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Innate IFN-γ Is Essential for Programmed Death Ligand-1–Mediated T Cell Stimulation following Listeria monocytogenes Infection

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Although best characterized for sustaining T cell exhaustion during persistent viral infection, programmed death ligand-1 (PDL-1) also stimulates the expansion of protective T cells after infection with intracellular bacterial pathogens. Therefore, establishing the molecular signals that control whether PDL-1 stimulates immune suppression or activation is important as immune modulation therapies based on manipulating PDL-1 are being developed. In this study, the requirement for PDL-1 blockade initiated before infection with the intracellular bacterium Listeria monocytogenes in reducing pathogen-specific T cell expansion is demonstrated. In turn, the role of proinflammatory cytokines triggered early after L. monocytogenes infection in controlling PDL-1–mediated T cell stimulation was investigated using mice with targeted defects in specific cytokines or cytokine receptors. These experiments illustrate an essential role for IL-12 or type I IFNs in PDL-1–mediated expansion of pathogen-specific CD8⁺ T cells. Unexpectedly, direct stimulation by neither IL-12 nor type I IFNs on pathogen-specific CD8⁺ cells was essential for PDL-1–mediated expansion. Instead, the absence of early innate IFN-γ production in mice with combined defects in both IL-12 and type I IFNR negated the impacts of PDL-1 blockade. In turn, IFN-γ ablation using neutralizing Abs or in mice with targeted defects in IFN-γR each eliminated the PDL-1–mediated stimulatory impacts on pathogen-specific T cell expansion. Thus, innate IFN-γ is essential for PDL-1–mediated T cell stimulation. The Journal of Immunology, 2012, 189: 000–000.
Materials and Methods

Mice

C57BL/6 (B6) (CD45.2<sup>+</sup>C90.2<sup>+</sup>; H-2<sup>Kb</sup>), Ly5.2 (CD45.1<sup>+</sup>C90.2<sup>+</sup>; H-2<sup>Kb</sup>), and C90.1 (CD45.2<sup>+</sup>C90.1<sup>+</sup>; H-2<sup>Kb</sup>) mice were purchased from The Jackson Laboratory or National Cancer Institute and used between 6 and 8 wk of age. Mice with targeted defects in PD-1, IL-12<sup>−</sup>, type I IFN<sub>R</sub>−, and PD-1<sup>−</sup> mice with combined defects in IL-12p40 and type I IFN<sub>R</sub> each on the B6 background have been described previously (26–31). OT-1 TCR transgenic mice containing CD8<sup>+</sup> cells with specificity to the OVA<sub>257−264</sub> H-2<sup>Kb</sup>-peptide were intercrossed with wild-type (WT) mice (C90.1), mice with combined defects in both IL-12 and type I IFN<sub>R</sub> (C90.2), or mice with targeted defects in IFN-γ<sup>−R</sup>- (C90.2) (32, 33). All experiments were performed under University of Minnesota Institutional Animal Care and Use Committee-approved protocols.

L. monocytogenes infection

Recombinant L. monocytogenes-OVA stably expresses OVA protein allowing the immune response to this surrogate L. monocytogenes Ag to be tracked using established cellular immunology tools (34). To bypass differences in susceptibility to virulent L. monocytogenes-OVA for mice with defects in PD-1, specific cytokines, and/or cytokine receptors, an attenuated L. monocytogenes-OVA strain containing targeted defects in actA that prevents intracellular and intercellular spread was used (14, 15, 24, 26, 35–39). The highly attenuated nature of L. monocytogenes ΔactA results in a nonproductive infection that is rapidly eliminated even in mice lacking cytokines such as IL-12 and IFN-γ required for innate resistance against virulent L. monocytogenes and normalizes the pathogen burden in IL-12 and type I IFN<sub>R</sub>-deficient mice where even relatively high inocula are eliminated with similar kinetics compared with control mice (26, 35, 40, 41). Similarly for anti–PD-1 Ab-treated mice, L. monocytogenes ΔactA is eliminated within the first 72 h postinfection with kinetics identical to mice treated with isotype control Ab (15). For infection, L. monocytogenes-OVA ΔactA was grown to early log phase (OD<sub>600</sub> 0.1) in brain–heart infusion media at 37°C, washed, and diluted with saline to a concentration of 10<sup>6</sup> CFUs per 200 μL, and this inoculum was used for i.v. injection as described previously (15).

