organs tested. Analysis of CD62L expression on expanded memory T cells confirmed that both $CD62L^{hi}$ and $CD62L^{lo}$ populations were expanded by $OVA_{257–264}$ and sCD70 injection (Fig. 3C and D).

As expected resting T_{EM} cells expressed less CD27 compared with T_{CM} cells (Fig. 3E; compare liver- and LN-derived T cells, respectively). However, after TCR stimulation, T_{EM} cells upregulated CD27 to levels similar to that expressed by T_{CM} cells. Additional activation with sCD70 further upregulated CD27 so that all secondary effector T cells became uniformly $CD27^{hi}$ in all tissues tested (Fig. 3E). To confirm that both reactivated T_{EM} and T_{CM} memory $CD8^+$ T cells express significant levels of CD27 after activation, we incubated splenic (a mix of T_{EM} and T_{CM} cells; Fig. 3C) memory $CD8^+$ T cells with anti-CD3 prior to staining for CD27. In contrast to resting memory $CD8^+$ T cells that expressed heterogeneous levels of CD27, all activated memory cells became uniformly $CD27^{hi}$ (Fig. 3F). These data cannot be attributed to preferential expansion of one memory $CD8^+$ T-cell subset over another, as cells were only stimulated for 24 h prior to the first cell division [39]. Thus, both T_{EM} and T_{CM} cells upregulate CD27 in response to TCR triggering, potentially enabling them to respond to Ag and sCD70.

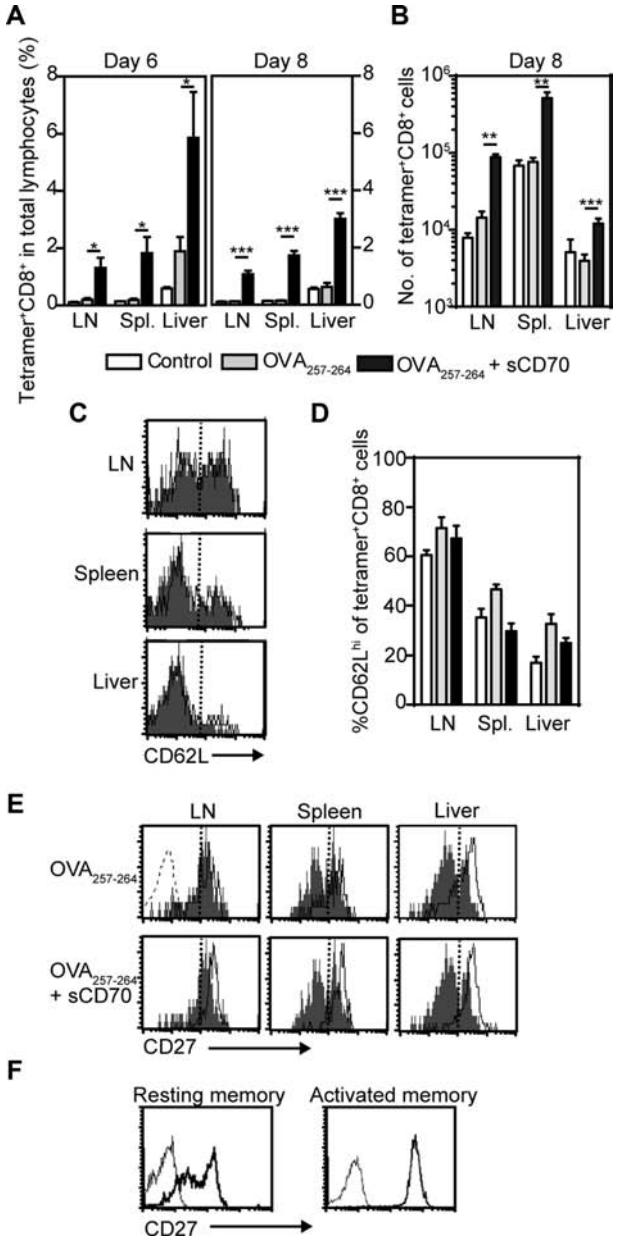

Figure 3. sCD70 and Ag induces memory $CD8^+$ T-cell accumulation in secondary lymphoid organs and peripheral tissues. B6 mice were immunized with OVA plus anti-CD40. Fifteen to 48 days later mice were restimulated with $OVA_{257–264}$ alone or with sCD70 or were left untreated (control). (A) OVA-specific $CD8^+$ T-cell frequency and (B) absolute number per organ were determined in LN, spleen (spl.), and liver on days 6 (A; left panel) and 8 (A; right panel, B) after secondary stimulation by staining with H-$2K^b$/$OVA_{257–264}$ tetramer and anti-CD8. Bars represent (A) the mean percentage of tetramer$^+$$CD8^+$ T cells in the lymphocyte population or (B) the mean number of tetramer$^+$$CD8^+$ cells per organ +SEM (n = 3–6 mice per group) and are representative of (A) two experiments or (B) show data from one of three independent experiments performed. $^*p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0005$, Student's t-test. (C–E) The phenotype of memory $CD8^+$ T cells (C, E) 6 or (D) 8 days after restimulation with $OVA_{257–264}$ alone or $OVA_{257–264}$ and sCD70 was analyzed in LN, spleen, and liver by staining with H-$2K^b$/$OVA_{257–264}$ tetramer and anti-CD8 in conjunction with anti-CD62L (C, D) or anti-CD27 (E). (C) Expression on resting memory tetramer$^+$$CD8^+$ T cells (filled histograms) and $OVA_{257–264}$ + sCD70 restimulated memory tetramer$^+$$CD8^+$ T cells (open histograms) is shown; dotted lines represent staining with isotype control antibodies. (C, E) Data are representative of two independent experiments with at least three mice per group or (D) show data from a separate third experiment. (F) Splenic memory $CD8^+$ T cells were cultured alone (resting memory) or in the presence of 10 μg/mL anti-CD3 (activated memory). Expression of CD27 at 24 h is shown on the H-$2K^b$/$OVA_{257–264}$$^+$$CD8^+$-gated population (heavy lines) compared with staining by an isotype control antibody (weak lines). Data are representative of two experiments with three mice per group.

