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J Immunol 2011; 186:6280-6286; Prepublished online 27 April 2011; doi: 10.4049/jimmunol.1003870
http://www.jimmunol.org/content/186/11/6280

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/04/27/jimmunol.1003870.DC1

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Programmed Death 1 Regulates Development of Central Memory CD8 T Cells after Acute Viral Infection

S. Rameeza Allie,* Weijun Zhang,* Shinchiro Fuse,† and Edward J. Usherwood*

The T cell response possesses a number of inhibitory receptors to regulate the extent of the antiviral response and prevent immune pathology. These receptors are generally transiently upregulated during an effector response and then downregulated during memory. Some inhibitory receptors, such as programmed death 1 (PD-1) and LAG-3, were shown to be aberrantly upregulated during memory to chronic lymphocytic choriomeningitis virus infection, limiting functional capabilities. However, little is known about the impact of inhibitory receptors on memory development during a normal CD8 T cell response to acute virus infection. Our previous data showed that PD-1 is aberrantly upregulated during a secondary response by memory CD8 T cells that were generated without CD4 T cell help. Therefore, we examined the role of PD-1 in memory differentiation during acute vaccinia virus infection in intact mice. In the absence of PD-1, the primary and memory CD8 T cell responses were enhanced. Moreover, there were distinct phenotypic and functional changes in the memory PD-1−/− CD8 T cells. Higher levels of CD62L, CD27, and CCR7 were detected; cells produced more IL-2 and made an enhanced secondary response. These changes indicate a skewing of the memory population toward the central memory phenotype in the absence of PD-1 signaling. The Journal of Immunology, 2011, 186: 6280–6286.

In most acute viral infections, the CD8 T cell response is critical for resolving the infection. After resolution of the acute infection, the CD8 population undergoes a contraction phase, and a large proportion of the effector T cells die. Cells that survive the contraction phase are maintained in the absence of Ag as memory cells (1). During activation of the CD8 response, a number of cell-intrinsic protocols are put in place to control the magnitude of the response. One of the mechanisms of control is the upregulation of inhibitory receptors immediately after activation of the CD8 T cell (2, 3). Some of the well-studied inhibitory receptors that are upregulated are programmed death 1 (PD-1), LAG-3, and CTLA-4 (4–7). Upregulation of the receptors is likely an attempt to inhibit uncontrolled activation and possible tissue destruction mediated by large numbers of highly activated CD8 T cells. PD-1 is a member of the B7 family and was discovered as a death receptor in programmed cell death by Honjo and colleagues in 1992 (8). It is upregulated on CD8 and CD4 T cells upon activation (9, 10), and it inhibits protein kinase C0 (PKC0) by exerting inhibitory effects on signaling molecules leading to PKC0 (11).

For the formation of optimal CD8 memory cells, reduction in the expression of the aforementioned inhibitory receptors is crucial (2). In chronic-infection models, continuous expression of Ag was shown to lead to dysfunctional CD8 cells that exhibit an exhausted phenotype because of the expression of a plethora of inhibitory receptors (2, 12, 13). The exhaustion phenotype results in CD8 cells that have reduced effector functions and/or a reduced capacity for multifunctionality. Recent work with chronic lymphocytic choriomeningitis virus showed that PD-1 is expressed at a higher level than the other inhibitory receptors in these exhausted cells (13). In HIV infection, Zhang et al. (14) showed that PD-1 on CD8 T cells is upregulated in typical progressors but not in long-term nonprogressors, and these cells exhibit an exhausted phenotype. Multifunctional CD8 T cells are correlates of improved immunity to HIV, and the factors that program these cells have great therapeutic potential (15, 16).

Less is known about the role of PD-1 in acute-infection models compared with the chronic models. Previous work from our laboratory showed that PD-1 blockade leads to rescue of the defective recall response of memory CD8 T cells generated in the absence of CD4 help in acute vaccinia virus infection (17). This work led us to infer that PD-1 signaling may not be limited to an inhibitory function but extends to a role in memory programming in CD8 T cells. It was important to determine whether this role included programming the multifunctionality of these cells. Because work done by Fuse et al. (17) showed a larger recall response after PD-1 blockade, we hypothesized that the absence of PD-1 would alter the differentiation of memory CD8 T cells following acute virus infection. To test this hypothesis, we monitored CD8 memory formation in animals infected with the Western Reserve strain of vaccinia virus (VV-WR), in the presence or absence of PD-1.

