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PD-L1 Limits the Mucosal CD8$^+$ T Cell Response to *Chlamydia trachomatis*

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***Chlamydia trachomatis* infection is the most common bacterial sexually transmitted disease in the United States. Repeated infections with *C. trachomatis* lead to serious sequelae, such as infertility. It is unclear why the adaptive immune system, specifically the CD8$^+$ T cell response, is unable to protect against subsequent *C. trachomatis* infections. In this article, we characterize the mucosal CD8$^+$ T cell response to *C. trachomatis* in the murine genital tract. We demonstrate that the immunoinhibitory ligand, PD-L1, contributes to the defective CD8$^+$ T cell response. Deletion or inhibition of PD-L1 restores the CD8$^+$ T cell response and enhances *C. trachomatis* clearance. *The Journal of Immunology*, 2014, 192: 1079–1090.**

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that infects 1.4 million people in the United States each year, and the incidence of infection has continued to rise since 2000 (1). Adolescent girls and young women infected with *C. trachomatis* face reproductive tract damage and increased risk for ectopic pregnancy and infertility (2).

During primary infection of mice, *C. trachomatis* infection stimulates a 100-fold expansion of CD8$^+$ T cells that subsequently contract to form a memory population (3, 4). However, upon rechallenge, the response of these cells is significantly smaller in magnitude than the primary response, with fewer cytokine producing CD8$^+$ T cells (4). This impaired secondary CD8$^+$ T cell response is reminiscent of infections with chronic viral pathogens, such as HIV and lymphocytic choriomeningitis virus (LCMV) Clone 13. The memory CD8$^+$ T cells that develop after HIV and LCMV Clone 13 infections exhibit an exhausted phenotype defined by low cytokine production, expression of proapoptotic genes, and low replicative potential, all of which lead to an extremely deficient secondary CD8$^+$ T cell response (5–7).

A significant cause of these defective CD8$^+$ T responses in chronic viral infections is the engagement of immunoinhibitory pathways (8–11). A well-described immunoinhibitory pathway is made up of the receptor PD-1, which is expressed on CD8$^+$ T cells, and its ligand PD-L1, which is expressed on professional APCs or on infected target cells. The engagement of the PD-L1/PD-1 pathway can antagonize the T cell signaling mediated by stimulatory molecules, as well as affect downstream-signaling pathways that decrease cytokine production and reduce memory potential (12, 13). It has not been explored whether PD-L1/PD-1 signaling plays a role in the lack of CD8$^+$ T cell recall potential resulting from *C. trachomatis* infection. In this article, we show that the CD8$^+$ T cell response to genital infection with *C. trachomatis*, as with chronic viral infections, is negatively affected by the immunoinhibitory receptor PD-1 and its ligand, PD-L1. We also show that deletion or inhibition of this pathway improves the CD8$^+$ T cell response and results in enhanced bacterial clearance.

Materials and Methods

Mice

C57BL/6, B6.PL-Thy1a (CD90.1/Thy1.1 congenic), and B6.129S7-IFNγtm1Agt (IFNγ$^{-/-}$) mice were purchased from The Jackson Laboratory. PD-L1 (Thy1.2 background) and PD-1 (Thy1.1 background)–deficient mice were described previously and were generously provided by Arlene Sharpe. Anti–PD-L1–blocking Ab (clone 10F.9G2) was generously provided by Gordon Freeman; anti–B7-1–blocking Ab (clone 1G10) was purchased from Bio X Cell. For transient-inhibition experiments, mice were treated with 200 μg anti–PD-L1 or anti–B7-1 each day for 3 d prior to infection and then every other day postinfection. All animals were maintained and cared for within the Harvard Medical School Center for Animal Resources and Comparative Medicine (14, 15). All mice were treated with 2.5 mg medroxyprogesterone s.c. 7 d prior to infection with *C. trachomatis* to synchronize the murine estrous cycle. All experiments were approved by the Institutional Animal Care and Use Committee.

Growth, isolation, and detection of bacteria

C. trachomatis serovar L2 (434/Bu) was propagated within McCoy cell monolayers grown in Eagle's MEM (Invitrogen, Grand Island, NY) supplemented with 10% FCS, 1.5 g/l sodium bicarbonate, 0.1 M nonessential amino acids, and 1 mM sodium pyruvate. Infected monolayers were dissociated from plates using sterile glass beads and were sonicated to disrupt the inclusion. Elementary bodies were purified by density gradient centrifugation, as described previously (16). Aliquots were stored at −80°C in medium containing 250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid and were thawed immediately prior to use. To quantify the levels of *C. trachomatis*, quantitative PCR (qPCR) with 16S primers specific for *C. trachomatis* was performed as previously described (16).

Flow cytometry

Tissues were mechanically disaggregated and immediately stained for surface markers or stimulated for 5 h with 50 ng/ml PMA (Alexis Biochemical) and 500 ng/ml ionomycin (Calbiochem) in the presence of brefeldin A (GolgiStop; BD Biosciences) for intracellular cytokine staining. Cells were preincubated with anti-FcRγ (Bio X Cell) before staining with anti–CrpA-allophycocyanin (National Institutes of Health Tetramer Core) or anti–PD-L1–allophycocyanin, anti–CD4 Q-Dot, anti–CD8–allophycocyanin–Cy7, or anti–CD90.2-PeCy7 (BioLegend). Cells also were incubated with anti–CD11b-PB, anti–CD11c-PB, anti–CD19-PB, and anti–B220-PB to exclude these populations. For activation marker analysis, we examined anti–CD62L-FITC and anti–CD127-PerCP (BD Biosciences). Anti–IFN-γ PE

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Abbreviations used in this article: DC, dendritic cell; dLN, draining lymph node; IFU, inclusion-forming unit; LCMV, lymphocytic choriomeningitis virus; qPCR, quantitative PCR; qRT-PCR, quantitative RT-PCR; Tcm, central memory T; Tem, effector memory T; WT, wild-type.

(BD Biosciences) was used for intracellular cytokine staining, and cells were permeabilized with the Cytofix/Cytoperm Plus Kit (BD Biosciences), according to the manufacturer's instructions. The absolute cell number in each sample was determined using AccuCheck Counting Beads (Invitrogen). Data were collected on an LSR II (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Inhibitory gene transcript expression

Mice were transcervically infected with 10^6 inclusion-forming units (IFU), as previously described (17). Five days postinfection, tissues were mechanically disaggregated in 2 ml PBS, and aliquots were immediately frozen at −20°C. RNA was extracted from 80-μl aliquots by phenol-chloroform precipitation. Quantitative RT-PCR (qRT-PCR) was performed using 25 ng purified RNA and amplified using TaqMan SYBR Green Master Mix. The following primers were used: CTLA4 Sense: 5′-GTTGGGGGCATTTTCACATA-3′, CTLA4 Antisense: 5′-TTTTACAGTTTCCTGGTCTC-3′; Tim3 Sense: 5′-GAACTGAAATTAGACATCAAAGCAGC-3′, Tim3 Antisense: 5′-GGTTCTTGGAGAAGCTGTAGTAGAGTC-3′; Lag3 Sense: 5′-TCCGCCTGCGCGTCG-3′, Lag3 Antisense: 5′-GACCCAATCAGACAGCTTGAGGAC-3′; CD160 Sense: 5′-GGCCACTTTCTCTCCGTTCTAG-3′, CD160 Antisense: 5′-GGTGTGACCTTTGTCTCTGTCTTATC-3′; 2B4 Sense: 5′-GTTGCCACAGCAGACTTTC-3′, 2B4 Antisense: 3′-TTCCAACCTCCTCGTACACGGTAC-3′; PD-1 Sense: 5′−CCCTCAGTCAAGAGGAGCAT-3′; PD-1 Antisense: 5′-TCCCAGCTTGTGGTAAACCT-3′; PD-L2 Sense: 5′-GTACCGTTGCCTGGTCATCT-3′, PD-L2 Antisense: 5′−GCCAGGACACTTCTGCTAGG-3′; B7-1 Sense: 5′-ATGGCTTGCAATTGTCAGTTGA-3′, B7-1 Antisense: 5′-ATCAGGAGGGTCTTCTGGGGGT-3′; PD-L1 Sense: 5′-TGGACAAACAGTGACCACCAA-3′, PD-L1 Antisense: 5′-CCCCTCTGTCCGGGAAGT-3′; and GAPDH Sense: 5′-GGTGCTGAGTATGTCGTGGA-3′, GAPDH Antisense: 5′-CGGAGATGATGACCCTTTTG-3′.

T cell depletion and transfer

For $CD8^+$ and $CD4^+$ T cell–depletion experiments, mice were injected i.p. with 200 μg anti-$CD8^+$ (clone 2.43), 200 μg anti-$CD4^+$ (clone GK1.5), or isotype control (clone LTF-2). Mice were treated with Ab every day starting 3 d prior to primary challenge and then every other day after challenge. Mice were sacrificed 5 d after challenge. For secondary challenge, mice were treated every day 3 d prior to secondary challenge and then every other day after secondary challenge.