Abs and flow cytometry

Fluorophore-conjugated Abs and other reagents for flow cytometry were purchased from BD Biosciences (San Jose, CA) or eBioscience (San Diego, CA). For PD-1 blockade, anti-mouse PD-1 (10F10G2) or rat IgG<sub>2b</sub> (LTF2) Abs were purchased from BioXCell (West Lebanon, NH) and injected i.p. 1 d before (500 μg/mouse) and 4 d postinfection (250 μg/mouse) or in separate mice beginning only 4 d postinfection (500 μg/mouse) (5, 15). For IFN-γ neutralization, anti-mouse IFN-γ (XM1G1.2) or rat IgG1 (HRPN) Abs (BioXCell) were injected i.p. 1 d prior to infection (1.0 mg/ mouse). L. monocytogenes-OVA specific CD8<sup>+</sup> T cells were identified among endogenous splenocytes by staining with H-2K<sup>b</sup> dimer X (BD Biosciences) loaded with OVA<sub>257−264</sub> peptide as described previously (15, 35). For cytokine production by Ag-specific CD8<sup>+</sup> cells, splenocytes isolated directly ex vivo were cultured in 96-well round-bottom plates (5 × 10<sup>4</sup> cells/ml) and stimulated with either OVA<sub>257−264</sub> peptide (10<sup>−6</sup> M) or no stimulation in media supplemented with brefeldin A (GolgiPlug; BD Biosciences) at 37°C for 5 h as described previously (15, 26, 35). For innate cytokine production, splenocytes isolated directly ex vivo within the first 12, 24, and 48 h postinfection were stained using anti–IFN-γ Ab without additional stimulation. For enumerating PDL-1 and PD-1 expression, splenocytes resuspended in saline supplemented with albumin (1%) and Fc block (anti-CD16/32) were cell surface stained using anti–CD11c (clone N418), anti-CD8 (clone 53.6.7), anti–PDL-1 (clones 2D11c, 2D11h), and anti–PD-1 (clone 2D343) Abs.

Adoptive cell transfer

For cell transfers, 1 × 10<sup>7</sup> CD8<sup>+</sup> cells from donor WT OT-1 (CD45.2<sup>+</sup>C90.1<sup>+</sup>) mice were mixed at a 1:1 ratio with CD8<sup>+</sup> cells from either IL-12<sup>−</sup>-, type I IFN<sub>R</sub>-deficient OT-1 (CD45.2<sup>+</sup>C90.2<sup>+</sup>), or IFN-γ<sup>−R</sup>-deficient OT-1 (CD45.2<sup>+</sup>C90.2<sup>+</sup>) TCR transgenic mice and i.v. transferred into recipient (CD45.1<sup>+</sup>) mice 1 d prior to infection. For tracking the expansion of endogenous OVA-specific T cells, one mouse equivalent of CD8<sup>+</sup> cells (~10<sup>5</sup> cells) purified by negative selection from PD-1<sup>−</sup>-deficient (C90.2) mice were adoptively transferred into WT C90.1 mice treated with anti–PDL-1 or isotype control Abs and infected with L. monocytogenes-OVA ΔactA the following day.

Statistics

The number and percent cells were first analyzed and found to be normally distributed, and thereafter, differences between groups were evaluated using the Student t test with p ≤ 0.05 taken as statistical significance (Prism software; GraphPad).

Results

PDL-1 blockade initiated prior to infection blunts pathogen-specific CD8<sup>+</sup> T cell expansion

