

# CD43 Expression Regulated by IL-12 Signaling Is Associated with Survival of CD8 T Cells

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**Background:** In addition to TCR and costimulatory signals, cytokine signals are required for the differentiation of activated CD8 T cells into memory T cells and their survival. Previously, we have shown that IL-12 priming during initial antigenic stimulation significantly enhanced the survival of activated CD8 T cells and increased the memory cell population. In the present study, we analyzed the mechanisms by which IL-12 priming contributes to activation and survival of CD8 T cells. **Methods:** We observed dramatically decreased expression of CD43 in activated CD8 T cells by IL-12 priming. We purified CD43<sup>lo</sup> and CD43<sup>hi</sup> cells after IL-12 priming and analyzed the function and survival of each population both *in vivo* and *in vitro*. **Results:** Compared to CD43<sup>hi</sup> effector cells, CD43<sup>lo</sup> effector CD8 T cells exhibited reduced cytolytic activity and lower granzyme B expression but showed increased survival. CD43<sup>lo</sup> effector CD8 T cells also showed increased *in vivo* expansion after adoptive transfer and antigen challenge. The enhanced survival of CD43<sup>lo</sup> CD8 T cells was also partly associated with CD62L expression. **Conclusion:** We suggest that CD43 expression regulated by IL-12 priming plays an important role in differentiation and survival of CD8 T cells.

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## INTRODUCTION

T cells can be classified into three separate classes: naïve, effector and memory. Antigen-specific naïve CD8 T cells exist at very low frequencies. But during an immune response, these cells undergo massive clonal expansion and finally form

memory T cells. Memory T cells are well suited to combat pathogens because they are present at higher numbers than naïve CD8 T cells, persist for extended periods due to antigen-independent homeostatic turnover, and can mediate a substantially accelerated recall response to secondary infection (1,2). Thus, many efforts have been made to elucidate how to efficiently elicit greater numbers and longer-lived memory T cells.

It has been proposed that a brief period of antigen encounter can commit naïve cells to a program of effector and memory differentiation (2-6). However, it has been shown that the duration and strength of antigenic and costimulatory signals can also regulate the fate of developing effector and memory T cells (7,8). In this case, memory cells are proposed to differentiate from naïve cells by virtue of qualitative and quantitative differences in the perceived signal. In particular, early inflammatory cytokine signals known as “third signal” have been shown to be involved in the induction of effector response and the generation and maintenance of effector and memory cells (9-14). However, the exact mechanisms regulating the fate of activated T cells still remain undefined. Previously, we showed that IL-12 priming during antigenic stimulation dramatically increased the population of effector and memory CD8 T cells mainly by preventing activation-induced cell death (9), suggesting that development of memory cells could be regulated by early IL-12 signaling.

CD43 is a cell-surface sialoglycoprotein expressed by a variety of hematopoietically derived cells, including T lymphocytes (15-17). Posttranslational modifications result in two gly-

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coforms of CD43: 115 kDa and 130 kDa. The mAb 1B11 recognizes the activation-associated glycoform (130 kDa) of CD43 (18,19). The 130 kDa glycoform of CD43 bears two core O-glycans, an oligosaccharide structure that can be created by the action of the core  $\beta$ -1-6-N-acetylglucosaminyltransferase. 1B11 binding is low on naïve CD8<sup>+</sup> T cells and high on Ag-specific effector CD8<sup>+</sup> T cells, and becomes reduced again on Ag-specific memory CD8<sup>+</sup> T cells (20). Although CD43 is one of the most abundant T cell surface glycoproteins, its function is still debated. It has been suggested to have anti-adhesive functions in T cell trafficking and homing (21-23). Some evidence suggests that CD43 provides a highly charged barrier to cell-cell interaction (24,25). A topological view of T cell-APC interactions has been proposed (26), suggesting that CD43 may be a cell surface regulatory protein that damps T cell responses by its physical presence (27). This hypothesis could be supported by the fact that CD43 is excluded from the immunological synapse during T cell activation (27-29). Additionally, CD43 has been also shown to play a role in T cell activation. Some studies showed that CD43 transduces multiple activating signals that ultimately induce expression of certain genes during T cell activation (30-32) and plays a costimulatory role *in vitro* and *in vivo* (33-35). In contrast, other groups have shown that CD43<sup>-/-</sup> T cells are hyperproliferative *in vitro* (21,36).

In the present study, we analyzed the mechanisms by which IL-12 priming contributes to the activation and the enhanced survival of CD8<sup>+</sup> T cells and observed dramatically decreased expression of CD43 in activated CD8<sup>+</sup> T cells primed by IL-12. To determine the role of CD43 expression in the survival of activated CD8 T cells, we purified CD43<sup>lo</sup> and CD43<sup>hi</sup> cells after IL-12 priming and analyzed the function and survival of each population. CD43<sup>lo</sup> effector CD8<sup>+</sup> T cells exhibited reduced cytolytic activity, lower granzyme B expression and reduced IFN- $\gamma$  production but showed significantly increased survival both *in vivo* and *in vitro* compared to CD43<sup>hi</sup> cells. These CD43<sup>lo</sup> effector CD8 T cells are associated with higher expression of CD62L than CD43<sup>hi</sup> effector CD8 T cells. Together, these results suggest that the expression of the activated form of CD43 is significantly down-regulated by IL-12 priming, which gives rise to a preferential, long-lived CD8<sup>+</sup> T cell memory population that is partly associated with the levels of CD62L expression.

## MATERIALS AND METHODS

### Mice

Female C57BL/6 mice were purchased from The Charles River Japan (Shizuoka, Japan). OT-I TCR transgenic mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed under specific pathogen-free conditions and were used between 6 and 12 weeks of age following institutional animal care and use committee protocols.

### Antibodies and reagents

All antibodies were purchased from BD Bioscience-Pharmingen (San Diego, CA), unless specified otherwise. Recombinant human IL-2 and murine IL-12 were purchased from R&D Systems (Minneapolis, MN). CD8 $\alpha$ <sup>+</sup> T cell isolation kits and anti-PE microbeads were obtained from Miltenyi Biotec (Auburn, CA).