Materials and Methods

Mice

C57BL/6 and congenic B6-Ly5.2-Cr mice (Ly5.1/CD45.1+) were purchased from The National Cancer Institute (Bethesda, MD). Congenic B6, FL-Thy1.1Cr (Thy1.1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 PD-1−/− mice were used by permission from Dr. Tasuku Honjo (Department of Immunology and Genetic Medicine, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan) (15). Programmed cell death ligand 1 (PD-L1)−/− mice were provided by Dr. William R. Green (Dartmouth Medical School) (18).

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Received for publication November 23, 2010. Accepted for publication March 29, 2011.

The work was supported in part by National Institutes of Health Grants AI069943 and CA103642.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BM, bone marrow; BMC, bone marrow chimeric; DC, dendritic cell; i.n., intranasal; LTR, long terminal repeat; KLRG1, killer cell lectin-like receptor subfamily G member 1; PD-1, programmed death 1; PD-L1, programmed cell death 1 ligand 1; PKC0, protein kinase C0; Tcm, central memory T cell; Tem, effector memory T cell; VV-WR, Western Reserve strain of vaccinia virus; WT, wild-type.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003870

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Viral infection

VV-WR was obtained from Dr. William Green (Dartmouth Medical School, Lebanon, NH). Primary infection (1000 PFU) was administered intranasally (i.n.). Secondary infection was administered (2 × 10^3 PFU) i.p.

Generation of mixed bone marrow chimeric mice

Bone marrow (BM) from donor B6-Ly5.2-Cr mice (1 × 10^6 cells) and C57BL/6 PD-1−/− mice (1 × 10^6 cells) was mixed 1:1 and transferred i.v. into recipient B6-Ly5.2-Cr mice. The recipient mice had been lethally irradiated (1200 rad) the day before BM transfer with a split dose (600 rad twice) given 24 h apart. Reconstitution of the BM was checked 50 d post-BM transfer by staining blood cells for the congenic marker CD45.2. The mice that had an approximate 1:1 reconstitution of CD45.2/CD45.2− were used in the study.

Tissue preparation

Blood was processed and the RBCs were lysed. The spleen and lymph nodes were homogenized, and the RBCs were lysed. The liver and lung were digested with collagenase (2.33 mg/ml; Sigma-Aldrich) and DNase (0.2 mg/ml; Roche Diagnostics) for 30 min. The WBCs were enriched using a Percoll (GE Healthcare) gradient.

Flow cytometry

An MHC/peptide tetramer for the VV-WR epitope B8R20-27 (TSYKFESV)/K was obtained from the National Institutes of Health Tetramer Core Facility (Emory University, Atlanta, GA). Cells were stained for 1 h at room temperature with tetramer, washed, and stained for surface markers for 30 min at 4˚C. The surface markers used were PerCP CD8 (BioLegend, San Diego, CA), FITC CD27 (BioLegend), PE CD62L (Invitrogen, Carlsbad, CA), FITC CD127 (BioLegend), PE KLRG1 (Abcam, Cambridge, MA), and PE CCR7 (BioLegend).

Intracellular cytokine staining

Splenocytes were restimulated with 1 μg/ml B8R peptide, 10 U/ml IL-2, and 10 μg/ml Brefeldin A for 5 h at 37˚C. Unstimulated splenocytes were used as a negative control, and the background subtracted. In the presence of 10 μg/ml brefeldin A, the cells were stained for surface expression of PerCP CD8 (BioLegend) for 20 min on ice. Following surface staining, the cells were fixed with 2% formaldehyde (Ted Pella, Redding, CA) for 20 min at room temperature and permeabilized with 1× permeabilization buffer (eBiosciences, San Diego, CA) for 10 min. Washed and permeabilized cells were stained with allophycocyanin-labeled anti–IFN-γ and PE-labeled anti–IL-2 (both from BioLegend).