Given the discordance between PDL-1 blockade prior to, compared with after, in vivo stimulation on CD8<sup>+</sup> cell expansion recently demonstrated for purified protein administered with polyinosinic-polycytidylic acid [poly(I:C)] (42), we investigated how the timing of PDL-1 blockade would impact the expansion of pathogen-specific T cells after recombinant L. monocytogenes infection. Consistent with the results of other studies (14, 15), PDL-1 blockade initiated prior to L. monocytogenes-OVA infection (day −1) triggered sharp reductions in the overall expansion of OVA-specific CD8<sup>+</sup> T cells. Seven days postinfection, which corresponds to the peak expansion of endogenous OVA-specific CD8<sup>+</sup> T cells, ~70% reductions in OVA-specific CD8<sup>+</sup> T cells were identified both by staining with OVA<sub>257−264</sub>-loaded H-2K<sup>b</sup> MHC dimer and intracellular cytokine staining after stimulation with OVA<sub>257−264</sub> peptide for anti–PDL-1 compared with isotype Ab-treated mice (Fig. 1). Furthermore, this reduction in L. monocytogenes-specific CD8<sup>+</sup> T cell expansion with PDL-1 blockade required PD-1, because the impacts of PDL-1 blockade were eliminated for mice with targeted defects in PD-1 in all cells or among PD-1<sup>−</sup>-deficient CD8<sup>+</sup> cells after adoptive transfer into PD-1<sup>−</sup>-sufficient recipients (Supplemental Fig. 1). Interestingly, however, when anti–PDL-1 Ab was administered later postinfection (day +4), the impacts of PDL-1 blockade were reversed as OVA-specific CD8<sup>+</sup> T cells expanded ~2.5-fold more in anti–PDL-1 compared with isotype control Ab treated mice (Fig. 1). Taken together, these results suggest the stimulatory effects of PDL-1 are controlled by signals triggered within the first few days after L. monocytogenes infection.

IL-12 or type 1 IFNs are required for PDL-1–mediated T cell stimulation

Given the efficiency whereby L. monocytogenes induces IL-12 and type 1 IFN production (24–26) and the importance of these cytokines in regulating PDL-1/PD-1 expression during viral infections (13, 20–23), the requirement for IL-12 or type 1 IFNs in PDL-1–mediated T cell stimulation were investigated with L. mono-
cytogenes infection. We found PDL-1 blockade in mice with individual defects in either IL-12 or type I IFNR caused significant reductions the expansion of L. monocytogenes-OVA–specific CD8+ T cells identified by staining with either OVA257-264-loaded H-2Kb MHC dimer or based on IFN-γ production after in vitro stimulation with cognate peptide similar to that observed in B6 control mice (Fig. 2). On the other hand and in sharp contrast to these reductions in L. monocytogenes-OVA–specific CD8+ T cell expansion in B6 or mice with individual defects in IL-12 or type I IFN, the impacts of PDL-1 blockade were eliminated in mice with combined defects in both IL-12 and type I IFNR (Fig. 2). Specifically, OVA257-264-specific CD8+ T cells identified using either OVA257-264-H-2Kb dimer or intracellular cytokine staining each expanded to a similar extent in anti–PDL-1 or isotype Ab-treated mice lacking both IL-12 production and type I IFN responsiveness. These findings illustrate the T cell stimulatory effects of PDL-1 during pathogen-specific T cell expansion. These findings indicate important nonredundant roles for these cytokines in stimulating PDL-1–mediated pathogen-specific CD8+ T cell expansion.

Cell-intrinsic stimulation by neither IL-12 nor type I IFNs is essential for PDL-1–mediated T cell expansion

Related experiments explored how IL-12 and type I IFNs may control the stimulatory effects of PDL-1. Given the potency whereby IL-12 and type I IFNs can directly stimulate T cell activation (44–47), we first investigated the requirement for cell-intrinsic stimulation with IL-12 and type I IFNs on Ag-specific T cells in overriding PDL-1–mediated T cell expansion. Specifically, we compared the impacts of PDL-1 blockade initiated 1 d before L. monocytogenes-OVA ΔactA infection on the subsequent expansion of Ag-specific CD8+ T cells from IL-12R, type I IFNR double-deficient (which cannot respond to direct cell-intrinsic stimulation by these cytokines, CD45.2*CD90.2*), and WT (CD45.2*CD90.1*) OT-1 TCR transgenic mice each after adoptive transfer into CD45.1+ recipient mice. The discordant expression of CD90.1/90.2 congenic markers allows these two subsets of adoptively transferred Ag-specific CD8+ T cells to be discriminated from each other, and expression of the CD45.2 marker allows both subsets of donor cells to be identified among recipient cells (Fig. 4A). With this approach, if cell-intrinsic stimulation via either IL-12 or type I IFNs on OVA-specific CD8+ T cells is essential for PDL-1–mediated expansion, the effects of PDL-1 blockade would be eliminated for cells with combined defects in receptors for both IL-12 and type I IFNs but preserved for WT cells that can respond to stimulation with each of these cytokines. Interestingly and in sharp contrast to this prediction, PDL-1 blockade caused reductions in expansion for both subsets of adoptively transferred Ag-specific CD8+ T cells to a similar extent (~50%) (Fig. 4B, 4C). Furthermore, the magnitude of these reductions among both subsets of OVA-specific T cells each paralleled the expansion defects among endogenous OVA-specific
CD8+ cells with PDL-1 blockade in B6 and mice with individual defects in IL-12 and type I IFNR (Figs. 1, 2) (14, 15). Taken together, these results demonstrate the negated impacts of PDL-1 blockade in mice with combined defects both IL-12 and type I IFNs are not due to defects in cell-intrinsic stimulation by IL-12 or type I IFNs on CD8+ T cells. Instead, other environmental differences resulting from the combined absence of both IL-12 and type I IFNs override the immune stimulatory effects of PDL-1 following *L. monocytogenes* infection.