### *In vitro* T cell activation, isolation, adoptive transfer and infection

Spleen cells from OT-I TCR transgenic mice were stimulated with OVA<sub>257-264</sub> peptide (SIINFEKL; referred to as OVAp) in complete IMDM supplemented with 2 mM L-glutamine, 50  $\mu$ M 2-ME, and 10 U/ml human rIL-2 in the presence of rIL-12 (5 ng/ml). After *in vitro* stimulation, the CD8<sup>+</sup> T cells were purified by negative selection using magnetic bead separation (MACS) according to the manufacturer's instructions (Miltenyi Biotec). CD43<sup>lo</sup> and CD43<sup>hi</sup> cells were then purified by negative selection and positive selection using anti-CD43-PE and anti-PE microbeads. Purified CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8<sup>+</sup> T cells ( $2 \times 10^6$  in 200  $\mu$ l of PBS) were transferred into naïve C57BL/6 mice via tail vein injection. For recall response,  $1 \times 10^5$  purified CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8<sup>+</sup> T cells in 200  $\mu$ l of PBS were transferred into naïve C57BL/6 mice via tail vein injection, and at day 1 after transfer, the mice were intranasally challenged with  $2 \times 10^7$  pfu of recombinant adenovirus expressing OVA (rAd/OVA).

### Surface staining, intracellular staining, and flow cytometric analysis

In order to count the total number of donor T cells, recipient mice were sacrificed, and cells from spleens were re-suspended in FACS buffer (1% FBS, 0.03% sodium azide in PBS) at a concentration of  $1 \times 10^7$  cells/ml. A total of 100  $\mu$ l of these cells ( $1 \times 10^6$  cells) was stained for CD8 (clone 53-6.7), CD43 (1B11), CD62L (MEL-14) or CD127 (A7R34),

and samples were acquired on FACS Calibur™ (BD Biosciences, San Jose, CA). PE or APC-conjugated OVA-specific MHC I tetramer, Kb/OVA-Tet, was produced as described elsewhere (9), and the optimal concentration was determined by titration. Cells were stained for 40 min at 4°C using fluorochrome-conjugated Abs and Kb/OVA-Tet, washed, and fixed in PBS containing 2% formaldehyde before analysis by flow cytometry. For intracellular staining, purified CD43<sup>hi</sup> and CD43<sup>lo</sup> cells were co-cultured at 37°C for 5 h with OVA peptide and BFA. After culture, the cells were first stained for surface markers, then washed, fixed and permeabilized with FACS buffer containing 0.5% saponin (Sigma-Aldrich, Seoul, Korea). The cells were then stained with anti-IFN- $\gamma$  (XMG1.2). For granzyme B staining, cells were fixed and permeabilized with FACS buffer containing 0.5% saponin after surface marker staining. Cells were stained with anti-granzyme B (GB12) or isotype control antibody conjugated to PE (Caltag Laboratories). Gates were set on lymphocytes by forward and side scatter profiles, and the data were analyzed using CellQuest™ Pro (BD Biosciences), FlowJo™ software (Windows version 5.7.2, TreeStar, San Carlos, CA), and WinMDI version 2.8 software (The Scripps Research Institute, La Jolla, CA).

#### Detection of apoptotic death

Apoptosis of *in vitro* activated T cells was determined by Annexin V and 7-amino-actinomycin D (7-AAD) staining, as recommended by the manufacturer (BD Bioscience, San Diego, CA). In brief, purified CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8 T cells were seeded in 24-well flat-bottom plates ( $1 \times 10^6$  cells/well) with IL-2 in 2 ml of complete IMDM. At the indicated time points, these cells were washed, resuspended in Annexin V binding buffer at a concentration of  $1 \times 10^6$  cells/ml, incubated with Annexin V-FITC and 7-AAD, and analyzed by flow cytometry.

#### Cell-mediated cytotoxicity assay

MACS-separated CD43<sup>lo</sup> and CD43<sup>hi</sup> effector cells were used in the CTL assay *in vitro*. Peptide-pulsed EL4 target cells ( $1 \times 10^4$ /well) were added to serial dilutions of effector cells (prepared as described above) in 96-well round-bottom plates at E : T cell ratio of 1 : 1 to 20 : 1. After 4 h at 37°C, cytotoxicity was quantified by measurement of cytosolic lactate dehydrogenase (LDH) in the culture supernatant ( $n=3$ ) using cytotoxicity detection kit (Roche Diagnostics). Specific lysis for each E : T cell ratio was expressed as: specific lysis=[(ex-

perimental release) – (spontaneous release)/(target maximum – target spontaneous release)]. Spontaneous LDH release in the absence of CTL was <10% of the maximal cellular release by detergent lysis. All experimental procedures and assays were performed two or more times with similar results.