sCD70 promotes the survival of proliferating memory CD8$^+$ T cells during the recall response

To understand how sCD70 enhances CD8$^+$ T-cell accumulation during the recall response, we examined the effect of sCD70 on the proliferation of restimulated memory CD8$^+$ T cells. To this end, enriched CD8$^+$ T cells from primed mice containing OVA-specific CD8$^+$ memory T cells were CFSE labeled and adoptively transferred into RAG1$^{-/-}$ recipients prior to injection of $OVA_{257–264}$ alone or in combination with sCD70. We chose to use RAG1$^{-/-}$ recipients in these experiments to maximize our ability to detect dilution of CFSE in the relatively small population of endogenous Ag-specific memory T cells. After 3 days, a minority (approximately 6%) of the memory CD8$^+$ T cells had undergone homeostatic proliferation in the absence of specific Ag. However, greater cell division was clearly apparent in cells boosted with $OVA_{257–264}$ (13% CFSE^{lo}) and more so by cells boosted with $OVA_{257–264}$ plus sCD70 (34% CFSE^{lo}; Fig. 4A). By day 6, homeostatic proliferation was more pronounced, but could be distinguished from Ag-driven proliferation by the relatively high CFSE content of homeostatically proliferating cells (Fig. 4A). Nonetheless, significantly more CFSE^{lo} cells were observed in the $OVA_{257–264}$ and $OVA_{257–264}$ plus sCD70 groups with 78% and 90% of OVA-specific memory CD8$^+$ T cells having undergone extensive cell division, respectively. In addition, sCD70 drove increased accumulation of memory CD8$^+$ T cells in recipient mice so that by day 6, OVA-specific CD8$^+$ T cells accounted for 35% of the transferred CD8$^+$ T cells in the $OVA_{257–264}$ plus sCD70 group compared with only 6% in the $OVA_{257–264}$ group (data not shown). In a nonadoptive transfer experimental setting, sCD70 also increased the frequency of CD8$^+$ T cells that had taken up BrdU from 10% of OVA-specific cells in mice receiving $OVA_{257–264}$ alone compared with 32% in mice receiving $OVA_{257–264}$ and sCD70 (Fig. 4B).

To assess whether sCD70 promotes CD8$^+$ T-cell survival during secondary expansion, we used tetramer and annexin V staining to identify OVA-specific apoptotic cells. In mice that were restimulated with $OVA_{257–264}$ alone, the frequency of annexin V$^+$ OVA-specific CD8$^+$ T cells increased from 12 to 22% between days 5 and 6 but remained less than 3% in mice receiving $OVA_{257–264}$ and sCD70 (Fig. 4C and D). Similarly, approximately 20% of OVA-specific CD8$^+$ T cells stained positively with YO-PRO-1 on days 4–6 compared with only 5% of OVA-specific CD8$^+$ T cells following restimulation with $OVA_{257–264}$ and sCD70 (data not shown).

Thus, CD27 costimulation during the recall response drives accumulation of proliferating cells and confers protection from apoptosis.