Secondary responses

At 60 d postinfection, splenocytes were enriched for CD8+ cells using positive-selection kits from StemCell Technologies (Vancouver, BC). A total of 4–10 × 10^6 B8R tetramer+ CD8+ T cells was adoptively transferred into naive C57BL/6 mice.

In the PD-1−/− model, the number of Ag-specific cells was normalized prior to transfer. In the BM chimeric (BMC) model, recipients were given 1–2 × 10^7 CD8 T cells containing 5–10 × 10^6 B8R tetramer+ PD-1−/− and wild-type (WT) CD8 T cells. Simultaneously, the recipients were infected with 2 × 10^6 VV-WR i.p. The transferred cells were stained with MHC/peptide tetramer for the VV-WR epitope B8R20-27 (TSYKFESV)/Kc prior to transfer. The percentage of tetramer+ cells in each group was used to calculate the number of Ag-specific cells given to each group. This calculation was also performed at the end of the experiment to calculate the fold expansion of the transferred cells. Six to eight mice were used per experiment.

Statistical analysis

The distribution was analyzed for normality, and p values were determined using the Student t test or the Mann–Whitney U test. Paired t tests were used in the BM model to account for the same internal environment of the WT and PD1−/− CD8 T cell populations. One-way ANOVA, followed by the Dunn posttest, was carried out when greater than two variables were compared. A p value < 0.05 was considered significant.

Results

Increase in the primary Ag-specific response in the absence of PD-1 signaling

To determine the role of PD-1 in the magnitude of the Ag-specific CD8 response in an acute infection, we infected WT (C57/B6) and PD1−/− mice with VV-WR i.n. VV-WR is cleared in 14–15 d and elicits a robust CD8 response in mice, which can be measured using an MHC/peptide tetramer folded with the immunodominant B8R epitope [(TSYKFESV)/Kc]. The Ag-specific response was measured during the acute and memory phases following infection. PD1−/− animals showed a consistently increased Ag-specific CD8 response at the acute and memory phases (Fig. 1). Responses were significantly increased in the acute and early memory phase (days 21–30; Fig. 1), and they showed a significant increase in the late memory phase (>60 days) in repeated experiments (data not shown). To determine whether PD-1 was signaling via PD-L1 to produce this increase in the Ag-specific memory population, we infected WT and PD-L1−/− mice with VV-WR i.n.; we noted a similar significant increase at the memory stage of the response (Supplemental Fig. 1).

Increase in the recall response in the absence of PD-1 signaling

To test the functional capability of the memory CD8 T cell response in PD1−/− mice, we measured the recall response to VV-WR infection. CD8 T cells were purified from PD-1−/− or WT mice and adoptively transferred into naive WT hosts (that were congenically different from the donor) and infected with high-dose VV-WR i.p. Six days postchallenge, the Ag-specific (B8R tetramer+) response was measured using congenic markers to identify the donor cells. The Ag-specific PD1−/− CD8 cells expanded to a greater magnitude than did the WT cells (29-fold versus 8.5-fold; Fig. 2).

Enhanced IL-2 production from memory cells in the absence of PD-1 signaling

Next, we tested cytokine secretion from the Ag-specific PD-1−/− memory CD8 T cells. Spleen cells at the memory time point (≥55 days) were stimulated with B8R peptide to identify Ag-specific CD8 T cells by staining for IFN-γ. Intracellular cytokine staining after peptide stimulation confirmed the higher frequency of B8R-specific cells observed by tetramer staining (Fig. 3A). We did not observe an increase in the per-cell IFN-γ production (mean fluorescence intensity) (data not shown) or a significant difference in the ratio of tetramer+/IFN-γ− between the two groups (Supplemental Fig. 2). Importantly, staining for IL-2 showed a significant increase in the percentage of IL-2–producing Ag-specific (i.e., IFN-γ+) memory cells in PD-1−/− mice compared with WT mice (Fig. 3B).