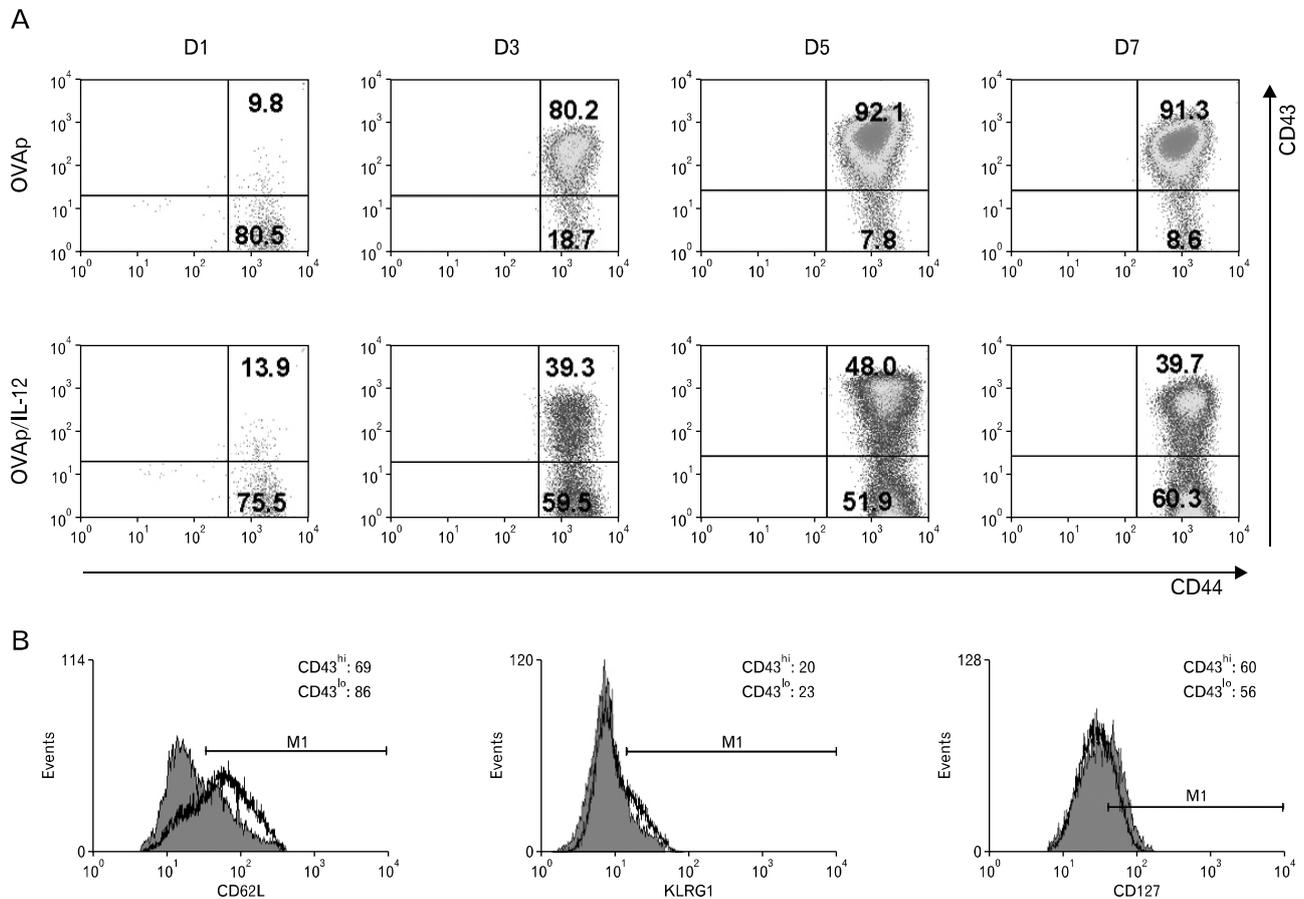
#### Statistical analysis

Comparisons between groups were analyzed using a Student's *t*-test (SAS8.2 software). Significance was accepted as a value of  $p < 0.05$ .

## RESULTS

### CD43 expression regulated by initial IL-12 priming during primary CD8<sup>+</sup> T cell stimulation

Previously we showed that IL-12 priming during initial antigenic stimulation significantly enhanced the survival of activated CD8 T cells and increased the memory cell population after adoptive transfer (9). Recent studies have suggested that activation markers, such as CD43 and CD27, define distinct subpopulations of memory CD8<sup>+</sup> T cells that differ in their capacities to mount recall responses (20,37). To determine whether IL-12 priming induces any changes in CD43 expression during *in vitro* stimulation, we analyzed CD43 expression on OT-I cells stimulated with cognate OVA (SIINFEKL) in the presence or absence of IL-12. The level of CD43 and CD44 on naïve cells was low, but after antigenic stimulation, most OT-I cells expressed CD44<sup>hi</sup> activated phenotype and some of them started to express CD43 on day 1 (Fig. 1). There was no significant difference in the CD43 expression between non-primed and IL-12-primed cells during this period. However, beginning on day 3, IL-12-primed cells showed a relatively lower proportion of CD43-positive cells than non-primed cells, and this selective down-regulation of CD43 by a subpopulation of effector cells was observed clearly on days 3, 5 and 7 (Fig. 1A). To determine whether CD43 is associated with the typical characteristics of memory precursors, we assessed CD43 expression together with other activation markers such as CD127 (38), KLRG-1 (39) and CD62L (40,41) that identify memory precursors. There was no significant difference in the CD127 expression between CD43<sup>hi</sup> cells and CD43<sup>lo</sup> cells (Fig. 1B). However, CD43<sup>lo</sup> cells showed a relatively higher expression of CD62L than CD43<sup>hi</sup> cells (Fig. 1B). These data suggest that IL-12 priming during antigenic stimulation induced lower CD43 expression than in non-primed cells and that this CD43<sup>lo</sup> pheno-