*L. monocytogenes*-induced IFN-γ production drives PDL-1–mediated T cell expansion

Because direct T cell stimulation by IL-12 and type I IFNs are jointly nonessential for PDL-1–mediated expansion of *L. monocytogenes*-specific T cells, we investigated the role other cytokines

**FIGURE 2.** IL-12 or type I IFNR is required for PDL-1–mediated expansion of pathogen-specific CD8+ T cells. (A) Percent OVA-specific CD8+ splenocytes identified by H-2Kb OVA257–264 dimer staining 7 d after *L. monocytogenes*-OVA ΔactA infection for each group of mice treated with either anti–PDL-1 or isotype control (IgG2b) Ab 1 d prior to infection. (B) Total number (top panel) and ratio (bottom panel) of OVA-specific dimer+CD8+ T cells for the mice described in (A). (C) Percent IFN-γ–producing CD8+ splenocytes 7 d after *L. monocytogenes*-OVA ΔactA infection and OVA257–264 peptide stimulation (top panel) or no stimulation controls (bottom panel) for each group of mice treated with either anti–PDL-1 or isotype Ab 1 d prior to infection. (D) Total number (top panel) and ratio (bottom panel) of IFN-γ–producing CD8+ T cells for the mice described in (C). These data reflect 7–12 mice/group from three independent experiments each with similar results. Bar, 1 SD. *p < 0.05.

**FIGURE 3.** Infection-induced shifts in PDL-1 expression by CD11c+ APC. Representative plots (top panel) and composite data (bottom panel) illustrating PDL-1 expression by CD11c+ splenocytes for each group of mice at the indicated time points after *L. monocytogenes*-OVA ΔactA infection (line histogram) compared with uninfected controls (shaded histogram). These data reflect six to eight mice per group from three independent experiments each with similar results. Bar, 1 SD.

**FIGURE 4.** T cell-intrinsic stimulation by neither IL-12 nor type I IFNs are essential for PDL-1–mediated pathogen-specific CD8+ T cell expansion. (A) Percent donor (CD45.2+) CD8+ cells among either WT (CD90.1+) or IL-12R, type I IFNR double knock-out (DKO) (CD90.2+) OT-1 cells 3 d after *L. monocytogenes*-OVA ΔactA infection, for cells adoptively transferred into CD45.1 recipient mice treated with either anti–PDL-1 or isotype control (IgG2b) Ab 1 d before infection. Total number (B) and ratio (C) of adoptively transferred OVA-specific CD8+ T cells from WT or IL-12R, type I IFNR DKO OT-1 transgenic mice for the mice described in (A). These data reflect six to eight mice per group from three independent experiments. Bar, 1 SD.
such as IFN-γ known to be stimulated by IL-12 and type I IFNs have in controlling the T cell stimulatory impacts of PDL-1 (48–50). Consistent with the results of our prior studies (35), innate IFN-γ production by T and NK cells among bulk splenocytes that peaks within the first 12 h after L. monocytogenes-OVA ΔactA infection in B6 mice was completely abolished in mice with combined defects in both IL-12 and type I IFN (Fig. 5). In contrast, although early IFN-γ production in IL-12–deficient mice was extinguished more rapidly from peak levels compared with B6 mice, L. monocytogenes-OVA ΔactA-induced indistinguishable levels of IFN-γ production within the first 12 h postinfection in mice with individual defects in IL-12 or type I IFN each compared with B6 mice (Fig. 5). Moreover, PDL-1 blockade did not significantly impact the magnitude or kinetics of IFN-γ production in mice with individual or combined defects in IL-12 or type I IFN each compared with B6 mice (data not shown). Thus, overriding effects of PDL-1 blockade in mice with combined defects in both IL-12 and type I IFN may reflect differences in production of immune modulatory cytokines such as IFN-γ.