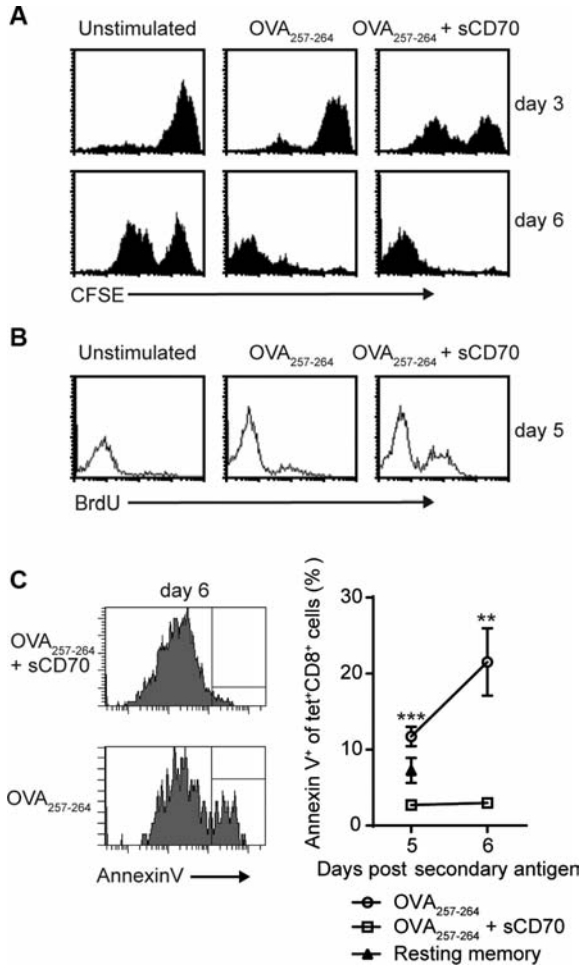

Figure 4. Ag and sCD70 promotes cell division and resistance to apoptosis in memory CD8$^+$ T cells. Groups of B6 mice were primed with OVA and anti-CD40. (A) Resting splenic memory CD8$^+$ T cells were isolated and stained with CFSE prior to injection into naive RAG1$^{-/-}$ recipients. Mice were then injected with $OVA_{257–264}$ alone or with sCD70 or were left untreated (unstimulated control). On days 3 and 6, the CFSE profile of CD8$^+$tetramer$^+$ cells was evaluated. (B, C) Mice were injected with $OVA_{257–264}$ alone, with sCD70 or were left unstimulated; some mice (B) additionally received BrdU on day 4 for analysis on day 5. Incorporation of BrdU (B) and binding to annexin V (C) by H-2K^b/$OVA_{264–257}$ tetramer$^+$CD8$^+$ cells was then determined on the days shown to determine cell division (B) and apoptosis (C), respectively. Data in (A) and (B) are representative of at least two experiments with three mice per group; data in (C) are from one experiment with four mice per group. $^{**}p < 0.005$, $^{***}p < 0.0005$, Student's *t*-test comparing $OVA_{257–264}$ with $OVA_{257–264}$ + sCD70 groups.

Memory CD8$^+$ T cells expanded by sCD70 are cytotoxic

To determine the functionality of memory CD8$^+$ T cells expanded by $OVA_{257–264}$ and sCD70, splenocytes were isolated at the peak of the secondary response and their ability to lyse $OVA_{257–264}$-pulsed target cells assessed directly ex vivo (Fig. 5A). Splenocytes from mice that received only the primary challenge were unable to kill target cells consistent with previous studies showing that resting memory CD8$^+$ T cells are poorly cytolytic as measured by ex vivo chromium release assays [40]. Mice boosted with $OVA_{257–264}$ alone also failed to kill peptide-pulsed target cells. In contrast, $OVA_{257–264}$- and sCD70-boosted memory CD8$^+$ T cells killed peptide-pulsed, but not control (data not shown) target cells, confirming that this boosting regimen expands memory CD8$^+$ T cells with Ag-specific cytotoxic function.

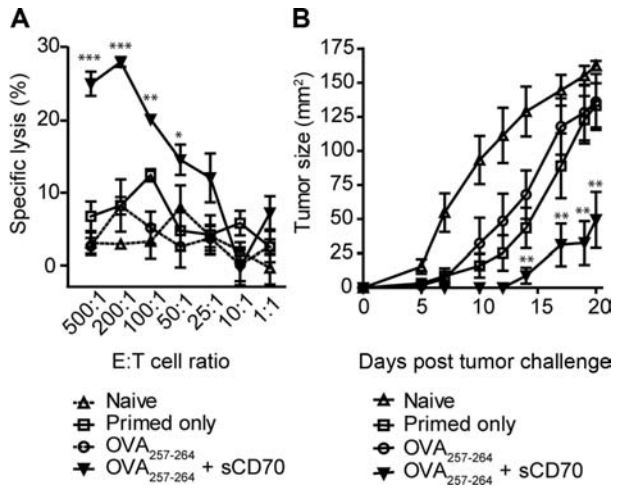

Figure 5. sCD70 promotes tumor rejection by memory CD8$^+$ T cells. Groups of mice were primed with OVA and anti-CD40 or left untreated (naive). (A) Twenty-one days later mice received a booster injection as indicated and were culled 6 days later to determine the cytolytic capability of splenocytes directly ex vivo by ^{51}Cr-release assay. Data indicate the mean (±SEM)% lysis of peptide-pulsed target cells in triplicate wells. (B) Fifteen days after priming mice were boosted as indicated prior to challenge 3 days later with E.G7 tumor cells s.c. Graphs represent the mean tumor area ± SEM over time (B). All data are representative of two independent experiments with three mice per group. $^*p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0005$, Student's *t*-test comparing $OVA_{257–264}$ with $OVA_{257–264}$ + sCD70 groups in each case.

Finally, to confirm that $OVA_{257–264}$- and sCD70-boosted memory CD8$^+$ T cells are cytolytic in vivo, prime-boosted mice were injected with the syngeneic OVA-positive tumor E.G7. Mice receiving the primary challenge alone, or mice boosted with $OVA_{257–264}$ boost, showed a slight delay in tumor growth compared with naive mice. However, significant inhibition of tumor growth was only seen in mice boosted with $OVA_{257–264}$ and sCD70 (Fig. 5B).

Discussion

We and others have reported previously that CD27 supplies a critical signal from CD40-licensed DC to drive CD8$^+$ T-cell priming and memory CD8$^+$ T-cell differentiation [4, 5, 12]. Data presented here additionally show first that CD27/70 is necessary for the expansion of memory CD8$^+$ T cells downstream of CD40, polyI:C or LPS, and describe sCD70 as a potent mediator of functional memory CD8$^+$ T-cell reactivation.