Studies in mixed BMC mice

PD1−/− animals were shown to have an increased risk for developing autoimmune disease (15, 16, 19). This raised the concern that the increased proinflammatory environment in PD-1−/− animals played a role in the observed increased Ag-specific response. To confirm that PD-1 signaling was acting in a cell-intrinsic manner, we created 1:1 WT/PD1−/− mixed BMC animals. In

![FIGURE 1](http://www.jimmunol.org/Downloadedfrom)

* p < 0.01, ** p < 0.001.
were isolated from PD1−/− mice at day 71 postinfection and transferred to naive C57BL/6 recipients by i.v. injection. The number of Ag-specific PD−/− or WT cells was determined using B8R tetramer staining and was normalized prior to adoptive transfer. In replicate experiments, 5−10 × 10^5 B8R tetramer+ CD8 T cells were adoptively transferred. The recipients of WT or PD1−/− B8R tetramer+ CD8 T cells received equal numbers of Ag-specific cells. The naive hosts were challenged with 2 × 10^6 VV-WR (i.p.) and monitored 6 d postinfection for the recall response. Ag-specific cell numbers at day 6 were determined by multiplying the total cell number of the specific donor population by the percentage of CD8+ cells and the percentage of B8R+/CD8+ cells. Each point represents data from an individual mouse. Data are representative of two experiments. The t test was performed, giving a p value < 0.0001.

Increase in the Ag-specific response in the absence of PD-1 signaling on CD8 T cells

To determine the role of PD-1 in the magnitude of the Ag-specific CD8 response, we infected BMC mice with VV-WR. The Ag-specific response was measured during the acute and memory phases using tetramer staining. The presence of WT and PD−/− cell populations in the same host allowed us to analyze the data using a paired test, increasing the statistical power. The PD−/− population showed a consistently increased Ag-specific CD8 response at the peak, contraction, and memory phases of the response (Fig. 4), confirming data obtained in PD1−/− mice.

**Enhanced recall response observed in the absence of PD-1 signaling on CD8 T cells**

The recall response was tested by adoptively transferring purified CD8 T cells into congenic Thy1.1+ hosts. The mice were then infected with VV-WR i.p. The number of total splenocytes and the proportion of WT and PD−/− Ag-specific CD8 cells were noted to determine the relative expansion after challenge for each population. Six days postchallenge, the Ag-specific (B8R tetramer+) response was measured using congenic markers to identify the WT and PD−/− donor cells. The Ag-specific PD1−/− CD8 cells expanded more than the WT cells (55-fold versus 4-fold; Fig. 5), thus recapitulating the results seen in PD1−/− mice.

**Enhanced IL-2 production in the absence of PD-1 signaling on CD8 T cells**

We stained for cytokine production by CD8 cells in chimeric mice at the memory time point. Intracellular staining for IFN-γ production following peptide stimulation confirmed a greater frequency of memory CD8 T cells in the PD−/− population (Fig. 6A). The cells were further stained for IL-2, which showed a significant increase in the Ag-specific PD−/− CD8 cells compared with the WT cells (Fig. 6B). We saw no differences in TNF-α production between the Ag-specific WT and PD−/− CD8 cells (data not shown). We observed a similar increase in the proportion of IFN-γ–producing cells and an increase in the proportion of Ag-specific CD8 cells in the PD−/− fraction compared with the WT fraction as early as 21 d postinfection (Supplemental Fig. 3).

The BMC model confirmed the results observed in WT and PD−/− mice, ruling out any effects inherent to the PD−/− mice that may nonspecifically enhance the CD8 T cell response.