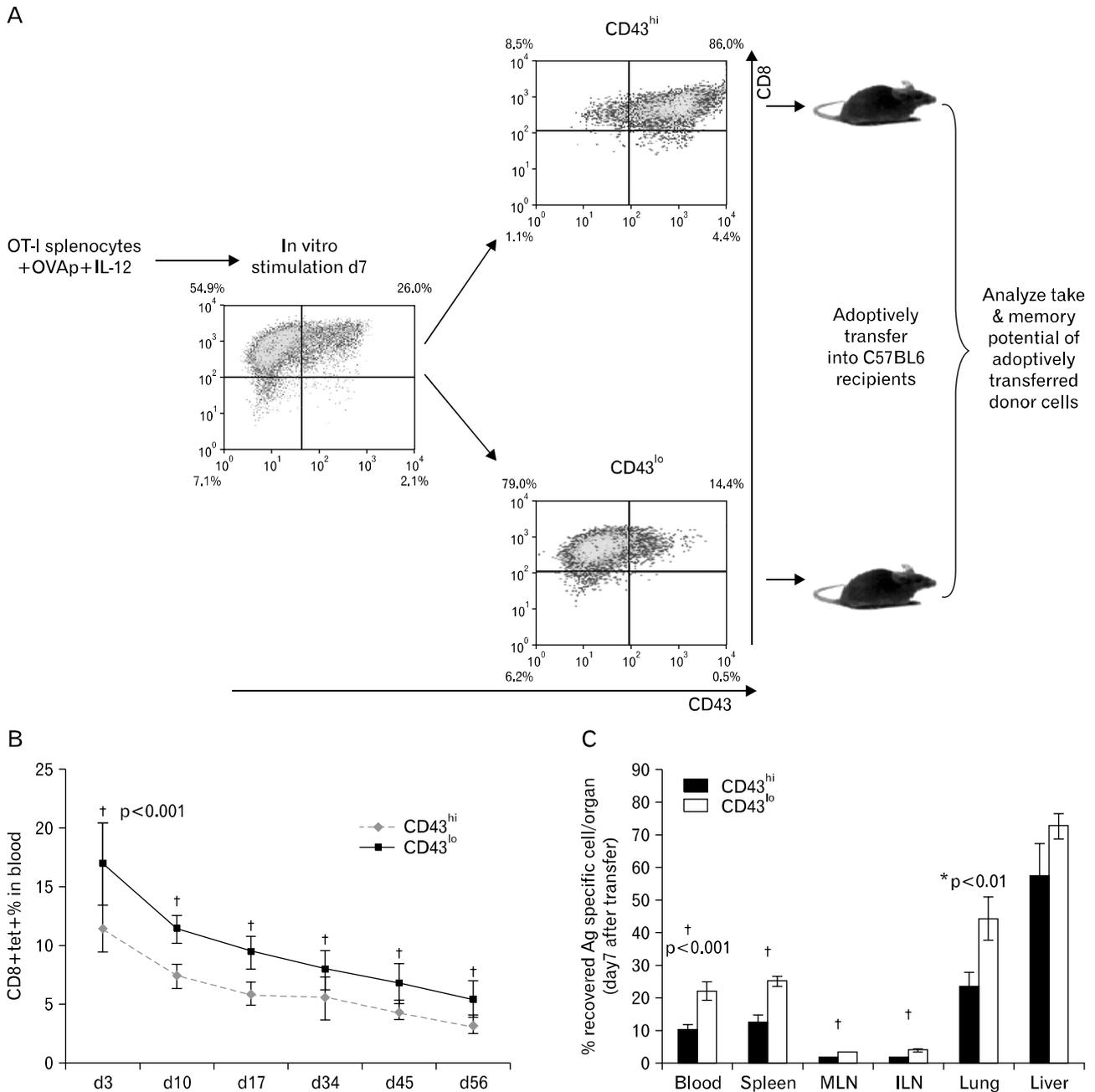
**Figure 1.** CD43 expression is regulated by initial IL-12 priming during primary CD8 T-cell stimulation. (A) OT-I TCR transgenic cells were stimulated with the OVA peptide (OVAp) in the presence or absence of IL-12. On days 1, 3 and 7, cells were harvested, stained with anti-CD8  $\alpha$ , CD44, and CD43, and analyzed by flow cytometry. (B) OT-I cells were stimulated with OVA peptide in the presence of IL-12 (5 ng/ml), and on day 7, cells were harvested, stained with anti-CD8  $\alpha$ , CD43 and CD127, CD62L or KLRG1, and analyzed by flow cytometry. Histograms show the expression levels of CD127, CD62L or KLRG1 in the gated CD43<sup>hi</sup> and CD43<sup>lo</sup> populations and are shown as the mean  $\pm$  SD. The results shown are representative of three independent experiments with similar results. Filled histogram, CD43<sup>hi</sup>; open histogram, CD43<sup>lo</sup>.

type among activated T cells may be associated with enhanced survival of activated CD8 T cells.

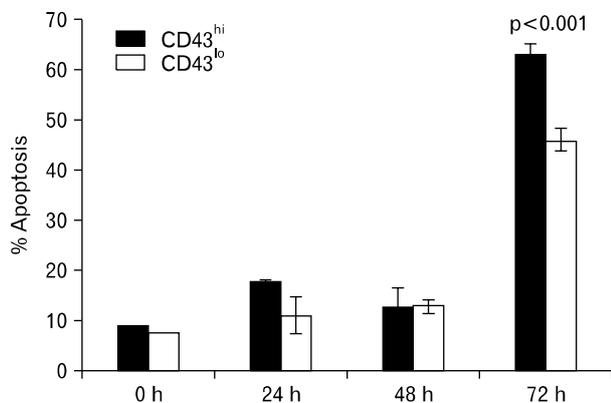
### Enhanced survival of CD43<sup>lo</sup> cells induced by IL-12

CD43 is one of the most abundant T cell surface glycoproteins. But the function of CD43 as a regulatory protein in T-cell activation remains unclear. To define the role of CD43 down-regulation by IL-12 priming, we analyzed the survival potential of IL-12-primed CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8 T cells by sorting the two populations by MACS and then adoptively transferring equal numbers into B6 recipient mice (Fig. 2A). We analyzed the “take” of donor cells in recipient mice at various time points after adoptive transfer. At all time points,  $\sim$ 1.5-2-fold higher levels of CD43<sup>lo</sup> donor cells were detect-

able in the peripheral blood (Fig. 2B). Some studies suggest that CD43 plays a role in the trafficking of T cells to sites of inflammation (23,42). In addition, a study using CD43-deficient mouse showed that CD43 negatively regulates T lymphocyte homing to secondary lymphoid organ (22). Thus, it was possible that the higher level of CD43<sup>lo</sup> donor cells in the blood was caused by differential homing to lymphoid organs. We therefore examined the trafficking of CD43<sup>hi</sup> and CD43<sup>lo</sup> donor CD8 T cells to the lymphoid organs and non-lymphoid organs in the recipient mice. At day 7 after adoptive transfer, CD43<sup>lo</sup> donor cells gave rise to 2-fold higher percentages of engraftment in all lymphoid organs examined such as spleen and mesenteric and inguinal lymph nodes and 1.5-fold higher percentages in non-lymphoid organs



**Figure 2.** CD43<sup>lo</sup> cells induced by initial IL-12 priming increase their survival rate *in vivo*, and enhanced survival of CD43<sup>lo</sup> cells is not due to differential trafficking of transferred cells to various organs. (A) For adoptive transfer experiments, the splenocytes from OT-I TCR transgenic mice were stimulated with the peptide in the presence of IL-12 for 7 days. CD8<sup>+</sup>CD43<sup>hi</sup> and CD8<sup>+</sup>CD43<sup>lo</sup> cells were MACS-purified and  $2 \times 10^6$  cells were i.v. injected into normal C57BL/6 mice. (B) After 3, 10, 17, 34, 45 and 56 days, donor OT-I cells in the blood of recipient mice were identified by CD8  $\alpha$ , K<sup>b</sup>/OVA-Tet and CD43 staining. (C) On day 7, the frequency of donor OT-I cells in lymphoid and non-lymphoid organs of the recipient mice were measured. The results shown are derived from spleens of 5 mice for each group and are representative of three independent experiments with similar results.