Our finding that memory T-cell reactivation by either anti-CD40 or sCD70 is dependent on CD80/86 confirms previous studies showing that CD28/80/86 are necessary for memory CD8$^+$ T-cell responses after infection with vaccinia, influenza, or lymphocytic choriomeningitis virus (LCMV) and for memory CD4$^+$ T-cell expansion and IL-2 secretion after rechallenge with antigenic protein [24–27]. However, whether CD27 similarly plays a role during memory T-cell activation has been less clear; many studies that sought to address this question have made use of CD27-deficient or CD70-overexpressing mice in which interpretation of the memory CD8$^+$ T-cell response is complicated by the impact of CD27/CD70 signals on CD8$^+$ T-cell priming/imprinting and memory differentiation [6, 9, 12, 41, 42]. Furthermore, the remaining studies have reported conflicting results. Blocking anti-CD70 mAb did not influence secondary memory CD8$^+$ T-cell accumulation during rechallenge with influenza [32] or vaccinia [12], suggesting that CD70 is dispensable for secondary T-cell expansion. However, others have reported that blocking anti-CD70 inhibited the expansion of LCMV-specific memory CD8$^+$ T cells after rechallenge with virus and abrogated memory CD8$^+$ T-cell-mediated cardiac graft rejection [30, 31]. Our data lend support for the view that CD27/CD70 interactions can be essential for maximal reactivation of memory CD8$^+$ T cells in vivo.

There are several potential explanations for the apparently contradictory results in these reports, inadequate antibody blocking and/or the use of different antibody clones being the most obvious. Another possibility is that CD70 has some intrinsic function on CD70-expressing DC, which is influenced by anti-CD70 mAb. This suggestion is made in light of findings that CD70 triggers reverse signaling in B cells [43] and that CD70 transport to the cell surface on DC is closely aligned with the transport of MHC class II [44]. Recently, Munitic et al. (2013) [33] have shown that transfer of memory LCMV-specific CD8$^+$ T cells to CD70$^{-/-}$ hosts has no detrimental effect on their reactivation, clearly indicating that in some circumstances CD70 can be dispensable for CD8$^+$ T-cell recall responses.

Instead, it seems likely that the relative importance of CD27/CD70 depends on the expression of CD70 induced by a particular pathogen/DC stimulus [33, 45] and/or upon the extent of inflammation. It is interesting that opposing results were obtained when boosting with the same infectious agent, LCMV [31, 33]. Different substrains and doses were used in these studies and could conceivably have modified either CD70 expression or the quantity/quality of innate activation via, e.g., TLR agonists or type I interferons. Our data reveal that memory CD8$^+$ T-cell reactivation remains largely dependent on CD70 after restimulation with peptide and either polyI:C or LPS, suggesting that TLR agonists alone cannot render secondary CD8$^+$ T-cell expansion independent of CD70. With regard to type I interferon, LCMV infection is known to drive high levels of this cytokine that can trigger T-cell proliferation and differentiation to memory [46]. In addition, we have previously reported that augmented T-cell responses induced by type I interferon are not inhibited by blockade of CD70 [5], indicating that type I interferon may compensate for CD70 in some circumstances. Perhaps the levels of type I interferon dictate the CD70 dependence of secondary CD8$^+$ T-cell responses; indeed, much greater doses of LCMV were used in the study that showed CD70 independence at boosting, fitting well with this hypothesis [33].

Our data also show that minimal peptide Ag and sCD70 at boosting is sufficient to drive accumulation and increased survival of memory CD8$^+$ T cells in the absence of other exogenous costimulation. It is possible that sCD70 exerts its effects indirectly through activation of other CD27-expressing cells (NK, iNKT, CD4$^+$, and B cells). However, we think this unlikely given

that sCD70-driven secondary $CD8^+$ T-cell accumulation is not inhibited by NK-cell depletion (data not shown) or the absence of $CD4^+$ or B cells (Fig. 4A). Importantly, sCD70-expanded memory $CD8^+$ T cells were bone fide effector cells capable of direct cytotoxicity in vitro and of retarding tumor growth in vivo. In humans, CD27 expression has been associated with increased survival, proliferation and persistence of T cells after adoptive immunotherapy for HIV, and persistence of tumor infiltrating lymphocytes in melanoma, suggesting that CD70 signals similarly drive memory T-cell maintenance in patients [47, 48]. Given the modest responses induced by many cancer immunotherapies [49], we anticipate that CD27 agonists used as part of a prime-boost protocol may be of clinical benefit. This notwithstanding, persistent CD27 stimulation during LCMV infection increases IFN-γ and TNF-α production, decreases both B-cell frequency and neutralizing antibody titers, and prevents viral clearance [50], suggesting that CD27 stimulation should be used with caution and in a temporally controlled manner. Unlike mAb, sCD70 has a restricted in vivo half-life and may therefore prove to be an attractive agent for exploitation of the therapeutic potential of this costimulatory molecule.

Surprisingly, sCD70 (plus $OVA_{257–264}$) was unable to prime a significant endogenous $CD8^+$ T-cell response, although the efficacy of the same vaccine combination for boosting memory $CD8^+$ T cells is clear. This finding stands in apparent contrast with our previous work in which naïve OT-I transgenic $CD8^+$ T cells responded strongly to $OVA_{257–264}$ and sCD70 [2]. We hypothesize that the decreased activation threshold requirements of high-affinity transgenic T cells afforded OT-I transgenic cells greater sensitivity to sCD70 and/or that their increased precursor frequency facilitated detection of the expanded cells [51]. Interestingly, provision of whole OVA protein and sCD70 is sufficient to prime a detectable endogenous naïve $CD8^+$ T-cell response (data not shown), suggesting that CD27 costimulation is sufficient to prime naïve T cells, provided that Ag presentation is optimal.