To investigate the requirement for IFN-γ in PDL-1–mediated T cell stimulation, the impacts of IFN-γ neutralization combined with PDL-1 blockade on pathogen-specific CD8+ T cell expansion were enumerated. Remarkably, anti–IFN-γ neutralization efficiently eliminated the defects in L. monocytogenes-OVA CD8+ T cell expansion associated with PDL-1 blockade. Specifically, the reductions in OVA-specific T cell expansion tracked using OVA257–264 H-2Kb dimer or intracellular cytokine staining associated with PDL-1 blockade were eliminated in mice treated with anti–IFN-γ neutralizing but preserved in mice treated with each respective isotype Ab (Fig. 6). In a similar fashion, the impacts of PDL-1 blockade on pathogen-specific CD8+ T cell expansion were also eliminated in mice with targeted defects in the IFN-γR but preserved in IFN-γR–sufficient control mice (Fig. 7). Therefore, although specific cytokine–Ab complexes have the potential to stabilize the biological activity of some cytokines in vivo (51–53), the paralleled elimination of PDL-1 stimulation using anti–IFN-γ-neutralizing Ab and in IFN-γR–deficient mice demonstrates an essential role for IFN-γ in PDL-1–mediated pathogen-specific CD8+ T cell expansion after L. monocytogenes infection. Taken together with the absence of IFN-γ production in mice with combined defects in IL-12 and type I IFN (Fig. 5) (35), these findings suggest IFN-γ production through either IL-12 or type I IFN-dependent pathways early after L. monocytogenes infection dictates PDL-1–mediated expansion of pathogen-specific CD8+ T cells.

FIGURE 6. IFN-γ neutralization eliminates PDL-1–mediated expansion defects for pathogen-specific CD8+ T cells. (A) Percent OVA-specific CD8+ splenocytes identified by H-2Kb OVA257–264 dimer staining 7 d after L. monocytogenes-OVA ΔactA infection for B6 mice treated with anti-PDL-1 and/or anti–IFN-γ or each respective isotype control Ab 1 d prior to infection. (B) Total number (top panel) and ratio (bottom panel) of OVA dimer+CD8+ T cells for the mice described in (A). (C) Percent IFN-γ–producing CD8+ splenocytes 7 d after L. monocytogenes-OVA ΔactA infection and OVA257–264 peptide stimulation (top panel) or no stimulation controls (bottom panel) for each group of mice, treated with anti-PDL-1, and/or anti–IFN-γ, or each respective isotype control Ab 1 d prior to infection. (D) Total number (top panel) and ratio (bottom panel) of IFN-γ–producing CD8+ T cells for the mice described in (C). These data reflect 7–12 mice/group from three independent experiments each with similar results. Bar, 1 SD. *p < 0.05.

FIGURE 5. IL-12 or type I IFNs are essential for innate IFN-γ production after L. monocytogenes infection. Representative FACS plots illustrating percent (top panel) and composite data demonstrating total number (bottom panel) of IFN-γ–producing splenocytes for each group of mice at the indicated time points after L. monocytogenes-OVA ΔactA infection. These data reflect six to eight mice per group from three independent experiments each with similar results. Bar, 1 SD.
Ag-specific CD8+ T cell expansion in IFN-γR-deficient mice are not explained by differences in infection induced PDL-1/PD-1 expression.

Cell-intrinsic stimulation by IFN-γ is nonessential for PDL-1–mediated T cell expansion

The requirement for IFN-γ in PDL-1–mediated expansion of Ag-specific CD8+ T cells following L. monocytogenes infection led us to further investigate the importance of IFN-γ responsiveness by CD8+ T cells and how PDL-1 blockade may control the expansion of these cells. Using a similar adoptive transfer strategy for investigating the requirement for cell-intrinsic IL-12 and type I IFN stimulation on OVA-specific CD8+ T cell expansion (Fig. 4), the impact of PDL-1 blockade initiated 1 d before L. monocytogenes-OVA ΔactA infection on the subsequent expansion of Ag-specific CD8+ T cells from IFN-γR-deficient (CD45.2+CD90.2+) compared with WT (CD45.2+CD90.1+) OT-1 TCR transgenic mice each after adoptive transfer into CD45.1 recipient mice was evaluated. We found PDL-1 blockade in recipient mice prior to L. monocytogenes-OVA ΔactA infection caused reductions in the expansion for both subsets of adoptively transferred OT-1 CD8+ T cells each to a similar extent (Fig. 9). Furthermore, the overall magnitude of these reductions (~50%) were similar to the reductions among endogenous OVA-specific CD8+ T cells and among adoptively transferred CD8+ cells from TCR transgenic mice with defects in the receptors for both IL-12 and type IFNs (Figs. 2, 4). Taken together, these results indicate that although IFN-γ produced early after L. monocytogenes infection is essential for PDL-1–mediated CD8+ T cell expansion, and direct cell-intrinsic IFN-γ stimulation on effector T cells has been described to be important for their expansion in other infections (54–57), IFN-γ stimulation on CD8+ T cells is nonessential for their expansion following L. monocytogenes infection.