**Phenotypic changes on CD8 T cells in the absence of PD-1 signaling**

We observed an increase in the proportion of cells that expressed CD27 in the absence of PD-1 signaling as early as 21 d postinfection (Fig. 7A). In addition, we observed an increase in the amount of CD27 expressed per cell by the Ag-specific PD-1–deficient cells (Fig. 7B). We also stained for CD62L expression, which showed a greater proportion of PD-1–deficient Ag-specific CD8 T cells that were CD62Lhigh compared with those with WT PD-1 expression (Fig. 7C). In contrast, the levels of the receptor indicative of an effector phenotype, killer cell lectin-like receptor subfamily G member 1 (KLRG1), were significantly increased in WT CD8 T cells compared with PD-1–deficient Ag-specific CD8 T cells at 21 d postinfection (Fig. 7D). This significant difference was maintained as late as 56 d postinfection, indicating a more effector-like phenotype in the WT CD8 T cells compared with the PD−/− CD8 T cells.

**Central memory T cell skewing in the absence of PD-1 signaling**

The fact that we observed a greater proportion of cells producing IL-2, as well as higher expression of CD27 and CD62L, implied a skewing toward central memory cells in the absence of PD-1. A cardinal feature of central memory T cells (Tcm) is their ability to preferentially enter noninflamed lymph nodes, which requires...
expression of CCR7 and CD62L. Therefore, we calculated the proportions of Ag-specific CD8 T cells positive for CD62L and CCR7. At day 21 postinfection, there was a significantly greater proportion of PD-1−/− cells coexpressing both molecules compared with WT cells (Fig. 8A) and a correspondingly lower population of effector memory T cells (TEm; CD62L−/−CCR7−) (Fig. 8B). These significant differences were also observed during the memory phase (>55 d postinfection; data not shown). We also noted an increase in CD27+ Ag-specific memory cells and an increase in the amount of CD27 expressed per cell (Fig. 9), consistent with results obtained at day 21 postinfection (Fig. 7A, 7B).

**Discussion**

In this report, we showed an increased proportion of Ag-specific CD8 T cells at the primary and memory phases of the antiviral CD8 response in the absence of PD-1 signaling. Most importantly, we observed an enhancement in the qualities that define central memory cells when PD-1 signaling was not present. The increase in central memory phenotype cells was seen as early as 21 d postinfection. This indicated that the absence of PD-1 was generating more central memory cells, as well as skewing them toward this phenotype, at early times postinfection. Selective enrichment of PD-1−/− virus-specific cells in lymphoid tissues is consistent with the preferential recirculation through lymphoid tissue displayed by central memory cells.

After T cell activation, along with the upregulation of costimulatory molecules there is a coordinated upregulation of inhibitory receptors (21). In an attempt to control the magnitude of activation, T cells upregulate inhibitory receptors, such as PD-1, which engage with their ligands on the APCs to counterbalance activation of the T cell. Normally, PD-1 expression decreases when viral Ag is cleared; however, this does not occur in some high-load chronic virus infections. Constant exposure to Ag and prolonged...
signaling via the TCR elicits long-term expression of PD-1 and other inhibitory receptors, leading to functional exhaustion of the T cells (22–24). Blockade of PD-1 can restore function to these T cells, improving control of the chronic infection (25).

Although PD-1 can be upregulated on T cells and B cells upon signaling via the TCR or BCR, the ligand is constitutively expressed on T cells, B cells, dendritic cells (DCs), BM-derived mast cells, and macrophages. Signaling to the T cell via PD-1 gives an inhibitory signal, whereas a recent study showed that signaling via PD-1 on the DCs leads to DC maturation (26). PD-1 signaling was shown to have different roles in DC development and function. Although studies showed that PD-1 blockade enhances DC function, thereby conferring protection from bacterial infection, another study showed that PD-1 signaling on the DC is needed for normal homeostasis and, in its absence, the DCs cannot present Ag efficiently (26, 27). Despite these important roles for PD-1 on DCs, in our studies in BMC mice, the effects of PD-1 were intrinsic to the CD8 T cells; WT and PD-1/−/CD8 T cells were primed in an environment containing a mixture of PD-1/− and WT APCs.

Although there is a plethora of work on PD-1/PD-L1 interactions in chronic infections, there has been limited work elucidating the role of this interaction in acute-infection models.