With respect to T-cell phenotype, high expression of CD27 was more evident in the resting T_{CM} population as defined by both CD62L expression ($CD62L^{hi}$) and anatomical location (predominantly secondary lymphoid tissues), as expected [16]. However, both populations became $CD27^{hi}$ after TCR activation and accumulated to similar extents after peptide and sCD70 rechallenge. This result is reminiscent of previous data showing decreased memory $CD8^+$ T-cell frequency but unchanged T_{EM}/T_{CM} ratios in primed $CD27^{-/-}$, compared with WT, mice [15]. Thus, CD27 signaling appears to have little effect on memory $CD8^+$ T-cell skewing during either primary or secondary expansion. Whether the lack of secondary memory T-cell skewing following sCD70 treatment results from equal division of T_{EM} and T_{CM} cells or is due to unequal proliferation/survival of the two subsets but compensatory differentiation from one subset to another is currently unclear and the subject of future studies.

The increased frequency of memory $CD8^+$ T cells after boosting with sCD70 was due to increased survival of proliferating secondary $CD8^+$ T cells. We and others have previously reported that CD27 signals contribute to both increased cell survival and proliferation during priming, although the dominant effect in a viral setting appears to be on the survival pathway [2, 52]. In common with other members of the TNFRSF, CD27 upregulates apoptosis-inhibiting Bcl-2 family members including Bcl-X_L [53, 54]; CD27 pro-survival effects are also thought to be mediated through upregulation of Pim-1 kinase [54].

In summary, our data show first that the CD27/70 axis can make a nonredundant contribution to memory $CD8^+$ T-cell expansion after secondary Ag encounter, and second that this costimulatory signal at boost is sufficient to drive significant accumulation of cytolytic memory $CD8^+$ T cells. We additionally describe sCD70 as a potent adjuvant for boosting memory T-cell responses and suggest that this reagent may be of clinical benefit in immunotherapeutic prime-boost protocols.

Materials and methods

Antibodies, reagents, and cells

The blocking anti-CD70 mAb (TAN 1.6, rat IgG2a) that does not deplete CD70-expressing cells in vivo was described previously [4, 11]. Purified mAb against A31 (Mc39–16, rat IgG2a) and BCL1 lymphoma (Mc106A5, rat IgG2a) idiotypes (used as controls), CD80 (1610A1, hamster 1gG), CD86 (GL-1, rat IgG2a), CD40 (3/23, rat IgG2a), Fcγ receptors II/III (2.4G2), CD3 (145.2C11), and FITC-conjugated anti-human Fc (SB2H2) were prepared in house and kindly provided by Prof. M. Glennie (University of Southampton). A multimeric recombinant protein comprising the extracellular domain of CD70 fused to the Fc region of human IgG1 (sCD70) was generated in house as previously described, and human IgG used as a control [2]. Allophycocyanin-conjugated anti-CD8α (53–6.7), FITC-conjugated anti-CD44 (IM7), FITC-conjugated anti-CD62L (MEL-14), allophycocyanin-labeled anti-CD27 (LG.3A10), FITC-conjugated anti-CD90.2 (53–2.1), allophycocyanin-labeled annexin V, and isotype-matched control mAb were purchased from BD Pharmingen (Oxford, UK). OVA, LPS, and polyI:C were purchased from Sigma-Aldrich (Dorset, UK). OVA peptide 257–264 ($OVA_{257–264}$) and PE-conjugated H-$2K^b$/$OVA_{257–264}$ tetramer were obtained from Peptide Protein Research Ltd. (Fareham, UK) and Beckman Coulter (Berks, UK), respectively; α-galactosylceramide was synthesized as described [38].

Mice and in vivo experiments

C57BL/6 (B6), C57BL/6 $RAG1^{-/-}$, and C57BL/6 CD90.1 congenic mice were bred and maintained in a pathogen-free environment. OVA-specific T cells were primed by immunization with 5 mg OVA plus 0.5 mg anti-CD40 i.p. For restimulation, mice were injected on days 21–90 after primary immunization with 30 nmoles $OVA_{257–264}$ i.v. alone or with 0.25–0.5 mg sCD70 i.v. on day 0 of the secondary response. Additional injections of

0.25–0.5 mg of sCD70 i.v. were given on days 1 and 2 of the secondary response. Blocking antibodies to CD70, CD80, and CD86 or isotype controls were administered at 0.5 mg i.p. on days 0 and 1 of the primary or secondary response.

Evaluation and phenotyping of Ag-specific T-cell responses

OVA-specific $CD8^+$ T cells were tracked in peripheral blood samples, spleen, LN (combined inguinal, brachial, and mesenteric), or liver. Tissues were homogenized through a 100 μm cell strainer and lymphocytes further enriched from liver by Percoll density gradient (GE Healthcare, Bucks, UK). Cells were stained with H-$2K^b$/$OVA_{257–264}$ tetramer and allophycocyanin-conjugated anti-CD8α mAb in the presence of Fcγ receptor II/III blocking mAb. Some tissue samples were additionally stained for CD44, CD62L, CD90.2, or stained with sCD70 followed by secondary detection with FITC-conjugated anti-human Fc. Flow cytometric analyses were performed using BD Biosciences FACS Calibur or FACS Canto II (Oxford, UK) and CellQuest or FACsDiva software; $CD8^+$tetramer$^+$cells were gated as indicated (Supporting Information Fig. 4).

CFSE dilution and BrdU incorporation

For CFSE dilution, $CD8^+$ T cells were isolated from spleen and LN of primed mice using the $CD8^+$ Cellect Immunocolumn Kit (Cedarlane, Ontario, Canada), labeled with 10 μM CFSE, and transferred into C57BL/6 $RAG1^{-/-}$ mice. Recipients were challenged with $OVA_{257–264}$ alone or with sCD70 i.v. or were left unstimulated; CFSE dilution of splenic OVA-specific $CD8^+$ cells was analyzed 3 and 6 days later. In some experiments, mice were given 1 mg BrdU (Sigma-Aldrich) twice, 8 h apart on day 4 after secondary stimulation. BrdU incorporation by OVA-specific $CD8^+$ cells was determined 12 h later using the BrdU Flow Kit (BD Pharmingen).