FIGURE 7. Expansion defects for pathogen-specific CD8+ T cells with PDL-1 blockade are eliminated in IFN-γR-deficient mice. (A) Percent OVA-specific CD8+ splenocytes identified by H-2Kb OVA 257–264 dimer staining 7 d after L. monocytogenes-OVA ΔactA infection for B6 (WT) or IFN-γR–deficient mice treated with either anti–PDL-1 or isotype control (IgG2b) Ab 1 d prior to infection. (B) Total number (top panel) and ratio (bottom panel) of OVA dimer+CD8+ T cells for the mice described in (A). (C) Percent IFN-γ–producing CD8+ splenocytes 7 d after L. monocytogenes-OVA ΔactA infection and OVA 257–264 peptide stimulation (top panel) or no stimulation controls (bottom panel) for each group of mice treated with anti–PDL-1 or isotype (IgG2b) Ab 1 d prior to infection. (D) Total number (top panel) and ratio (bottom panel) of IFN-γ–producing CD8+ T cells for the mice described in (C). These data reflect 7–10 mice/group from three independent experiments each with similar results. Bar, 1 SD. *p < 0.05.

FIGURE 8. IFN-γR plays nonessential roles for infection-induced up-regulation of PDL-1 expression. Representative plots (top panel) and composite data (bottom panel) illustrating PDL-1 expression by CD11c+ splenocytes for each group mice at the indicated time points after L. monocytogenes-OVA ΔactA infection (line histogram) compared with uninfected controls (shaded histogram). These data reflect six to eight mice per group from three independent experiments each with similar results. Bar, 1 SD.

FIGURE 9. T cell-intrinsic stimulation by IFN-γ is nonessential for PDL-1–mediated expansion of pathogen-specific CD8+ T cells. (A) Percent donor (CD45.2+) CD8+ T cells among WT (CD90.1+) or IFN-γR–deficient (CD90.2+) OT-1 cells 3 d after L. monocytogenes-OVA ΔactA infection for cells adoptively transferred into CD45.1 recipient mice treated with either anti–PDL-1 or isotype control (IgG2b) Ab 1 d before infection. Total number (B) and ratio (C) of adoptively transferred OVA-specific CD8+ T cells from WT or IFN-γR–deficient OT-1 transgenic mice for the mice described in (A). These data reflect six mice per group from three independent experiments each with similar results. Bar, 1 SD.
Discussion
The balance between immune stimulation and suppression signals that together control T cell activation and expansion is intricately regulated. Although PDL-1 has been mostly characterized to mediate suppression and functional T cell exhaustion during persistent viral infections (5, 9–12), the interaction between PDL-1 and PD-1 can also stimulate T cell activation most notably post-infection with intracellular bacterial pathogens. For example, PD-1–deficient mice have increased pathogen burden and readily succumb to even relatively low inocula of aerosol M. tuberculosis (17). Moreover, during M. tuberculosis infection, PD-1 expression marks highly proliferative pathogen-specific T cell precursors that replenish protective cytokine-producing effector CD4+ cells (17, 58). Similarly, PDL-1 disruption using mice with targeted defects in this molecule or blocking Abs administered prior to infection augments infection susceptibility and impedes the expansion of pathogen-specific T cells following infection with other intracellular bacterial pathogens such as S. enterica and L. monocytogenes (14, 15, 18). Accordingly, PDL-1 stimulation has the potential to provide either immune activation or suppression signals, and these discordant roles are most likely controlled by differences in the cytokine milieu or other infection-induced environmental differences between intracellular bacterial compared with viral pathogens. Our finding that PDL-1–mediated T cell stimulation that occurs with PDL-1 blockade initiated before L. monocytogenes infection is reversed with blockade initiated 4 d postinfection (Fig. 1) further illustrates discordant roles for PDL-1 at early and later time points within the same infection. These results are consistent with the discordant impacts resulting from PDL-1 blockade initiated before with after in vivo stimulation with purified protein plus poly(I:C) on T cell expansion (42) and together reinforce the potential importance of the cytokine milieu that shifts dramatically within the first few days after acute infection or immunization on the ensuing immune response through PDL-1.