For $CD8^+$ T cell–transfer experiments, lymphoid organs and uteri were collected from naive wild-type (WT) (Thy1.1) and PD-1–deficient (Thy1.1) mice. $CD8^+$ T cells were isolated using a Dynal Negative Selection $CD8^+$ T cell kit (Invitrogen), and 5×10^6 purified $CD8^+$ T cells were transferred i.v. into naive Thy1.2 IFN-γ–deficient mice.

Statistical analysis

The data represent the mean ± SEM and were calculated using GraphPad Prism version 4.0. The *p* values were determined using the nonparametric Mann–Whitney *U* test or an unpaired *t* test, as indicated.

Results

$CD8^+$ T cells are impaired during C. trachomatis *infection in the genital tact*

During systemic infection with *C. trachomatis*, the secondary $CD8^+$ T cell response is impaired (4). In this study, we used a transcervical route of infection to assess the $CD8^+$ T cell response in the genital mucosa, the site of natural human infection (17). We measured recruitment of endogenous *Chlamydia*-specific $CD8^+$ T cells to the genital tract throughout the course of infection. We infected mice transcervically with *C. trachomatis* and harvested the uteri at specific time points postinfection. Flow cytometry was used to measure the number of $CD8^+$ T cells bound by a previously described class I tetramer specific for the *C. trachomatis* immunodominant Ag CrpA (18). By day 7 postinfection, the number of CrpA-specific $CD8^+$ T cells in the uterus was 1000-fold higher compared with uninfected mice (Fig. 1A). This was followed by a steep contraction and a return to preinfection numbers by 4 wk postinfection (Fig. 1A). After rechallenge, the peak of the secondary response again occurred at day 7 postinfection, although the total number of $CD8^+$ T cells was significantly lower than during primary challenge (Fig. 1A, Supplemental Fig. 1). In summary, genital infection with *C. trachomatis* elicits a robust primary $CD8^+$ T cell response but a greatly diminished secondary response.

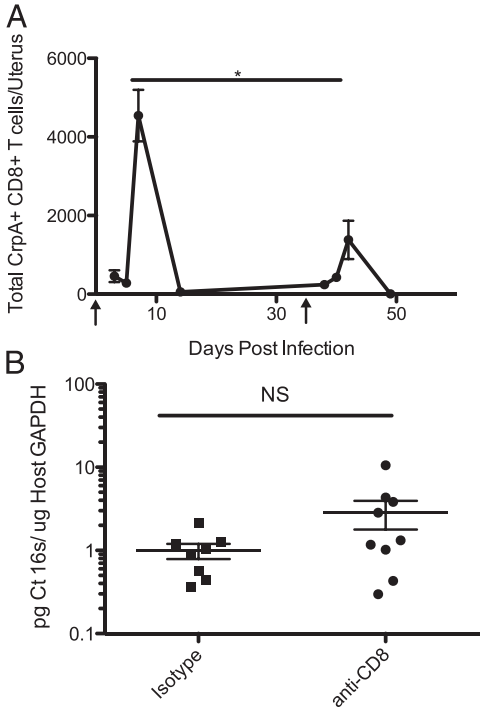

FIGURE 1. The $CD8^+$ T cell response to *C. trachomatis* infection is impaired. (**A**) Quantification of the *C. trachomatis*–specific $CD8^+$ T cell response in the genital tract over time using a tetramer specific for the *C. trachomatis* Ag CrpA. Mice were infected with 10^6 IFU of *C. trachomatis* at time points indicated by black arrows. At these time points, lymphocytes were isolated from uterine tissue and stained with the CrpA tetramer. Points represent the average number of $CrpA^+$ $CD8^+$ T cells obtained from each of five mice. Error bars indicate SEM. Statistical significance was calculated using the Mann–Whitney *U* test and is indicated between day 7 primary and day 7 secondary. (**B**) Mice were treated with anti-CD8–depleting Ab or isotype-control Ab for 3 d prior to and 2 d after secondary infection. Mice were transcervically infected with 10^6 IFU of *C. trachomatis*, and bacterial burden was measured by qPCR 5 d postsecondary infection. Data are representative of two independent experiments.

Although there are significantly fewer *C. trachomatis*–specific $CD8^+$ T cells during secondary infection compared with primary infection, it is possible that this small population could contribute to restricting *C. trachomatis* replication via potent cytokine production. We hypothesized that if memory $CD8^+$ T cells are important in controlling secondary infection, then loss of $CD8^+$ T cells should alter bacterial clearance during reinfection. To test this, we infected mice transcervically with *C. trachomatis* and allowed for bacterial resolution and immune memory establishment over the course of 4 wk. We then treated mice with anti-CD8–depleting Ab or isotype control 3 d prior to secondary infection. Depletion was confirmed by flow cytometry, showing a >100-fold reduction in the number of $CD8^+$ T cells in the draining lymph nodes (dLNs) and uterus (data not shown). We measured bacterial burden 5 d after secondary challenge and observed no differences in mice treated with anti-CD8–depleting Ab compared with mice treated with control Ab (Fig. 1B). Taken together, our data demonstrate that memory $CD8^+$ T cells have an impaired ability to expand and do not contribute to controlling *C. trachomatis* during secondary infection.

PD-L1 is upregulated in the genital mucosa upon C. trachomatis *infection*

It is clear that *C. trachomatis* is able to stimulate a $CD8^+$ T cell response. However, our data and that of other investigators showed

that this CD8$^+$ T cell response is impaired during *C. trachomatis* infection (4). Studies (19–22) with other chronic pathogens demonstrated that the activation of immunoinhibitory pathways can directly inhibit the CD8$^+$ T cell response. We hypothesized that the upregulation of one or more of these pathways during *C. trachomatis* infection might contribute to the defect that we observed in the CD8$^+$ T cell response to *C. trachomatis.* To test this, we infected mice transcervically and examined the expression of different immunoinhibitory receptors and ligands by qRT-PCR in whole uterus at the peak of bacterial burden (5 d postinfection) (Fig. 2A). Of the genes tested, the immunoinhibitory ligand PD-L1 was expressed >10-fold higher in the uteri of infected mice compared with uninfected mice (Fig. 2A). Because previous work suggested that PD-L1 expression plays a role in the anti-inflammatory response to *C. trachomatis*, we started by investigating whether PD-L1 expression was dependent on live *C. trachomatis* infection or just the result of a general inflammatory response (23). We infected mice with either live or UV-inactivated *C. trachomatis* and assessed PD-L1 expression in the uterus by qRT-PCR 5 d postinfection. PD-L1 expression in mice infected with UV-inactivated *C. trachomatis* was similar to mock-infected mice, confirming that the increase in PD-L1 expression is dependent on infection with live *C. trachomatis* (Fig. 2B).

PD-L1 is expressed on a variety of cell types, such as dendritic cells (DCs), CD4$^+$ T cells, endothelial cells, and epithelial cells. Expression on different cell types can result in different PD-L1–signaling properties (24–26). Therefore, we wanted to examine the expression of PD-L1 on different cellular populations within the uterus and the iliac lymph nodes that drain the uterus. We infected mice transcervically with *C. trachomatis*, harvested uteri and dLNs at different time points during primary and secondary infection, and measured PD-L1 surface expression by flow cytometry. Five days after both primary and secondary infection, PD-L1 surface expression on uterine epithelial cells increased 5-fold in infected mice compared with uninfected mice (Fig. 2C, *upper left panel*, 2D). PD-L1 surface expression was also significantly higher on DCs in the dLNs 5 d after primary and secondary infection (Fig. 2D, *right panel*). However, 27 d after primary infection, during the memory phase, there was no difference in PD-L1 expression compared with uninfected mice (Fig. 2D, *middle panel*). These data demonstrate that PD-L1 is highly upregulated in uterine epithelial cells and dLN DCs during primary and secondary *C. trachomatis* infection, but this elevated expression is not maintained after primary infection is cleared.

Two receptors of PD-L1 have been described, PD-1 and B7-1, both of which can be expressed on T cells. Engagement of PD-L1 with either receptor on CD8$^+$ T cells can negatively regulate T cell function, and the upregulation of PD-1 on memory CD8$^+$ T cells is often used as a marker of T cell exhaustion (6, 27). Although neither receptor was upregulated during *C. trachomatis* infection of mice as measured by qRT-PCR (Fig. 2A), we reasoned that the qRT-PCR assay of the whole uterine tissue might not be sensitive enough to detect subtle differences in PD-1 and B7-1 expression on specific cell types. Therefore, we measured PD-1 and B7-1 surface expression by flow cytometry on different cell subsets during primary infection, during the memory phase, and following secondary infection. There were no differences in the expression of PD-1 or B7-1 in primarily infected mice compared with uninfected mice in any cell type that we examined (Fig. 2E, Supplemental Fig. 2). However, during the memory response, 27 d postinfection, both PD-1 and B7-1 surface expression were upregulated ~1.5-fold on CD8$^+$ T cells within the dLNs (Fig. 2E, Supplemental Fig. 2). There was also significant upregulation of B7-1 surface expression on CD8$^+$ T cells in the dLNs after the secondary infection. The PD-1 and B7-1 upregulation that we observed during the memory phase suggests that CD8$^+$ memory T cells become exhausted following primary *C. trachomatis* transcervical infection.