CTL assay

Ex vivo evaluation of CTL activity was determined by ^{51}Cr-release assay essentially as described [2] using pooled splenocytes as effectors and 10 μM $OVA_{257–264}$ pulsed EL4 cells as targets. Lytic activity against unpeptide-pulsed EL4 cells never exceeded 3%.

Tumor challenge and cell transfer experiments

E.G7, a derivative of EL4, transfected with chicken OVA cDNA was obtained from the ATCC-LGC (Middlesex, UK). Three days after restimulation, mice were challenged with 2×10^6 E.G7 cells s.c. Tumor growth was monitored and animals culled when tumor exceeded 150 mm^2. For transfer of $CD90.2^+$ $CD8^+$ T cells to $CD90.1^+$ congenic recipients, 1×10^7 $CD8^+$ T cells purified from primed $CD90.2^+$ mice were transferred to $CD90.1^+$ recipients and mice treated as indicated. All animal experiments were conducted in accordance with U.K. Home Office guidelines and approved by the University of Southampton's Ethical Committee.

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References

1 **Croft, M.**, The role of TNF superfamily members in T-cell function and diseases. *Nat. Rev. Immunol.* 2009. **9**: 271–285.

2 **Rowley, T. F. and Al-Shamkhani, A.**, Stimulation by soluble CD70 promotes strong primary and secondary CD8+ cytotoxic T cell responses in vivo. *J. Immunol.* 2004. **172**: 6039–6046.

3 **Tesselaar, K., Xiao, Y., Arens, R., van Schijndel, G. M., Schuurhuis, D. H., Mebius, R. E., Borst, J. et al.**, Expression of the murine CD27 ligand CD70 in vitro and in vivo. *J. Immunol.* 2003. **170**: 33–40.

4 **Taraban, V. Y., Rowley, T. F. and Al-Shamkhani, A.**, Cutting edge: a critical role for CD70 in CD8 T cell priming by CD40-licensed APCs. *J. Immunol.* 2004. **173**: 6542–6546.

5 **Taraban, V. Y., Rowley, T. F., Tough, D. F. and Al-Shamkhani, A.**, Requirement for CD70 in CD4+ Th cell-dependent and innate receptor-mediated CD8+ T cell priming. *J. Immunol.* 2006. **177**: 2969–2975.

6 **Hendriks, J., Xiao, Y., Rossen, J. W., van der Sluijs, K. F., Sugamura, K., Ishii, N. and Borst, J.**, During viral infection of the respiratory tract, CD27, 4–1BB, and OX40 collectively determine formation of CD8+ memory T cells and their capacity for secondary expansion. *J. Immunol.* 2005. **175**: 1665–1676.

7 **Bullock, T. N. and Yagita, H.**, Induction of CD70 on dendritic cells through CD40 or TLR stimulation contributes to the development of CD8+ T cell responses in the absence of CD4+ T cells. *J. Immunol.* 2005. **174**: 710–717.

8 **Schildknecht, A., Miescher, I., Yagita, H. and van den Broek, M.**, Priming of CD8+ T cell responses by pathogens typically depends on CD70-mediated interactions with dendritic cells. *Eur. J. Immunol.* 2007. **37**: 716–728.

9 **Dong, H., Franklin, N. A., Roberts, D. J., Yagita, H., Glennie, M. J. and Bullock, T. N.**, CD27 stimulation promotes the frequency of IL-7 receptor-expressing memory precursors and prevents IL-12-mediated loss of CD8(+) T cell memory in the absence of CD4(+) T cell help. *J. Immunol.* 2012. **188**: 3829–3838.

10 **Lefrancois, L., Altman, J. D., Williams, K. and Olson, S.**, Soluble antigen and CD40 triggering are sufficient to induce primary and memory cytotoxic T cells. *J. Immunol.* 2000. **164**: 725–732.

11 **French, R. R., Taraban, V. Y., Crowther, G. R., Rowley, T. F., Gray, J. C., Johnson, P. W., Tutt, A. L. et al.**, Eradication of lymphoma by CD8 T cells

following anti-CD40 monoclonal antibody therapy is critically dependent on CD27 costimulation. *Blood* 2007. **109**: 4810–4815.

12 **Feau, S., Garcia, Z., Arens, R., Yagita, H., Borst, J. and Schoenberger, S. P.**, The CD4(+) T-cell help signal is transmitted from APC to CD8(+) T-cells via CD27-CD70 interactions. *Nat. Commun.* 2012. **3**: 948.

13 **Sallusto, F., Lenig, D., Forster, R., Lipp, M. and Lanzavecchia, A.**, Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999. **401**: 708–712.

14 **Wherry, E. J., Teichgraber, V., Becker, T. C., Masopust, D., Kaech, S. M., Antia, R., von Andrian, U. H. et al.**, Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol.* 2003. **4**: 225–234.

15 **Yamaura, K., Boenisch, O., Watanabe, T., Ueno, T., Vanguri, V., Yang, J., Tanaka, K. et al.**, Differential requirement of CD27 costimulatory signaling for naive versus alloantigen-primed effector/memory CD8+ T cells. *Am. J. Transplant* 2010. **10**: 1210–1220.