By initiating PDL-1 blockade prior to infection in mice with targeted defects in cytokines such as IL-12 and type I IFNs that are readily induced by L. monocytogenes and other intracellular bacterial pathogens, we sought to identify how PDL-1–mediated T cell stimulation is controlled. Although IL-12 and type I IFNs have each been described to control PDL-1/PD-1 expression in various other contexts (13, 20–23), we found mice with individual defects in each cytokine (or cytokine receptor) showed no significant differences in PDL-1 expression by CD11c+ APCs or PD-1/PD-1 expression by Ag-specific CD8+ T cells (Fig. 2. Supp. Fig. 2). Interestingly, however, L. monocytogenes infection-induced upregulation of PDL-1 expression was blunted in mice with combined defects in both IL-12 and type I IFNs. Although the reduced levels of PDL-1 expression in mice lacking both IL-12 production and type I IFN signaling may contribute to the negated impacts of PDL-1 blockade, the requirement for IFN-γ in PDL-1–mediated T cell expansion of L. monocytogenes-OVA specific CD8+ T cells suggests these results are more likely explained by the absence of IFN-γ triggered early after L. monocytogenes infection in these mice. In turn, the comparable magnitude and tempo whereby PDL-1 expression is upregulated in IFN-γ–deficient and control mice indicate the requirement for IFN-γ in PDL-1–mediated T cell expansion are not simply due to differences in PDL-1 expression. Instead, other IFN-γ–induced molecules such as NO that are upregulated with PDL-1 blockade and suppress T cell proliferation may explain the requirement for IFN-γ in PDL-1–mediated T cell stimulation (59, 60). This notion is supported by the sharp upregulation of reactive nitrogen intermediates after L. monocytogenes infection that occurs in an IFN-γ–dependent fashion (60, 61) and suggest establishing how PDL-1 controls NO production after in vivo infection are important areas for future investigation.

The requirement for IFN-γ in PDL-1–mediated T cell expansion during infection with intracellular bacterial pathogens is consistent with the essential role this cytokine plays in host defense against these infections (40, 62, 63). Our results suggest that during infection with these pathogens, early IFN-γ, in addition to activating innate host defense, also promotes the expansion of protective pathogen-specific T cells via PDL-1. This association between intracellular pathogens that stimulate a Th1/Tc1 dominated response and the reversal of PDL-1 from immune suppression to activation signals are also consistent with T-bet–mediated repression of PD-1 expression that stimulates the activation of viral-specific CD8+ T cells (64). Reciprocally, for other pathogens such as LCMV clone 13 or Mycobacterium bovis bacillus Calmette-Guérin that cause persistent infection and where early IFN-γ is less critical for innate host defense, PDL-1 stimulates T cell suppression instead of activation signals (5, 65–67). A notable exception in this study is Toxoplasma gondii infection in which IFN-γ is critically required for innate protection, and yet PDL-1 actively inhibits protective CD8+ T cell responses (68, 69). Whether this is unique for Toxoplasma or more generalizable for other parasitic pathogens remains undefined but also represents important areas for future investigation. Finally, given the discordant roles in host defense resulting from PDL-1/PD-1 disruption, developing PDL-1 blockade for therapeutically boosting immunity against some infections needs to be carefully weighed against the potential for increased susceptibility to infection with other types of pathogens. The finding that early IFN-γ is essential for PDL-1–mediated T cell stimulation suggests PDL-1 disruption will most severely impede host defense against infections where IFN-γ is produced early and presumably essential for innate resistance. Our ongoing studies are aimed at further investigating the interplay between PDL-1 and IFN-γ in stimulating pathogen-specific adaptive responses using other models of experimental infection and dissecting the molecular basis whereby IFN-γ redirects PDL-1 stimulation into T cell activation signals.

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Disclosures
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