**Figure 3.** Attenuation of apoptosis in CD43<sup>lo</sup> population. Spleen cell suspensions from naïve OT-I mice were cultured for 7 days with 100 nM OVA<sub>p</sub> in the presence of IL-12. Then, activated OT-I cells were MACS-purified and rested for 3 additional days without any stimulant and with IL-2. At the indicated time points, the cells were harvested and apoptotic cell death was determined by Annexin V and 7-AAD double-staining. The samples were assayed in triplicate and the error bars represent the SD values of the mean.

such as lung and liver (Fig. 2C). These data exclude the possibility that increased frequency of CD43<sup>lo</sup> donor cells in the peripheral blood was due to differential trafficking to various organs.

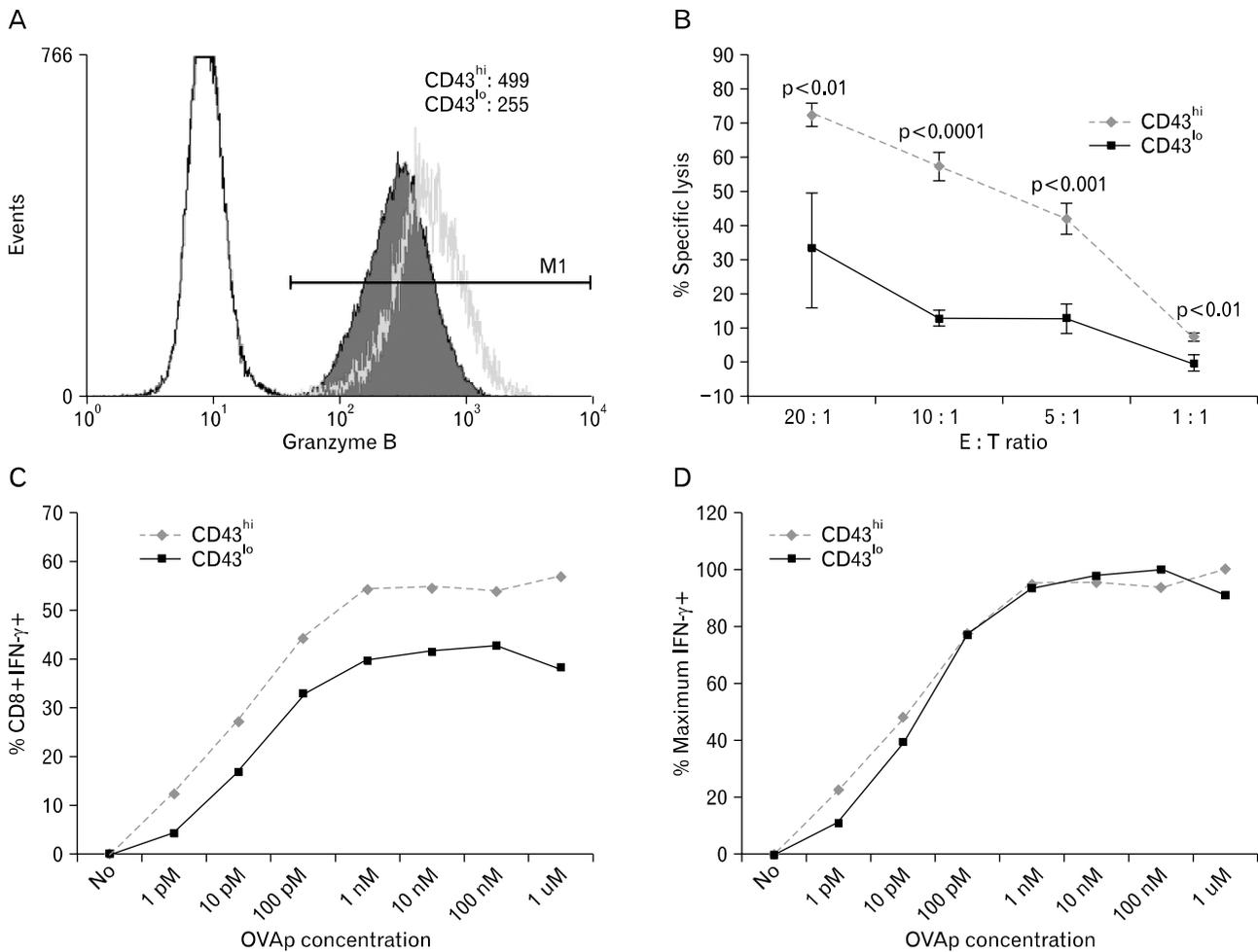
Next, we investigated whether the phenotypic distinction of activated CD8 T cells by CD43 affected the susceptibility to apoptosis. To this end, activated OT-I cells were sorted to CD43<sup>hi</sup> and CD43<sup>lo</sup> cells and each subset was rested with IL-2 and monitored for apoptotic cell death at various time points (Fig. 3). CD43<sup>lo</sup> T cells showed a similar death rate to CD43<sup>hi</sup> T cells by 48 h into the resting period, but exhibited a significant reduction in apoptotic death by 72 h ( $p < 0.001$ ). The proliferation of CD43<sup>lo</sup> effector cells was similar to that of CD43<sup>hi</sup> effector cells (data not shown). These results were consistent with the report that CD43<sup>-/-</sup> mice showed decreased death of Ag-specific CD8 T cells (35). Taken together, our results indicate that the phenotypic change in CD43 expression induced by IL-12 was associated with reduced cell death and the subsequent enhanced survival of effector and memory CD8 T cells.