**FIGURE 6.** Increased cytokine production by Ag-specific memory CD8 T cells in the absence of PD-1 signaling in the BMC model. Spleen cells from chimeric mice were incubated for 5 h with or without B8R peptide in the presence of brefeldin A. IFN-γ (A) or IL-2 (B) (in the IFN-γ+ CD8 T cells) production was measured with intracellular cytokine staining, and cells were stained for the congenic marker CD45.2 to determine PD-1 status. A, Representative dot plots of WT and PD-1/− CD8 T cells. The numbers are the percentages of IFN-γ-producing cells in the WT (CD45.2+) or PD-1/− (CD45.2−) compartment. The data are presented graphically (lower panel). The background was subtracted using the no-peptide control values. B, Representative dot plots of WT and PD1/− CD8 T cells. The numbers are the percentage of IL-2 produced in the IFN-γ-producing CD8 population in the WT (CD45.2+) or PD-1/− (CD45.2−) compartment. A graphical representation of the data is shown (lower panel). Each point represents data from an individual mouse, and data are representative of three experiments. *p < 0.01, paired t test. NP, no peptide stimulation; WP, with peptide stimulation.

**FIGURE 7.** Early skewing toward a central memory surface marker phenotype. Mixed BMC mice were infected with VV-WR (1000 PFU i.n.). Twenty-one days postinfection, spleen cells were stained with B8R tetramer plus the activation/memory markers indicated. A, Percentage of CD27+ve cells among the Ag-specific CD8 population. B, Fluorescence intensity per cell of CD27 staining in the Ag-specific CD8 populations. C, Percentage of CD62Llow cells among the Ag-specific CD8 population. D, Percentage of KLRG1+ cells among the Ag-specific CD8 population. Each pair of points represents data from an individual chimeric mouse; data are representative of three experiments. *p < 0.05, **p < 0.005; paired t test.

**FIGURE 8.** Early Ag-specific WT CD8s have more effector memory phenotype CD8 T cells compared with PD1/−/CD8 T cells. Mixed BMC mice were infected with VV-WR (1000 PFU i.n.). Twenty-one days postinfection, B8R tetramer staining was performed on spleen cells together with staining for CCR7 and CD62L. A, CD62L and CCR7 double-positive population stained to indicate Tcm Ag-specific CD8 T cells. B, CD62L and CCR7 double-negative cells stained to indicate Tem Ag-specific CD8 T cells. Each pair of points represents data from an individual chimeric mouse; data are representative of two experiments. ***p < 0.0001, paired t test.
model showed that PD-1\(^{-/-}\) mice were markedly protected from lethality, which was accompanied by a decreased bacterial burden and suppressed inflammatory cytokine responses. By depleting peritoneal macrophages, the investigators showed that the loss of PD-1 on peritoneal macrophages, which expressed significantly higher levels of PD-1 during sepsis, was associated with the development of cellular dysfunction (28). In a study using a mouse model of acute infection with *Histoplasma capsulatum*, Lázár-Molnár et al. (29) showed an increase in PD-L1 expression by macrophages, which also showed an increased suppression of CD8 proliferation; they hypothesized this to be the mechanism for the lethality to *H. capsulatum* infection. In addition, two studies using acute hepatitis C virus and acute rabies encephalitis showed the role for the PD-1 pathway in viral persistence and lethality, respectively (30, 31). Further they showed that PD-1 played a role in the dysfunction and migratory capacity of the CD8 T cells.

Although the above studies in acute infections showed an inhibitory role for the PD-1 pathway in acute infections, a model with *Listeria monocytogenes* showed that blockade of this pathway led to reductions in the magnitude and kinetics of T cell expansion to primary (attenuated) and secondary (virulent) *L. monocytogenes* infection, indicating an immune-stimulatory role for the PD-1 pathway in infections with an intracellular bacterial pathogen (32).

In addition to the work on the role of PD-1 signaling on DCs, the above studies delved into the role of PD-1 on CD8 T cells, focusing on the acute response to the pathogen; however, they did not address memory differentiation following acute viral infection (27–32). Our study elucidates a key role for PD-1 in restraining the development of memory cells, and central memory cells, in particular.