Finally, we wanted to understand whether PD-L1 upregulation was dependent on the surrounding tissues and the cytokine milieu. To test this, we infected a cultured thymic epithelial cell line with live *C. trachomatis* or UV-inactivated *C. trachomatis*, and we compared the expression of PD-L1 on these cells with uninfected control cells by flow cytometry 18 h later. Similar to our in vivo data, PD-L1 was highly expressed on cells infected with live *C. trachomatis* but not on cells infected with UV-inactivated *C. trachomatis* or uninfected cells (Fig. 2F). These data provide evidence suggesting that *C. trachomatis* may directly upregulate PD-L1 expression.

Taken together, these data demonstrate that PD-L1 expression is highly upregulated on uterine epithelial cells and DCs of the dLNs following *C. trachomatis* infection and provide evidence for the role of PD-L1 in the inhibition of the CD8$^+$ T cell response during *C. trachomatis* infection of the uterus.

PD-L1 and PD-1 contribute to control of C. trachomatis *burden in the uterus*

The significant upregulation of PD-L1 expression upon *C. trachomatis* infection led us to consider whether expression of PD-L1 may alter the clearance of *C. trachomatis*. To test this, we examined bacterial burden of WT mice or PD-L1–deficient mice 5 d after primary transcervical infection. PD-L1–deficient mice exhibited a 10-fold lower bacterial load compared with WT mice (Fig. 3A, Supplemental Fig. 3A). We also tested whether the loss of PD-L1 affected protection during secondary challenge. WT and PD-L1–deficient mice were transcervically infected with *C. trachomatis*, allowed to recover for 4 wk, and then reinfected transcervically. Bacterial levels 5 d after secondary infection were similar between WT and PD-L1–deficient mice (Fig. 3A). These data demonstrate that loss of PD-L1 improves bacterial clearance during primary infection, but it has no effect on the ability of the host to clear secondary infection.

Because PD-L1–deficient mice clear primary infection more efficiently, we sought to determine whether therapeutically blocking PD-L1 would also enhance bacterial clearance. To test this, we transiently blocked PD-L1 by administering anti–PD-L1 Ab 3 d prior and 2 d after primary infection. Five days after primary infection, we measured bacterial burden in the uterus of mice and observed that mice treated with anti–PD-L1 Ab showed a >2-log reduction in bacterial burden compared with mice treated with isotype Ab (Fig. 3B). Similar to results observed in PD-L1–deficient mice, mice that were treated with anti–PD-L1 Ab prior to primary infection, and then subsequently reinfected 4 wk later, exhibited no difference in bacterial burden compared with mice treated with isotype Ab (Fig. 3B). These data indicate that transient blockade of PD-L1 during primary infection is sufficient to improve bacterial clearance, and it does not affect bacterial burden during secondary infection. These data suggest that protection during secondary infection may be mediated by PD-L1–independent adaptive immune responses (Fig. 3B).

PD-L1 can signal through the receptors PD-1 and B7-1. To determine which receptor PD-L1 was acting through to limit efficient bacterial clearance, we compared bacterial levels in mice deficient for PD-1 to Thy1.1 (WT) mice 5 d after primary transcervical infection. PD-1–deficient mice had significantly lower bacterial burden compared with Thy1.1–deficient mice (Fig. 3C, Supplemental Fig. 3B). To determine whether PD-L1 also acts through the receptor B7-1, we treated mice 3 d before and 2 d after primary infection with an Ab to block B7-1 or an isotype-control Ab. Five days after transcervical infection, we measured bacterial burden in the uterus and determined that there was no significant

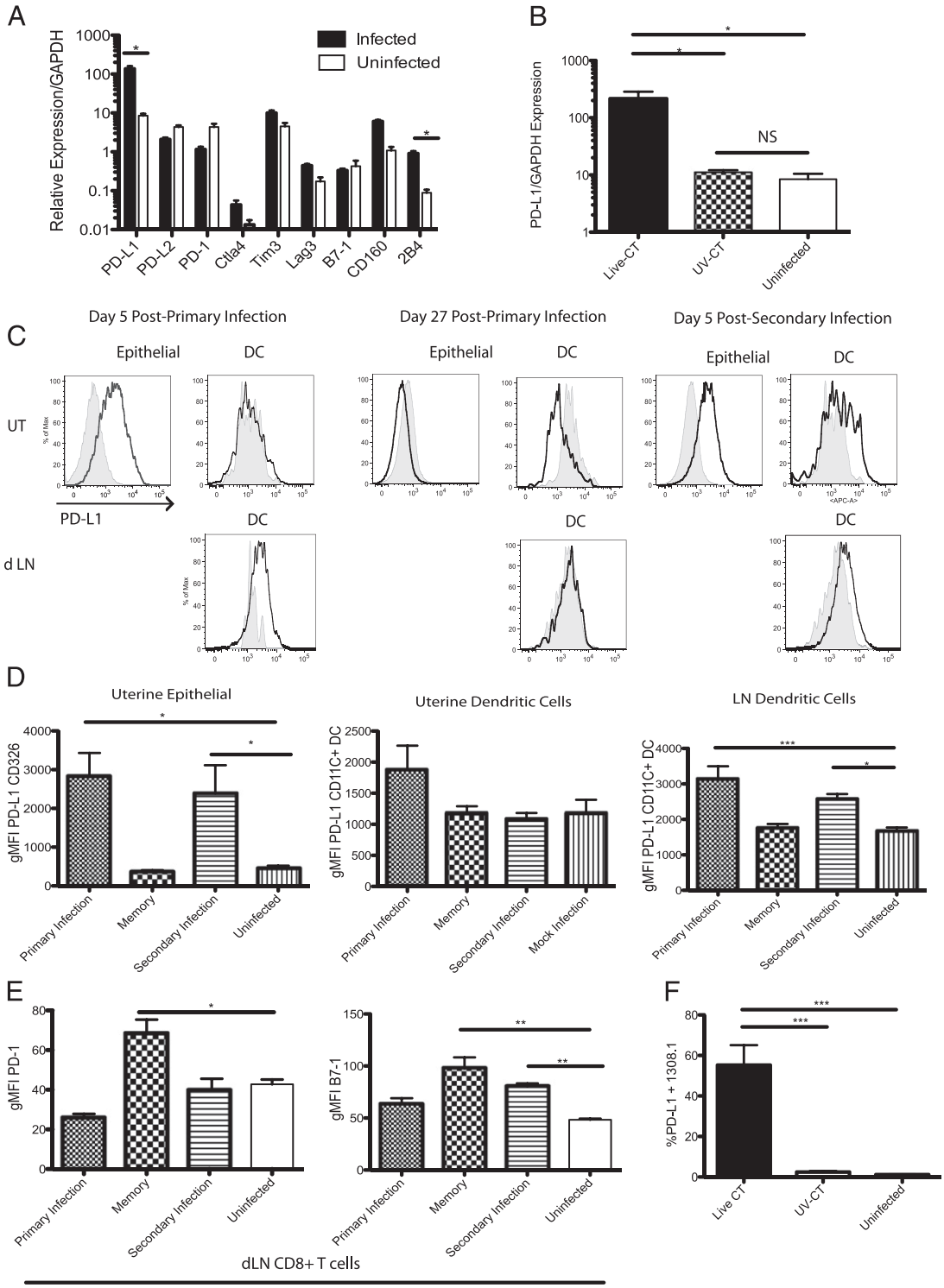

FIGURE 2. PD-L1 is highly expressed upon *C. trachomatis* infection. (**A**) Mice were transcervically infected with 10^6 IFU of *C. trachomatis*. RNA was extracted from uterine tissue 5 d postinfection, qRT-PCR was performed using primers specific for the indicated genes, and expression levels were compared with mock-infected mice. Bars represent the mean of five mice. Error bars indicate SEM. (**B**) Mice were infected with 10^6 IFU of live *C. trachomatis* or UV-inactivated *C. trachomatis.* RNA was extracted from uterine tissue 5 d postinfection, and qRT-PCR was performed to measure PD-L1 expression. Bars represent the mean of five mice. Error bars indicate SEM. (**C**) Mice were infected with 10^6 IFU of *C. Trachomatis*; at indicated time points postinfection, uteri (*upper panels*) and dLNs (*lower panels*) were examined for PD-L1 expression by flow cytometry. Epithelial cells were gated on live $CD326^+$ populations; DCs were gated on live $CD45.2^+CD11c^+CD4^-CD8^-$ populations. Representative data for PD-L1 expression are shown. The open graphs represent infected mice; the shaded graphs represent uninfected mice. (**D**) Average geometric mean fluorescence intensity (gMFI) of PD-L1 expression on uterine epithelial cells (*left panel*), uterine DCs (*middle panel*), and lymph node DCs (*right panel*) ($n = 5$ mice/time point). Error bars represent SEM. (**E**) gMFI of PD-1 expression (*left panel*) or B7-1 expression (*right panel*) on $CD3^+CD8^+$ T cells in the dLNs; shown is the average of five mice/time point, and error bars represent SEM. (**F**) 1308.1 thymic epithelial cells were infected at a multiplicity of infection of 1:1 with live *C. trachomatis* or UV-inactivated *C. trachomatis.* PD-L1 expression was measured by flow cytometry at 18 h postinfection. Shown is the mean of three independently infected wells; error bars indicate SEM. Data are representative of two independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, Mann–Whitney *U* test (**A**–**E**) or unpaired *t* test (**F**).