### Functional difference between CD43<sup>lo</sup> and CD43<sup>hi</sup> cells induced by initial IL-12 priming

As previously stated, the reported positive and negative roles of CD43 in T cells are inevitably opposed to each other. Thus, we investigated whether the CD43<sup>lo</sup> and CD43<sup>hi</sup> T cell subsets exhibited any difference in effector functions. To this

end, the subsets of CD43<sup>hi</sup> and CD43<sup>lo</sup> cells were sorted after antigenic stimulation in the presence of IL-12, and granzyme B expression and killing activity were measured (Fig. 4). A comparison of these effector properties between CD43<sup>lo</sup> and CD43<sup>hi</sup> T cells demonstrated that CD43<sup>lo</sup> CD8 T cells contained less granzyme B than CD43<sup>hi</sup> CD8 T cells (Fig. 4A), and exhibited significantly less killing of OVA peptide-pulsed targets (Fig. 4B). These results were consistent with the report that CD43 up-regulation shows a strong correlation with the acquisition of effector function (20). It is possible that CD43 expression affects functional avidity of CD8 T cells since CD43 can provide a charge barrier based on the topological view of cell-cell interactions (24). It is reported that TCR avidity of CD8 T cells to peptide/MHC complex correlates with ex vivo cytotoxicity and IFN- $\gamma$  secretion (43). So, we investigated whether CD43<sup>lo</sup> and CD43<sup>hi</sup> phenotype affected the functional avidity of OVA-specific effector CD8 T cells by measuring dose-responsive IFN- $\gamma$  production. CD43<sup>hi</sup> effector cells exhibited higher IFN- $\gamma$  expression than CD43<sup>lo</sup> effector cells in all peptide concentrations used (Fig. 4C). But when the data are normalized to % maximum response, dose-responsive IFN- $\gamma$  production of CD43<sup>hi</sup> and CD43<sup>lo</sup> effector cells was comparable (Fig. 4D). This indicates that functional avidity of CD43<sup>hi</sup> effector cells and CD43<sup>lo</sup> effector cells was similar. Together, these results suggest that the CD43<sup>lo</sup> phenotype induced by IL-12 priming was associated with lower granzyme B expression, killing activity and IFN- $\gamma$  secretion but not with TCR avidity.

One of the functional memory properties is recall response to the same antigen. So, we next examined recall responses of adoptively transferred CD43<sup>lo</sup> and CD43<sup>hi</sup> populations by challenging Ag. To test recall proliferation of memory cells generated from CD43<sup>hi</sup> and CD43<sup>lo</sup> donor cells, we adoptively transferred equal numbers of sorted CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8 T cells into naïve mice and challenged them with recombinant adenovirus expressing OVA (rAd/OVA) 1 day after adoptive transfer. We picked this early time point of challenge since CD43<sup>hi</sup> and CD43<sup>lo</sup> subsets displayed differential survival rates even a few days after transfer. We challenged mice with the rAd/OVA virus intranasally because the distribution of CD43<sup>lo</sup> and CD43<sup>hi</sup> donor T cells in the lung tissues was similar. Most of the CD8 T cells detected in the lungs at the peak (day 5 after challenge) were donor OT-I cells, and the number of OVA/Tet<sup>+</sup> cells among lymphocyte-gated mononuclear cells were 3~5 fold higher in mice that received CD43<sup>lo</sup> cells than in mice that received the equal number of



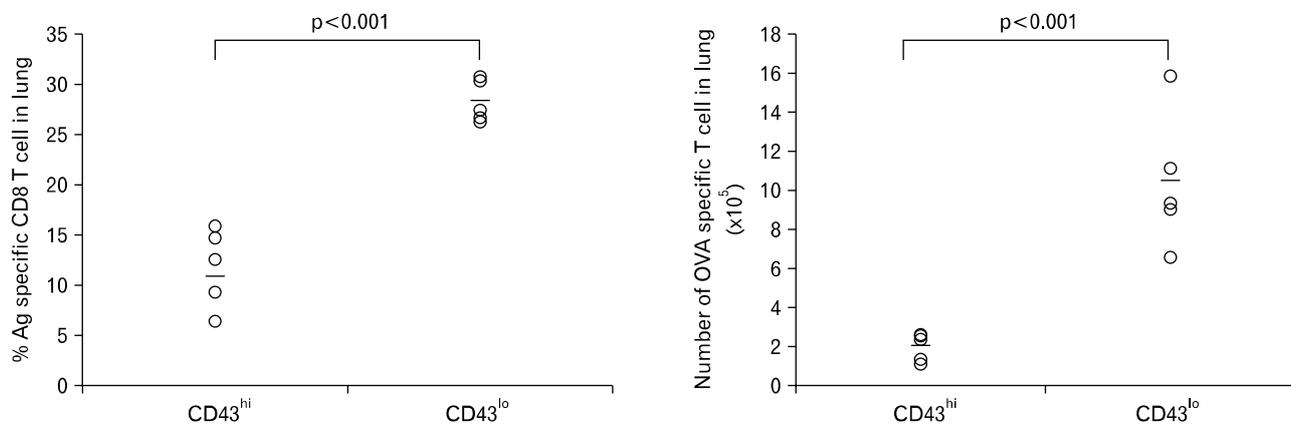
**Figure 4.** Decreased cytolytic functions in CD43<sup>lo</sup> population. Spleen cell suspensions from naïve OT-I mice were cultured for 7 days with 100 nM OVAp in the presence of IL-12. Then, activated OT-I cells were MACS-sorted into CD8<sup>+</sup>CD43<sup>hi</sup> and CD8<sup>+</sup>CD43<sup>lo</sup> populations. (A) The levels of granzyme B in each cell population were determined by intracellular staining. Histograms show the expression of granzyme B in the gated CD43<sup>hi</sup> and CD43<sup>lo</sup> subsets and are shown as the mean ± SD. The results shown are representative of three independent experiments with similar results. Filled histogram, CD43<sup>lo</sup>; open green histogram, CD43<sup>hi</sup>. (B) Peptide-pulsed EL4 target cells (1 × 10<sup>4</sup>/well) were added to serial dilutions of effector cells (prepared as described above) in 96-well round-bottom plates at E : T ratio of 1 : 1 to 20 : 1. After 4 h at 37°C, cytotoxicity was quantified by measurement of cytosolic lactate dehydrogenase (LDH) in the culture supernatant (n=3) using a cytotoxicity detection kit. All experimental procedures and assays were performed two or more times, with similar results. (C) IFN-γ production of purified CD43<sup>hi</sup> and CD43<sup>lo</sup> cells was assessed by intracellular IFN-γ staining following 5 h stimulation with 1 pM~1 μM OVAp. (D) Data for functional avidity of purified CD43<sup>hi</sup> and CD43<sup>lo</sup> cells have been normalized to equate the maximum response obtained after stimulation with the OVAp used to establish the line to 100%.