16 **Hikono, H., Kohlmeier, J. E., Takamura, S., Wittmer, S. T., Roberts, A. D. and Woodland, D. L.**, Activation phenotype, rather than central- or effector-memory phenotype, predicts the recall efficacy of memory CD8+ T cells. *J. Exp. Med.* 2007. **204**: 1625–1636.

17 **Fritsch, R. D., Shen, X., Sims, G. P., Hathcock, K. S., Hodes, R. J. and Lipsky, P. E.**, Stepwise differentiation of CD4 memory T cells defined by expression of CCR7 and CD27. *J. Immunol.* 2005. **175**: 6489–6497.

18 **Wirth, T. C., Xue, H. H., Rai, D., Sabel, J. T., Bair, T., Harty, J. T. and Badovinac, V. P.**, Repetitive antigen stimulation induces stepwise transcriptome diversification but preserves a core signature of memory CD8(+) T cell differentiation. *Immunity* 2010. **33**: 128–140.

19 **Sprent, J. and Surh, C. D.**, T cell memory. *Annu. Rev. Immunol.* 2002. **20**: 551–579.

20 **Sung, J. H., Zhang, H., Moseman, E. A., Alvarez, D., Iannacone, M., Henrickson, S. E., de la Torre, J. C. et al.**, Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell* 2012. **150**: 1249–1263.

21 **Pihlgren, M., Dubois, P. M., Tomkowiak, M., Sjogren, T. and Marvel, J.**, Resting memory CD8+ T cells are hyperreactive to antigenic challenge in vitro. *J. Exp. Med.* 1996. **184**: 2141–2151.

22 **Zammit, D. J., Cauley, L. S., Pham, Q. M. and Lefrancois, L.**, Dendritic cells maximize the memory CD8 T cell response to infection. *Immunity* 2005. **22**: 561–570.

23 **Belz, G. T., Wilson, N. S., Smith, C. M., Mount, A. M., Carbone, F. R. and Heath, W. R.**, Bone marrow-derived cells expand memory CD8+ T cells in response to viral infections of the lung and skin. *Eur. J. Immunol.* 2006. **36**: 327–335.

24 **Ndejembi, M. P., Teijaro, J. R., Patke, D. S., Bingaman, A. W., Chandok, M. R., Azimzadeh, A., Nadler, S. G. et al.**, Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. *J. Immunol.* 2006. **177**: 7698–7706.

25 **Borowski, A. B., Boesteanu, A. C., Mueller, Y. M., Carafides, C., Topham, D. J., Altman, J. D., Jennings, S. R. et al.**, Memory CD8+ T cells require CD28 costimulation. *J. Immunol.* 2007. **179**: 6494–6503.

26 **Fuse, S., Zhang, W. and Usherwood, E. J.**, Control of memory CD8+ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J. Immunol.* 2008. **180**: 1148–1157.

27 **Garidou, L., Heydari, S., Truong, P., Brooks, D. G. and McGavern, D. B.**, Therapeutic memory T cells require costimulation for effective clearance of a persistent viral infection. *J. Virol.* 2009. **83**: 8905–8915.

28 **MacLeod, M., Kwakkenbos, M. J., Crawford, A., Brown, S., Stockinger, B., Schepers, K., Schumacher, T. et al.**, CD4 memory T cells survive and proliferate but fail to differentiate in the absence of CD40. *J. Exp. Med.* 2006. **203**: 897–906.

29 **Hendriks, J., Gravestein, L. A., Tesselaar, K., van Lier, R. A., Schumacher, T. N. and Borst, J.**, CD27 is required for generation and long-term maintenance of T cell immunity. *Nat. Immunol.* 2000. **1**: 433–440.

30 **Yamada, A., Salama, A. D., Sho, M., Najafian, N., Ito, T., Forman, J. P., Kewalramani, R. et al.**, CD70 signaling is critical for CD28-independent CD8+ T cell-mediated alloimmune responses in vivo. *J. Immunol.* 2005. **174**: 1357–1364.

31 **Matter, M. S., Claus, C. and Ochsenbein, A. F.**, CD4+ T cell help improves CD8+ T cell memory by retained CD27 expression. *Eur. J. Immunol.* 2008. **38**: 1847–1856.

32 **Dolfi, D. V., Boesteanu, A. C., Petrovas, C., Xia, D., Butz, E. A. and Katsikis, P. D.**, Late signals from CD27 prevent Fas-dependent apoptosis of primary CD8+ T cells. *J. Immunol.* 2008. **180**: 2912–2921.

33 **Munitic, I., Kuka, M., Allam, A., Scoville, J. P. and Ashwell, J. D.**, CD70 deficiency impairs effector CD8 T cell generation and viral clearance but is dispensable for the recall response to lymphocytic choriomeningitis virus. *J. Immunol.* 2012. **190**: 1169–1179.

34 **van Kooten, C. and Banchereau, J.**, Functions of CD40 on B cells, dendritic cells and other cells. *Curr. Opin. Immunol.* 1997. **9**: 330–337.

35 **Maxwell, J. R., Campbell, J. D., Kim, C. H. and Vella, A. T.**, CD40 activation boosts T cell immunity in vivo by enhancing T cell clonal expansion and delaying peripheral T cell deletion. *J. Immunol.* 1999. **162**: 2024–2034.

36 **Martin, M. D., Wirth, T. C., Lauer, P., Harty, J. T. and Badovinac, V. P.**, The impact of pre-existing memory on differentiation of newly recruited naive CD8 T cells. *J. Immunol.* 2011. **187**: 2923–2931.