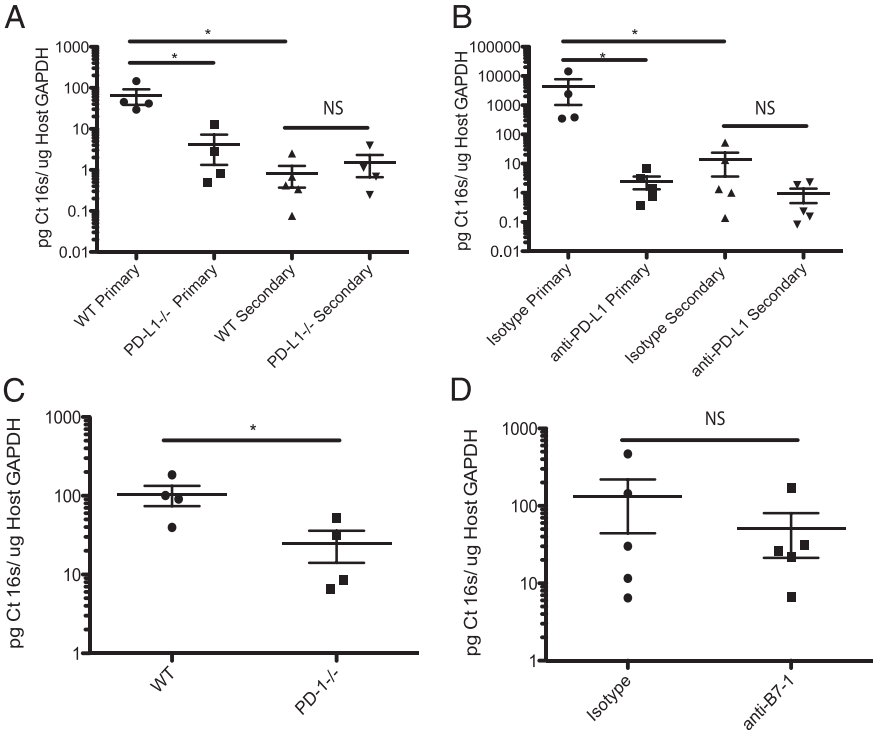

FIGURE 3. PD-L1 expression limits bacterial clearance. (**A**) WT and PD-L1–deficient mice were transcervically infected with 10^6 IFU of *C. trachomatis*. Five days after primary and secondary infection, bacterial levels in the uterus were measured by qPCR. Similar results were obtained in three independent experiments. (**B**) WT mice were treated with anti–PD-L1 Ab or isotype-control Ab 3 d prior to and 2 d after transcervical infection with 10^6 IFU. Bacterial levels were measured by qPCR 5 d after primary or secondary infection. Similar results were obtained in two independent experiments. (**C**) WT and PD-1–deficient mice were transcervically infected with 10^6 IFU of *C. trachomatis*. Bacterial levels in the uterus were measured by qPCR 5 d after primary infection. Similar results were obtained in two different experiments. (**D**) WT mice were treated with anti–B7-1 Ab or isotype control 3 d prior to and 2 d after transcervical infection with 10^6 IFU of *C. trachomatis*. The bacterial levels in the uterus were measured by qPCR 5 d after primary infection. Error bars represent SEM. Data are representative of two independent experiments. $*p < 0.05$, Mann–Whitney *U* test.

difference in bacterial levels in mice treated with anti-B7-1 Ab compared with mice treated with an isotype-control Ab (Fig. 3D). Together, these data suggest that PD-L1 acts through PD-1 and not B7-1 to inhibit the immune response in the uterus. Moreover, blockade or deletion of the interaction between PD-L1 and the receptor PD-1 leads to enhanced bacterial clearance.

$CD8^+$ T cells contribute to enhanced C. trachomatis *clearance in PD-L1–deficient mice*

PD-L1 and PD-1 signaling limits efficient *C. trachomatis* clearance from the genital tract. We hypothesized that the enhanced clearance in the absence of PD-L1/PD-1 signaling was due to an enhanced $CD8^+$ T cell response. To test this, we administered anti-CD8 or isotype-control Ab to PD-L1–deficient and WT mice 3 d before and 2 d after primary infection. We confirmed, by flow cytometry, a >100-fold decrease in $CD8^+$ T cells in the uterus, dLNs, and spleen of mice treated with the anti-CD8 Ab (Fig. 4B). We measured bacterial levels 5 d postinfection. $CD8^+$ T cell depletion in WT mice did not exacerbate bacterial burden compared with WT mice treated with control Ab (Fig. 4A, Supplemental Fig. 3C). As expected, PD-L1–deficient mice treated with control Ab showed significantly lower bacterial burden compared with WT mice treated with control Ab (Fig. 4A, Supplemental Fig. 3C). However, PD-L1–deficient mice treated with anti-CD8 Ab showed significantly higher bacterial levels compared with PD-L1–deficient mice treated with control Ab (Fig. 4A). Although $CD8^+$ T cells appear to be dispensable in WT mice, $CD8^+$ T cells contribute to the majority of *C. trachomatis* clearance observed in PD-L1–deficient mice. These data show that the enhanced bacterial clearance seen in PD-L1–deficient mice is mediated, in part, by an enhanced $CD8^+$ T cell response.

The PD-L1 pathway contributes to the inhibition of the uterine $CD8^+$ T cell response following transcervical C. trachomatis *infection*

Our data indicate that PD-L1 expression leads to an abrogated $CD8^+$ T cell response and results in the defect in bacterial clearance that we observed during primary infection (Fig. 4A). We hypothesized that PD-L1 signaling was responsible for altering the phenotype of the $CD8^+$ T cells. To test this, we assessed the phenotype of the $CD8^+$ T cells that developed during infection in WT and PD-L1–deficient mice. Upon priming and activation $CD8^+$ T cells can be broadly separated into two subsets: central memory T (Tcm) cells and effector memory T (Tem) cells (28, 29). These subsets are defined by the expression of the surface markers CD62L and CD127 (28–30). The lymph node–homing marker, CD62L, directs $CD8^+$ T cells to the lymphoid organs and is highly expressed on naive and Tcm cells but not on Tem cells (29). The IL-7R, CD127, is necessary for $CD8^+$ T cells to respond to IL-7, which promotes T cell development and persistence in the periphery. CD127 is highly expressed on central and effector memory subsets. To test whether PD-L1 expression affects the $CD8^+$ T cell phenotype during *C. trachomatis* infection, we transcervically infected WT and PD-L1–deficient mice and compared the Tem and Tcm populations by flow cytometry during primary infection (5 d after primary infection), memory phase (27 d after primary infection), or secondary infection (5 d after secondary infection). Five days after primary infection, we observed a 3-fold decrease in the percentage of Tcm cells ($CD62L^{high}/CD127^{high}$) in PD-L1–deficient mice compared with WT mice (Fig. 5A, 5B, *left panels*, Supplemental Fig. 4A). Additionally, there was a slight, but significant, increase in the percentage of Tem cells ($CD62L^{low}/CD127^{high}$) in the uteri of PD-L1–deficient mice (Fig. 5A, 5B, *left panels*). When we measured total numbers of Tem and Tcm cells in the uteri, there was a significant 3-fold increase in the ratio of Tem/Tcm cells in PD-L1–deficient mice compared with WT mice (Fig. 5C). The 3-fold increase in the ratio of Tem/Tcm cells was consistent when we compared PD-L1–deficient mice with WT mice during the memory phase (Fig. 5A–C, *middle panels*). These data indicate that PD-L1 expression skews the $CD8^+$ T cells toward a central memory phenotype during a primary *C. trachomatis* infection and that a loss of PD-L1 shifts $CD8^+$ T cells toward the effector memory phenotype.