CD43<sup>hi</sup> cells (Fig. 5). These results are consistent with the report that CD43<sup>lo</sup> CD8 T cells mediate stronger recall responses than CD43<sup>hi</sup> cells (37).

#### Enhanced survival of CD43<sup>lo</sup> CD8 T cells partly associated with CD62L expression

As shown in Fig. 1B, CD43<sup>lo</sup> cells showed a relatively higher expression of CD62L than CD43<sup>hi</sup> cells. High CD62L ex-

pression among CD8 T cell memory population has been shown to be a phenotypic characteristic of the central memory subset (44). To determine whether the higher expression of CD62L was involved in the enhanced survival of CD43<sup>lo</sup> cells, we analyzed the memory potential of IL-12-primed CD62L<sup>hi</sup> and CD62L<sup>lo</sup> CD8 T cells by MACS purification and a subsequent adoptive transfer experiment. After sorting, the ratio of CD43<sup>hi</sup> : CD43<sup>lo</sup> cells among CD62L<sup>hi</sup> population was



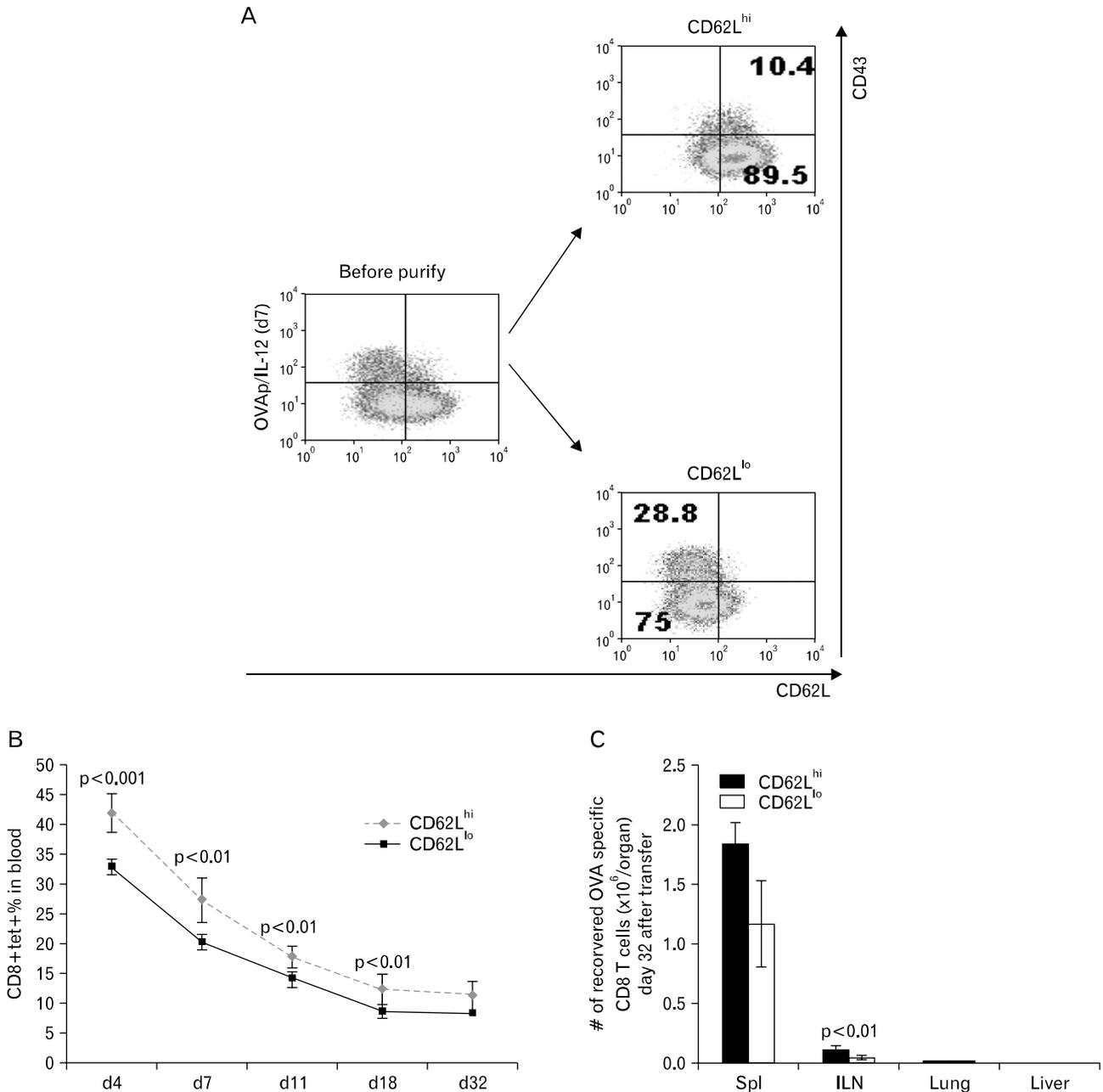
**Figure 5.** Recall response of CD43<sup>lo</sup> population is superior to that of CD43<sup>hi</sup> population. For recall response, the splenocytes from OT-I TCR transgenic mice were stimulated with the antigenic peptide (OVA<sub>p</sub>) in the presence of IL-12 for 7 days. Then, CD8<sup>+</sup>CD43<sup>hi</sup> and CD8<sup>+</sup>CD43<sup>lo</sup> cells were separated by MACS.  $1 \times 10^5$  purified CD43<sup>hi</sup> and CD43<sup>lo</sup> CD8<sup>+</sup> T cells in  $200 \mu\text{l}$  of PBS were transferred into naïve C57BL/6 mice via tail vein injection and then 1 day after transfer, the mice were intranasally challenged with  $2 \times 10^7$  pfu of recombinant adenovirus expressing OVA (rAd/OVA).