Previously, we showed that increased PD-1 expression after secondary Ag challenge leads to poor recall responses to VV-WR in the absence of CD4 T cell help (17). Because normal activation leads to downregulation of PD-1 after Ag removal, and increased expression leads to dysfunction (in chronic infections or in the absence of CD4 help), we hypothesized that the absence of PD-1 would result in quantitative and qualitative enhancement of the CD8 T cell response. In addition, previous work showing that only PD-1 low or intermediate cells can be rescued from exhaustion by PD-1 blockade suggests that PD-1 may need to be low during or soon after activation for the generation of multifunctional memory CD8 T cells (33–35). Recent work also showed that blockade of signaling via another inhibitory receptor (Tim-3) led to stronger acute and memory CD8 responses in HSV infection (36). Therefore, our data, together with these previous studies, strongly support the hypothesis that PD-1 regulates the magnitude of the CD8 T cell response, as well as the functional abilities of the resultant memory population.

Skewing toward a central memory phenotype in the absence of PD-1 is novel and unexpected, because it may be predicted that the removal of a signal inhibiting activation may lead to a more effector/effector memory phenotype. In addition to the expression of surface markers, such as CD62L, CD8 T\(_{cm}\) produce more IL-2, which likely enhances the recall response. CD27 signaling was shown to be involved in PI3K-mediated maintenance of the memory state in CD8 T cells, allowing their maintenance in the G1 stage of the cell cycle rather than the G0 stage, which occurs in naive T cells (37). The same group of investigators showed that maintenance of the G1 state allowed the rapid-response function of these memory cells. Our studies showed an elevated level of CD27 expression in the memory CD8 T cells in the absence of PD-1 signaling, which may be important for their functional enhancement (greater IL-2 production, more expansion) upon ex vivo recall to Ag.

Signal-transduction studies showed that PD-1 inhibits IL-2 signaling by blocking ZAP70/CD3\(\xi\) signaling, which attenuates PKC\(\theta\). Thus removing PD-1 would release this inhibition, resulting in more T cell activation, as seen by our results (11). Additionally, increased IL-2 may explain the enhanced recall response in the absence of PD-1 signaling on the CD8 T cells. This is supported by our previous work showing that supplementing IL-2 rescued the recall response in CD4 helpless CD8 T cells, in addition to preventing excessive PD-1 expression during the secondary response (17).

We observed a moderate quantitative enhancement in the CD8 response in the absence of PD-1; however, other inhibitory receptors may be working in conjunction with PD-1 to modulate the CD8 memory response. CTLA-4 was shown to work collectively with PD-1 in hepatitis C virus-specific CD8 T cells, supporting the idea that memory programming is additively affected by multiple inhibitory receptors (38). Further work is needed to address the role of other inhibitory receptors in memory CD8 T cell development. Also it remains to be determined whether there is fluidity in the phenotype of PD-1–deficient memory cells and...
whether they maintain their central memory skewing after multiple re-exposure to Ag. Because we observed phenotypic skewing of the CD8 population as early as 14–21 d postinfection in the absence of PD-1 signaling, and memory precursor cells can be identified very early during the primary response, the influence of PD-1 signaling may occur at very early stages of memory formation. Identification of the critical point at which PD-1–mediated suppression affects the characteristics of the CD8 memory population will help in therapeutic endeavors using PD-1 blockade. To our knowledge, this study is the first to identify a cell-intrinsic role for PD-1 signaling affecting the quality of CD8 memory T cells during acute viral infection. Although further work is needed to isolate the critical period of the response when PD-1 blockade is required for these effects, this work can be used as a foundation for enhancing memory responses induced by virus immunization.

Acknowledgments
We thank Dr. Patricia Ernst (Department of Genetics, Dartmouth Medical School) for providing advice on improvements to the BMC protocol. Dr. William Green (Department of Microbiology and Immunology, Dartmouth Medical School) provided the PD-1−/− mice, which were originally obtained from Dr. Tatsuki Honjo (Department of Immunology and Genetic Medicine, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan).

Disclosures
The authors have no financial conflicts of interest.

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