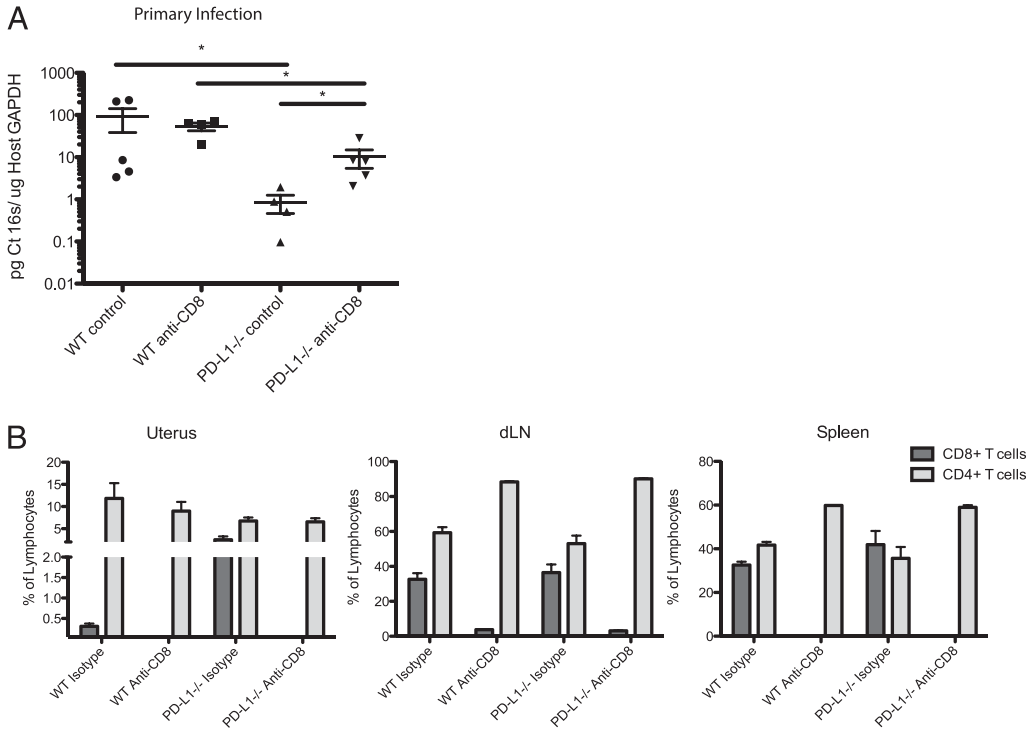

FIGURE 4. $CD8^+$ T cells contribute to protection in PD-L1–deficient mice. (**A**) WT and PD-L1–deficient mice were treated with anti-CD8–depleting Ab or isotype-control Ab 3 d prior to and 2 d after primary infection. Mice were infected with 10^6 IFU of *C. trachomatis*, and bacterial burden was measured in the uterus by qRT-PCR 5 d postinfection. (**B**) $CD8^+$ and $CD4^+$ T cell populations were measured by flow cytometry in the uteri, dLNs, and spleen of Ab-treated mice. Percentages of $CD8^+$ or $CD4^+$ T cells from $CD90.2^+$ lymphocytes are shown. Data are representative of two independent experiments. $*p < 0.05$, Mann–Whitney *U* test.

Next, we tested whether the shift toward the $CD8^+$ T cell effector memory phenotype in PD-L1–deficient mice would affect functions such as Ag-specific T cell abundance or IFN-γ production. Although PD-L1 deficiency did not alter secondary bacterial clearance, we hypothesized that the altered memory $CD8^+$ T cell phenotype would affect the secondary $CD8^+$ T cell response. Therefore, we assessed whether the absence of PD-L1 would restore the magnitude of the secondary $CD8^+$ T cell response that is blunted in WT *C. trachomatis*–infected mice. Five days after secondary infection, we observed a significant 1.5-fold increase in the number of *C. trachomatis*–specific $CD8^+$ T cells in the genital tract of PD-L1–deficient mice compared with WT mice (Fig. 5D, Supplemental Fig. 4B). Moreover, we observed that the number of *C. trachomatis*–specific $CD8^+$ T cells in PD-L1–deficient mice was significantly higher in secondary infection compared with primary infection (Fig. 5D). These data suggest that the absence of PD-L1 signaling results in memory $CD8^+$ T cells that are able to expand to greater numbers upon rechallenge or are more efficiently recruited to the genital tract.

The second functional consequence of PD-L1 deficiency that we assessed was the expression of IFN-γ, a cytokine critical for restricting *C. trachomatis* growth. We compared the ability of endogenous uterine $CD8^+$ T cells to produce IFN-γ in WT and PD-L1–deficient mice. In comparison with WT mice, we observed, by flow cytometry, that PD-L1–deficient animals had a significant increase in the number of IFN-γ–producing $CD8^+$ T cells during both primary and secondary responses (Fig. 5E, Supplemental Fig. 4C). This suggests that PD-L1 deficiency leads to enhanced IFN-γ production by $CD8^+$ T cells during both primary and secondary infection.

$CD8^+$ T cells contribute to secondary protection in the absence of PD-L1 signaling

Although the enhanced $CD8^+$ T cell recall response that we observed (Fig. 5) could potentially contribute to bacterial clearance, PD-L1 deficiency did not lead to improved *C. trachomatis* clearance during secondary infection (Fig. 3). We reasoned that it is possible that any secondary protection afforded by the enhanced $CD8^+$ T cells in PD-L1–deficient mice would be masked by the even more efficient $CD4^+$ T cell response. To decouple secondary protection mediated by $CD4^+$ T cells from protection via $CD8^+$ T cells, we depleted $CD4^+$ T cells prior to secondary challenge in WT and PD-L1–deficient mice. Mice were transcervically infected and examined for bacterial burden and T cell counts 5 d later. As expected, depletion of the $CD4^+$ T memory pool in WT mice led to exacerbated bacterial burden (Fig. 6A, Supplemental Fig. 4C). However, $CD4^+$ T cell depletion in PD-L1–deficient mice did not lead to exacerbated bacterial burden as in WT mice; in fact, there were significantly lower bacterial levels in PD-L1–deficient mice treated with anti-CD4 Ab compared with WT mice treated with anti-CD4 Ab (Fig. 6A). Thus, $CD4^+$ T cells are not necessary for protection against secondary challenge in PD-L1–deficient mice. These data also suggest that $CD8^+$ T cells can compensate for a lack of $CD4^+$ T cells during secondary infection in PD-L1–deficient mice.

We hypothesized that the enhanced secondary clearance that we observed in PD-L1–deficient mice was due to a superior $CD8^+$ T cell population. To determine whether Ag-experienced $CD8^+$ T cells from WT and PD-1–deficient mice were distinguishable in their ability to protect mice against infection, we collected $CD8^+$ T cells from the spleen, lymph nodes, and genital tracts of previously infected WT and PD-1–deficient mice (Fig. 6B). We then transferred equal numbers of purified $CD8^+$ T cells into groups of naive congenic IFN-γ–knockout mice, so that the only source of IFN-γ was from the transferred $CD8^+$ T cells. One day after $CD8^+$ T cell transfer, we transcervically infected recipient mice with *C. trachomatis* (Fig. 6B). We measured the quantity of $CD8^+$ T cells in the dLNs and uteri 5 d postinfection. Cells from both WT and PD-1–deficient mice were able to migrate to the uterus and the