37 **Alpan, O., Bachelder, E., Isil, E., Arnheiter, H. and Matzinger, P.**, 'Educated' dendritic cells act as messengers from memory to naive T helper cells. *Nat. Immunol.* 2004. **5**: 615–622.

38 **Taraban, V. Y., Martin, S., Attfield, K. E., Glennie, M. J., Elliott, T., Elewaut, D., Van Calenbergh, S. et al.**, Invariant NKT cells promote CD8+ cytotoxic T cell responses by inducing CD70 expression on dendritic cells. *J. Immunol.* 2008. **180**: 4615–4620.

39 **Stoll, S., Delon, J., Brotz, T. M. and Germain, R. N.**, Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science* 2002. **296**: 1873–1876.

40 **Selin, L. K. and Welsh, R. M.**, Cytolytically active memory CTL present in lymphocytic choriomeningitis virus-immune mice after clearance of virus infection. *J. Immunol.* 1997. **158**: 5366–5373.

41 **van Gisbergen, K. P., Klarenbeek, P. L., Kragten, N. A., Unger, P. P., Nieuwenhuis, M. B., Wensveen, F. M., ten Brinke, A. et al.**, The costimulatory molecule CD27 maintains clonally diverse CD8(+) T cell responses of low antigen affinity to protect against viral variants. *Immunity* 2011. **35**: 97–108.

42 **van Gisbergen, K. P., van Olffen, R. W., van Beek, J., van der Sluijs, K. F., Arens, R., Nolte, M. A. and van Lier, R. A.**, Protective CD8 T cell memory is impaired during chronic CD70-driven costimulation. *J. Immunol.* 2009. **182**: 5352–5362.

43 **Arens, R., Nolte, M. A., Tesselaar, K., Heemskerk, B., Reedquist, K. A., van Lier, R. A. and van Oers, M. H.**, Signaling through CD70 regulates B cell activation and IgG production. *J. Immunol.* 2004. **173**: 3901–3908.

44 **Keller, A. M., Groothuis, T. A., Veraar, E. A., Marsman, M., Maillette de Buy Wenniger, L., Janssen, H., Neefjes, J. et al.**, Costimulatory ligand CD70 is delivered to the immunological synapse by shared intracellular

trafficking with MHC class II molecules. *Proc. Natl. Acad. Sci. USA* 2007. **104**: 5989–5994.

45 **Salek-Ardakani, S., Flynn, R., Arens, R., Yagita, H., Smith, G. L., Borst, J., Schoenberger, S. P. et al.**, The TNFR family members OX40 and CD27 link viral virulence to protective T cell vaccines in mice. *J. Clin. Invest.* 2011. **121**: 296–307.

46 **Kolumam, G. A., Thomas, S., Thompson, L. J., Sprent, J. and Murali-Krishna, K.**, Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J. Exp. Med.* 2005. **202**: 637–650.

47 **Ochsenbein, A. F., Riddell, S. R., Brown, M., Corey, L., Baerlocher, G. M., Lansdorp, P. M. and Greenberg, P. D.**, CD27 expression promotes long-term survival of functional effector-memory CD8+ cytotoxic T lymphocytes in HIV-infected patients. *J. Exp. Med.* 2004. **200**: 1407–1417.

48 **Huang, J., Kerstann, K. W., Ahmadzadeh, M., Li, Y. F., El-Gamil, M., Rosenberg, S. A. and Robbins, P. F.**, Modulation by IL-2 of CD70 and CD27 expression on CD8+ T cells: importance for the therapeutic effectiveness of cell transfer immunotherapy. *J. Immunol.* 2006. **176**: 7726–7735.

49 **Zhou, G. and Levitsky, H.**, Towards curative cancer immunotherapy: overcoming posttherapy tumor escape. *Clin. Dev. Immunol.* 2012. **2012**: Article ID 124187, 12 pages.

50 **Matter, M., Odermatt, B., Yagita, H., Nuoffer, J. M. and Ochsenbein, A. F.**, Elimination of chronic viral infection by blocking CD27 signaling. *J. Exp. Med.* 2006. **203**: 2145–2155.

51 **Rees, W., Bender, J., Teague, T. K., Kedl, R. M., Crawford, F., Marrack, P. and Kappler, J.**, An inverse relationship between T cell receptor affinity and antigen dose during CD4(+) T cell responses in vivo and in vitro. *Proc. Natl. Acad. Sci. USA* 1999. **96**: 9781–9786.

52 **Hendriks, J., Xiao, Y. and Borst, J.**, CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *J. Exp. Med.* 2003. **198**: 1369–1380.

53 **van Oosterwijk, M. F., Juwana, H., Arens, R., Tesselaar, K., van Oers, M. H., Eldering, E. and van Lier, R. A.**, CD27-CD70 interactions sensitise naive CD4+ T cells for IL-12-induced Th1 cell development. *Int. Immunol.* 2007. **19**: 713–718.

54 **Peperzak, V., Veraar, E. A., Keller, A. M., Xiao, Y. and Borst, J.**, The Pim kinase pathway contributes to survival signaling in primed CD8+ T cells upon CD27 costimulation. *J. Immunol.* 2010. **185**: 6670–6678.

Abbreviations: **LCMV**: lymphocytic choriomeningitis virus · **sCD70**: soluble recombinant CD70 · **T_{CM}**: central-memory $CD8^+$ T cell · **T_{EM}**: effector-memory $CD8^+$ T cell · **TNFRSF**: TNF receptor superfamily

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