approximately 1 : 9 while that among CD62L<sup>lo</sup> population was  $\sim 3 : 7$  (Fig. 6A). At various time points after adoptive transfer, CD62L<sup>hi</sup> donor cells gave rise to 1.2-fold higher levels of surviving memory cells in peripheral blood (Fig. 6B). At day 32 after transfer, CD62L<sup>hi</sup> donor cells increased to 2-fold higher levels in lymph nodes, compared with CD62L<sup>lo</sup> donor cells, while there was no significant difference between CD62L<sup>hi</sup> cells and CD62L<sup>lo</sup> cells in other tissues including spleen, lung and liver (Fig. 6C). These data demonstrate that the enhanced survival of IL-12-primed CD43<sup>lo</sup> CD8 T cells is partly associated with CD62L expression.

## DISCUSSION

Despite progress over the last few years in elucidating the mechanisms of memory cell development in T cell immunity, it is still not well understood. The presence of proinflammatory cytokines in addition to TCR and costimulatory signals can have a profound effect on the outcome of immune responses. Our previous study suggests that initial IL-12 signaling strongly influences the programming of memory T cell development (9). In this study, we have identified a new surface marker, CD43, the expression of which is regulated by initial IL-12 signaling. CD43 expression was previously shown to be a marker that distinguishes between memory and effector T cells (18,20,35). Expression of CD43 is relatively low on naïve CD8 T cells but high on Ag-specific effector CD8 T cells, and becomes reduced again on Ag-specific memory

CD8 T cells (20). Our data indicate that expression of the activated form of CD43 (1B11) is down-regulated by IL-12 priming (Fig. 1A), and all adoptively transferred CD43<sup>lo</sup> and CD43<sup>hi</sup> cells eventually changed into CD43<sup>lo</sup> cells in the recipient mice. But these antigen-specific CD8 T cells exhibited CD43<sup>hi</sup> phenotype again when they met the same antigen (data not shown). What are the functional consequences of the changes in CD43 expression on activated T cells? Recent studies suggest that activation markers such as CD27 and CD43 define three distinct subpopulations of memory CD8 T cells that differ in their capacities to mount a recall response (37). By adoptively transferring purified CD43<sup>hi</sup> and CD43<sup>lo</sup> T cells, we have shown that CD43<sup>lo</sup> cells induced by IL-12 priming have enhanced survival (Fig. 2B) and mediate stronger recall responses (Fig. 5). Although all adoptively transferred CD43<sup>lo</sup> and CD43<sup>hi</sup> cells eventually turned into CD43<sup>lo</sup> cells in recipient mice, the CD43<sup>lo</sup> subset showed higher survival and recall potential than CD43<sup>hi</sup> subset. In addition, we found a strong correlation between decreased expression of CD43 on antigen-specific CD8<sup>+</sup> T cells and decreased functions such as granzyme B, cytolytic activity and IFN- $\gamma$  secretion. Until now, the exact role of CD43 has not been well defined due to its seemingly contradictory roles in several processes such as cell adhesion and costimulation (20,22,35,45). Our results support the idea that CD43 is promiscuously related to T cell activation, effector functions, survival and recall responses but not T cell homing. The previous works have shown that TCR signaling leads to the redis-



**Figure 6.** Enhanced survival of CD43<sup>lo</sup> cells is partly associated with CD62L expression. (A) OT-I cells were cultured for 7 days with OVAp in the presence of IL-12. After 7 days, purified CD8<sup>+</sup>CD62L<sup>hi</sup> T cells and CD8<sup>+</sup>CD62L<sup>lo</sup> T cells were adoptively transferred into C57BL/6 mice. (B) At the indicated time points, donor cell recovery from the peripheral blood of recipient mice was determined by tetramer staining, and (C) on day 32, the frequency of donor OT-I cells in lymphoid and non-lymphoid organs of the recipient mice was measured.

tribution of CD43 outside of the mature immunological synapse, and that relocalization of CD43 is required for optimal T cell activation (29,46). In addition, CD43 appears to provide intracellular signals that synergize with those of the TCR (47).

These previous reports and our results both support the idea that CD43<sup>hi</sup> cells have both TCR and CD43 signals at the same time, consequently CD43<sup>hi</sup> cells, which have a stronger signal, may exhibit more effector function than CD43<sup>lo</sup> cells. The

progressive T cell activation model proposes that signal strength regulates the progression of the T cell through the hierarchy of proliferation, differentiation and death (7). Thus, it is likely that down-regulation of CD43 by IL-12 priming diminishes the overall strength of T cell stimulation, which provides a distinct advantage for the survival of activated CD8 T cells.

In summary, the data presented here suggest that the effect of IL-12 priming on the prolonged survival of activated CD8 T cell is mediated by down-regulation of CD43 expression. And decreased expression of CD43 has the potential to regulate generation and survival of effector/memory CD8<sup>+</sup> T cell. Although further studies are needed to characterize the exact mechanisms of CD43 in the generation of memory CD8<sup>+</sup> T cells, our findings suggest that a decreased expression of CD43 on activated CD8<sup>+</sup> T cells by IL-12 priming is involved with enhanced generation of central memory precursors.

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## CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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