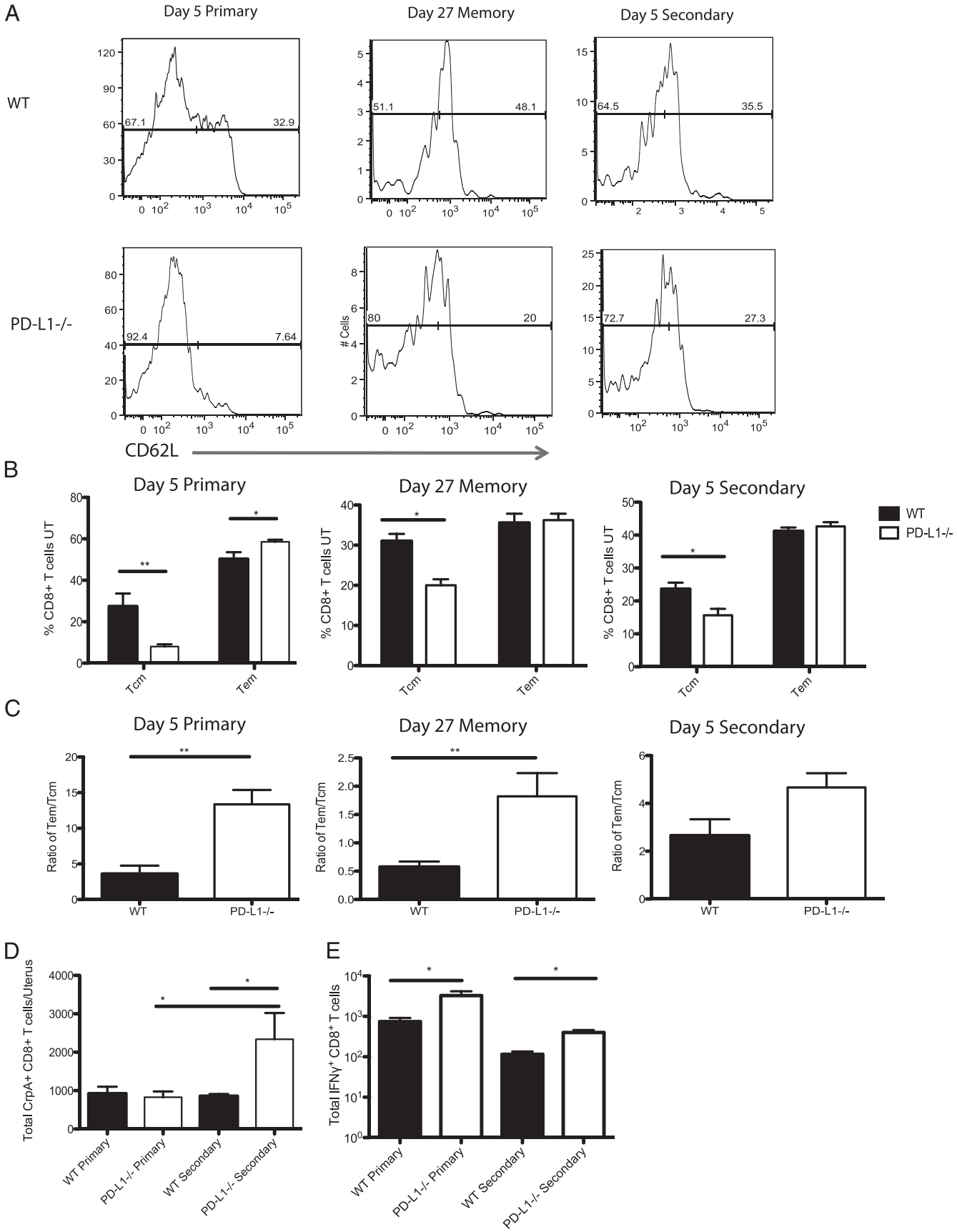

FIGURE 5. PD-L1 expression shifts the ratio of Tem/Tcm CD8$^+$ T cells during *C. trachomatis* infection. (**A**) WT and PD-L1–deficient mice were transcervically infected with 10^6 IFU of *C. trachomatis*. At the indicated time points, uteri were harvested and stained for CD62L and CD127 expression. CD8$^+$ memory populations were gated on live, CD90.2$^+$CD127$^+$CD4$^-$CD8$^+$ cells; graphs of CD62L expression of this population are shown. (**B**) Quantification of uterine Tem cells (CD127^{high}/CD62L^{low}) and Tcm cells (CD127^{high} /CD62L^{high}) at time points indicated in WT and PD-L1–deficient mice. Bars represent mean of four or five mice/group. (**C**) Ratio of the absolute numbers of Tem/Tcm CD8$^+$ T cells found in (*Figure legend continues*)

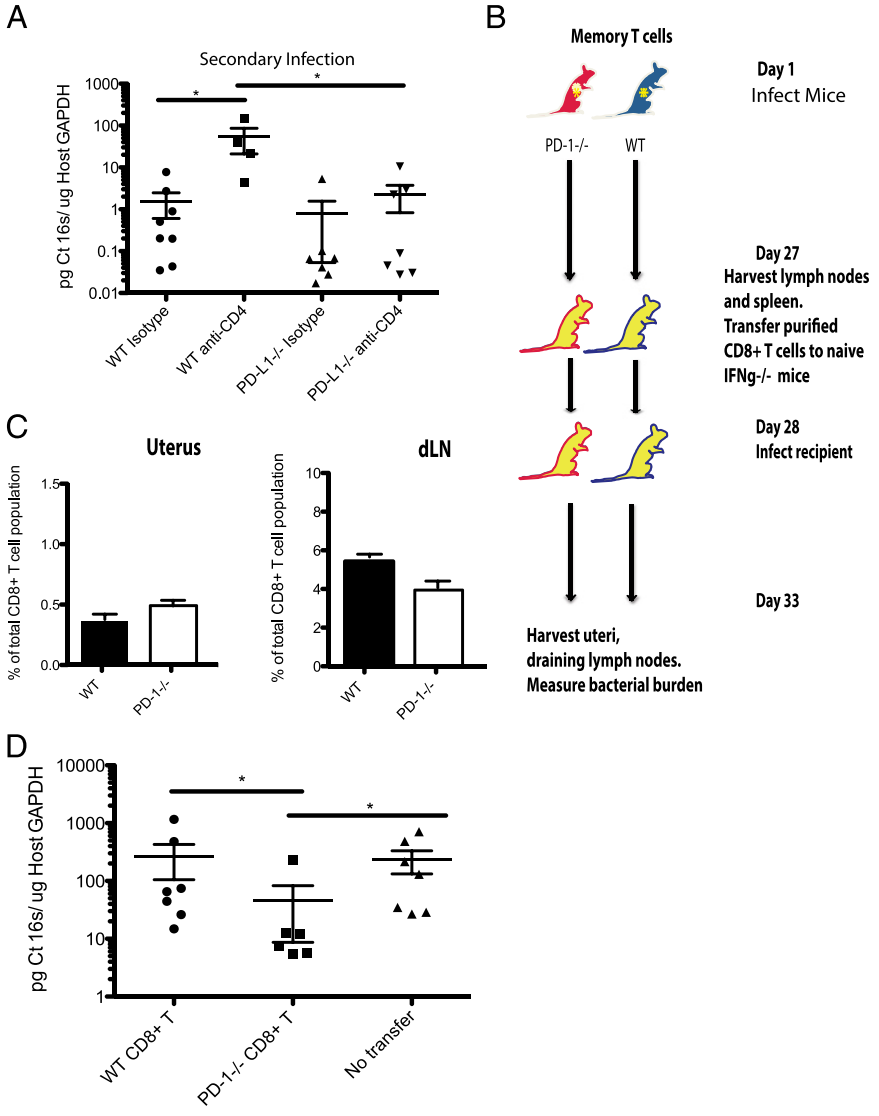

FIGURE 6. Ag-experienced CD8$^+$ T cells confer superior protection in the absence of PD-L1/PD-1 signaling. (**A**) WT and PD-L1–deficient mice were treated with isotype-control Ab or anti-CD4 Ab 3 d prior to and 2 d after secondary infection. Bacterial burden was measured in the uterus by qRT-PCR 5 d after secondary infection. (**B**) Schematic diagram of the experimental design of the T cell–transfer experiment; CD8$^+$ T cells were isolated from previously infected (*C. trachomatis*) WT and PD-1–deficient mice. Isolated CD8$^+$ T cells were then transferred to naive IFN-γ–knockout mice. One day after T cell transfer, recipient mice were transcervically infected with 10^6 IFU of *C. trachomatis*. Five days postinfection, bacterial burden and T cell recruitment were measured. (**C**) Transferred CD8$^+$ T cell recruitment was measured in the uteri and dLNs of recipient mice. (**D**) Bacterial burden in the uterus was measured by qRT-PCR in recipient mice 5 d after *C. trachomatis* transcervical infection. Data are representative of two independent experiments. $*p < 0.05$, $**p < 0.01$, Mann–Whitney *U* test.

dLNs of the recipient mice (Fig. 6C). We recovered almost equal percentages of WT and PD-1–deficient CD8$^+$ T cells in the uterus and dLNs (Fig. 6C). When we measured bacterial levels in the genital tract of infected recipient mice, we found that mice receiving CD8$^+$ T cells from previously infected WT mice had bacterial levels similar to mice that received no T cells. However, mice receiving CD8$^+$ T cells from previously infected PD-1–deficient mice had significantly lower bacterial levels compared with mice that received WT CD8$^+$ T cells (Fig. 6D, Supplemental Fig. 4D). Taken together, our data provide significant evidence that *C. trachomatis* infection severely impairs the CD8$^+$ T cell response via the PD-1/PD-L1 immunoinhibitory pathway, and disruption of the PD-1/PD-L1 pathway restores the protective capacity of these T cells.

PD-L1 expression is required on both hematopoietic and nonhematopoietic cells to inhibit protection against C. trachomatis

PD-L1 can be expressed on a variety of cell types, including DCs, T cells, endothelial cells, and epithelial cells. We sought to determine which of the cell populations expressing PD-L1 influenced the shift in the CD8$^+$ T cell response to a more central memory response. It seemed likely that that PD-L1 expression on APCs would be needed to bias the CD8$^+$ T cell response toward the central memory phenotype during priming in WT mice. To test this, we created reciprocal bone marrow chimeras from WT and PD-L1–deficient mice. After ensuring successful chimerism ≥ 96%, we infected mice transcervically and analyzed the CD8$^+$ T cell

genital tracts of WT and PD-L1–deficient mice. Bars represent mean data from four or five mice/group. (**D**) Five days after primary or secondary transcervical challenge, the number of *Chlamydia*-specific CD8$^+$ T cells was quantified in the genital tracts of WT and PD-L1–deficient mice using a tetramer specific for the *Chlamydia* Ag CrpA. Bars represent the mean of four or five mice/group with SEM. (**E**) Total numbers of IFNγ$^+$CD8$^+$ T cells in the uterus 5 d after primary or secondary transcervical infection of WT and PD-L1–deficient mice. Data are the mean of five mice/group. Mice were treated with anti–PD-L1–blocking Ab or isotype-control Ab 2 d prior and 2 d after primary transcervical infection with 10^6 IFU of *C. trachomatis*. Mice were allowed to recover for 4 wk, at which point they were rechallenged transcervically with 10^6 IFU of *C. trachomatis*. Five days after rechallenge, the ratio of CD8$^+$ Tem/Tcm cells was determined in the genital tract. Data are representative of at least two independent experiments. $*p < 0.05$, $**p < 0.01$, Mann–Whitney *U* test.

response 5 d later. As expected, PD-L1–deficient mice that received PD-L1–deficient bone marrow had a significantly higher ratio of Tem/Tcm cells in the uterus compared with WT mice that received WT bone marrow (Fig. 7A, 7B). WT mice that received PD-L1–deficient bone marrow also had a significantly higher ratio of Tem/Tcm cells compared with WT mice that received WT bone marrow. However, the shift toward an effector memory phenotype was still significantly lower than that observed in PD-L1–deficient mice that received PD-L1–deficient bone marrow (Figs. 6B, 7A). PD-L1–deficient mice that received WT bone marrow had a similar ratio of Tem/Tcm cells in the uterus compared with WT mice that received WT bone marrow (Fig. 7A, 7B). Overall, these data indicate that PD-L1 expression on hematopoietic cells largely influences the $CD8^+$ T cell response and drives $CD8^+$ T cells to a more central memory phenotype during *C. trachomatis* infection. However, the absence of PD-L1 is required on both hematopoietic and nonhematopoietic cells to fully recapitulate the $CD8^+$ T cell response observed in PD-L1–deficient mice.

The absence of PD-L1 expression on hematopoietic cells alone was able to promote a $CD8^+$ T cell response that closely resembled that of PD-L1–deficient mice; however, it was unclear whether it was sufficient to decrease bacterial burden. Therefore, we measured bacterial burden in the chimeric mice 5 d postinfection. As shown in Fig. 7D, the absence of PD-L1 on either hematopoietic or nonhematopoietic cells alone was not enough to confer protection against *C. trachomatis.* Therefore, these data suggest that the expression of PD-L1 on both hematopoietic and nonhematopoietic cells contributes to limiting *C. trachomatis* clearance.

Discussion

In this study, we sought to identify mechanisms that contribute to the defective $CD8^+$ T cell response to *C. trachomatis*. We hypothesized that one or more inhibitory mechanisms may be upregulated during infection that would affect the quality of the $CD8^+$ T cell response. We found that PD-L1 is strongly upregulated during *C. trachomatis* infection. Because PD-L1 plays a role in T cell exhaustion, we tested whether PD-L1 affects the $CD8^+$ T cell response during *C. trachomatis* infection. Our data demonstrate that PD-L1 expression alters the $CD8^+$ T cell memory phenotype and impairs the effector function of $CD8^+$ T cells, thus limiting their ability to restrict *C. trachomatis* replication.

The PD-1/PD-L1 pathway is a critical T cell regulatory pathway that is important in maintaining the balance between immune tolerance and effector function. In some pathogen models, engaging PD-1 on T cells can have detrimental effects on pathogen elimination. For example, infection with lower respiratory viruses, such as influenza A virus or metapneumovirus, induces PD-L1 expression on lung epithelial cells, which leads to $CD8^+$ T cell impairment. Blockade of PD-L1 reverses $CD8^+$ T cell impairment, improves IFN-γ production, and enhances viral clearance (31). In cancer models, contact of $CD8^+$ T cells with $PD\text{-}L1^+$ tumor cells results in a reduction of the cytolytic activity of the $CD8^+$ T cells and an impaired ability to clear the tumor cells (32). For bacterial pathogens, the role of PD-1/PD-1 is less clear. PD-L1 blockade in mice infected with *Listeria monocytogenes* impaired the $CD8^+$ T cell response and led to exacerbated bacterial burden, indicating that PD-L1 may act as a stimulatory signal in this context (33, 34). However, blockade of PD-L1 on monocytes infected with *Staphylococcus aureus* improved IL-2 production by $CD8^+$ T cells (35). Interestingly, our data indicate that blockade of PD-L1 improves the IFN-γ response of $CD8^+$ T cells during *C. trachomatis* infection, leading to the enhanced bacterial clearance that is observed during primary infection.

A major source of variability in previous studies has been the anatomical site of infection, which may give rise to the diversity of effects attributed to the PD-1/PD-L1 pathway. Some anatomic sites need protection from host immune responses, and inhibitory mechanisms may be necessary to limit inflammation damaging to the host. In the respiratory tract, for example, inhibitory PD-L1 signaling properly restricts T cells to avoid inflammation that could damage the lungs. The genital tract is also a privileged immune site, because excessive inflammation could result in reduced fertility. During *C. trachomatis* infection, PD-L1 expression may allow the host to avoid an exaggerated and damaging $CD8^+$ T cell response. In fact, evidence suggests that $CD8^+$ T cells contribute to the uterine pathology that results from *C. trachomatis* infection (36, 37). One recent study (23) demonstrated that blockade of PD-L1 in combination with the inhibitory receptor, Tim3, led to enhanced uterine pathology of mice infected with *Chlamydia muridarum*. Although it was unclear from that study what factors mediate the enhanced pathology, it provides evidence that immune-inhibitory pathways are important for limiting inflammation and pathology in the genital tract. Additional in-depth pathology studies will be required to understand whether the lack of PD-L1 alone leads to enhanced pathology in the genital tract during *C. trachomatis* infection. It is possible that multiple inhibitory pathways are engaged during *C. trachomatis* infection to avoid inflammation in the host, and deleting one of these pathways may not be sufficient to observe changes in gross pathology. We observed upregulated expression of other inhibitory molecules, 2B4 and CD160, in infected mice, and it will be interesting to investigate whether these molecules also contribute to reducing pathology and immune impairment during *C. trachomatis* infection.

Although upregulation of the PD-1/PD-L1 pathway may be important in limiting damage to the host, there is no doubt that impairing the $CD8^+$ T cell response would also be beneficial to the invading pathogen. Although $CD4^+$ T cells are the main mechanism of *C. trachomatis* clearance during natural infection, it was shown that *C. trachomatis* can be susceptible to the $CD8^+$ T cell response when these T cells are stimulated by vaccination or passively transferred (3). Therefore, *C. trachomatis* may benefit from mechanisms to subvert the $CD8^+$ T cells. The evidence presented in this article indicates that *C. trachomatis* is able to limit expansion and/or recruitment of $CD8^+$ T cells in the genital tract. Our data suggest that *C. trachomatis* is able to limit recruitment of secondary $CD8^+$ T cells to the genital tract by skewing the $CD8^+$ T cell response to a central memory phenotype. In fact, other studies (38) demonstrated that human papillomavirus excludes $CD8^+$ T cells from the genital epithelium by skewing $CD8^+$ T cells toward a central memory phenotype; however, additional experiments will investigate whether this hypothesis holds true for *C. trachomatis*. Future experiments will also be needed to determine whether other inhibitory mechanisms contribute to the impaired $CD8^+$ T cell response during *C. trachomatis* infection, because we have no evidence to suggest that PD-1/PD-L1 deficiency alone provides an optimum $CD8^+$ T cell response.

Our data illuminate one mechanism by which the $CD8^+$ T cell response is impaired during *C. trachomatis* infection. During the primary response to *C. trachomatis*, it is clear that PD-L1 expression limits the number of IFN-γ–producing $CD8^+$ T cells present in the uterus. *C. trachomatis* is highly susceptible to IFN-γ, and the additional IFN-γ–producing $CD8^+$ T cells in PD-L1–deficient mice may explain the enhanced clearance that we observe during primary infection. This is consistent with our data demonstrating that bacterial clearance during primary infection of PD-L1–deficient mice is dependent on $CD8^+$ T cells (Fig. 4). PD-

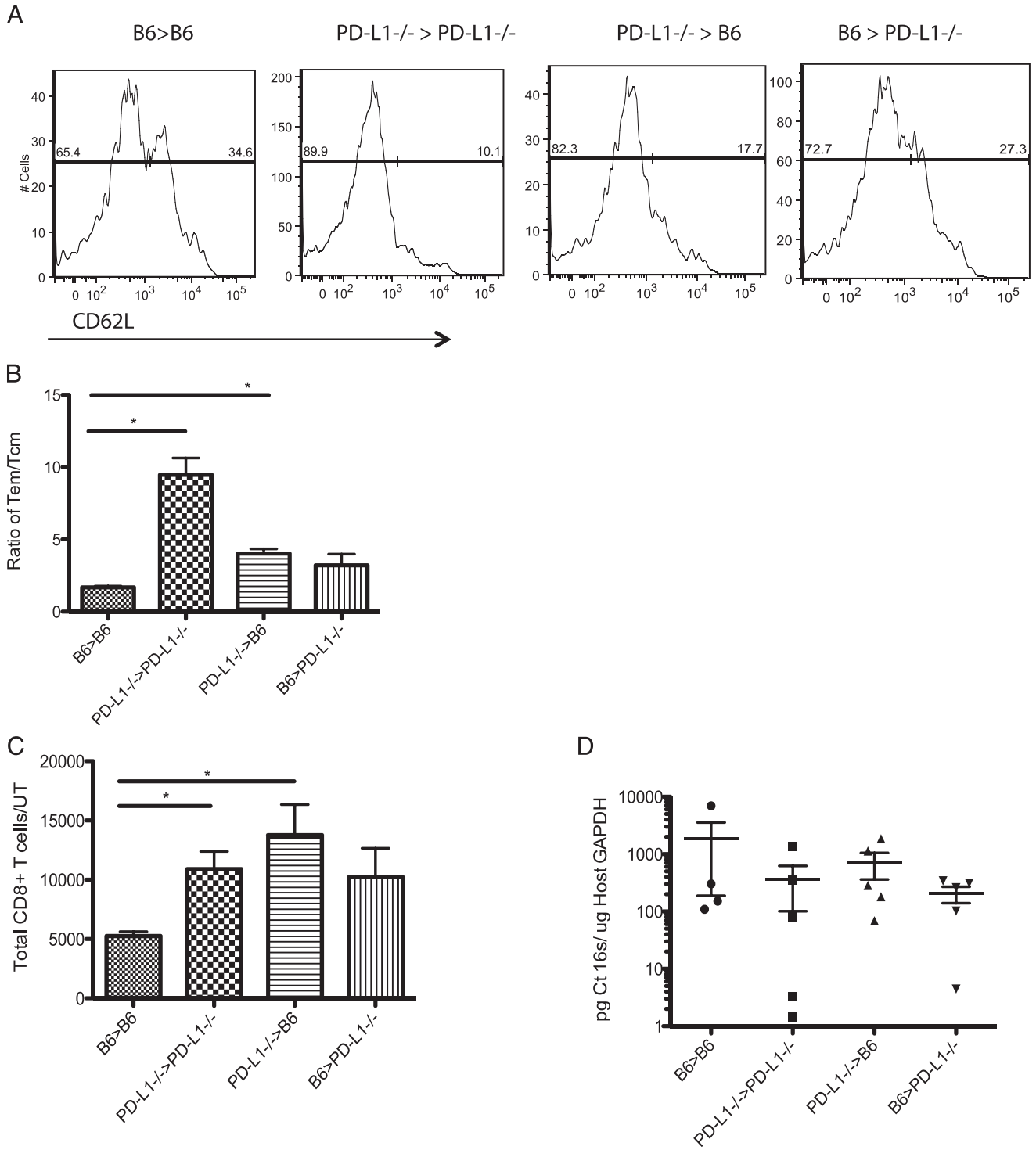

FIGURE 7. PD-L1 expression on hematopoietic and nonhematopoietic cells contributes to PD-L1 deficiency phenotype. (**A**) WT and PD-L1–deficient reciprocal chimeric mice were transcervically infected with 10^6 IFU of *C. trachomatis*. The uteri were harvested 5 d postinfection. The memory $CD8^+$ population was gated on live $CD90^+CD4^-CD127^+CD8^+$ T cells. Graphs of CD62L expression of each population are shown. (**B**) Ratio of effector/central memory $CD8^+$ T cells found in the uteri of chimeric mice. (**C**) Total number of $CD8^+$ T cells measured in the uterus 5 d postinfection. (**D**) Bacterial burden in the uterus was measured by qPCR in recipient mice 5 d after transcervical infection. $*p < 0.05$ determined by Mann–Whitney *U* test.

L1 expression during primary infection skews the memory $CD8^+$ T cell response toward a Tcm phenotype in the genital tract, and this may be one way to limit the number of IFN-γ–producing $CD8^+$ T cells in the genital tract. When Tem and Tcm cells were first described in the literature, data indicated that the Tcm cells were the more effective subset in terms of expansion and clearance capabilities (39). However, further studies (40) demonstrated that the contribution of Tcm and Tem cells to pathogen control depends on the particular pathogen and the site of infection. For pathogens that replicate in lymphoid organs, Tcm cells are required for controlling pathogen burden. For example, Tcm cells are more efficient than Tem memory $CD8^+$ T cells at lysing LCMV-infected cells (41). However, for mucosal infections, Tcm $CD8^+$ T cells are less effective because they are more confined to

lymphoid organs and slow to respond to peripheral challenge. In contrast, Tem cells reside in the tissue and, thus, are closer to the site of the pathogen upon reinfection; therefore, they can immediately engage the pathogen and limit its replication (42–44). PD-L1 expression during *C. trachomatis* infection results in a reduction in the number of effector memory, IFN-γ–producing $CD8^+$ T cells and results in incomplete bacterial clearance.

Importantly, our data show that $CD8^+$ T cells generated during *C. trachomatis* infection in the absence of PD-1/PD-L1 signaling are able to provide superior protection compared with memory cells from WT mice. Although the major difference between Tcm and Tem cells may be in their capacity to migrate to peripheral tissues, our data indicate that there is a functional difference as well, which is mediated by the PD-1/PD-L1 interaction. As such, these data provide an interesting model that could explain how PD-L1 deficiency alters the $CD8^+$ T cell response to improve bacterial clearance during both primary and secondary infections. During primary infection, PD-L1 expression on epithelial cells may directly protect these cells from cytolytic killing by $CD8^+$ T cells by disengaging the $CD8^+$ T cell. Alternatively, when $CD8^+$ T cells are engaged by PD-L1, it may cause transcriptional changes in the $CD8^+$ T cell that lead to the Tcm phenotype and a dampening of the cytolytic response. The interaction with PD-L1–expressing epithelial cells leads to inefficient bacterial clearance during primary infection, which can be reversed by PD-L1 deficiency or blockade. In the absence of PD-L1, $CD8^+$ T cells have an enhanced ability to recognize and engage infected cells or have an improved cytolytic capacity that contributes to bacterial clearance. Future experiments will test the cytolytic response of $CD8^+$ T cells to infected cells in vitro, with and without PD-L1 blockade, to investigate whether $CD8^+$ T cell cytokine production is reduced in the presence of PD-L1.

Once engaged by PD-L1, the $CD8^+$ T cell may be headed down a transcriptional course that results in the increased expression of PD-1 and the development of the Tcm phenotype. PD-1 expression indicates that $CD8^+$ T cells may be exhausted as infection is cleared and memory is established, thus T cells are more prone to apoptosis and have a decreased ability to produce cytokines. Furthermore, the PD-L1/PD-1 interaction drives $CD8^+$ T cells to a Tcm phenotype, which further disables the secondary response by sequestering Ag-experienced $CD8^+$ T cells from the genital tract. Upon reinfection, the secondary $CD8^+$ T cells that are able to migrate to the genital tract have lost the capacity to produce IFN-γ and other inflammatory cytokines; thus, these $CD8^+$ T cells do not contribute to secondary bacterial control. During secondary infection, adaptive immune responses other than $CD8^+$ T cells are able to quickly control infection. However, in the absence of PD-L1, $CD8^+$ T cells become sufficient for controlling infection. Future studies will investigate the transcriptional changes that occur in $CD8^+$ T cells following *C. trachomatis* infection in the presence and absence of PD-L1. It will be interesting to test whether the transcriptional profile of memory $CD8^+$ T cells from *C. trachomatis* is similar to what has been described for exhausted $CD8^+$ T cells in other models (45).

Finally, these data provide insight into peripheral $CD8^+$ T cell responses in general. Unlike systemic infections, an efficient secondary $CD8^+$ T cell response in the periphery is defined by the effector phenotype of the $CD8^+$ T cell population and not by their capacity to greatly expand. In this study, we provide evidence that the PD-1/PD-L1 pathway is an important factor in regulating peripheral $CD8^+$ T cell responses by skewing the $CD8^+$ T cells to a more central memory phenotype, ultimately impairing the ability of these $CD8^+$ T cells to respond upon rechallenge. Little is known about the development of Tcm and Tem cells in the genital tract. We show that the Tem cell subset can be an important memory population in the genital tract during *C. trachomatis* infection. Mechanisms to promote the expansion and retention of effector memory $CD8^+$ T cells, whether by inhibiting PD-L1 or by other means, will be an important concept in the design of *Chlamydia* vaccines. An effective *C. trachomatis* vaccine may have to overcome inhibition mediated by PD-L1 to improve the $CD8^+$ T cell response without causing immunopathologies in the genital tract.

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Disclosures

The authors have no financial conflicts of interest.

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