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Dual Control of Antitumor CD8 T Cells through the Programmed Death-1/Programmed Death-Ligand 1 Pathway and Immunosuppressive CD4 T Cells: Regulation and Counterregulation

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Tumors have evolved multiple mechanisms to evade immune destruction. One of these is expression of T cell inhibitory ligands such as programmed death-ligand 1 (PD-L1; B7-H1). In this study, we show that PD-L1 is highly expressed on mesothelioma tumor cells and within the tumor stroma. However, PD-L1 blockade only marginally affected tumor growth and was associated with the emergence of activated programmed death-1+ ICOS+ CD4 T cells in tumor-draining lymph nodes, whereas few activated CD8 T cells were present. Full activation of antitumor CD8 T cells, characterized as programmed death-1+ ICOS+ Ki-67+ and displaying CTL activity, was only observed when CD4 T cells were depleted, suggesting that a population of suppressive CD4 T cells exists. ICOS+ Foxp3+ regulatory T cells were found to be regulated through PD-L1, identifying one potentially suppressive CD4 T cell population. Thus, PD-L1 blockade activates antitumor CD8 T cell most potently in the absence of CD4 T cells. These findings have implications for the development of PD-L1-based therapies.

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by PD-1, and have implications for the design of PD-L1-based immunotherapies.

Materials and Methods

Reagents and Abs

The anti-mouse mAbs conjugated to FITC, Alexa488, PE, PerCP-Cy5.5, allophycocyanin, PE-Cy7, or allophycocyanin-Alexa750, as required, along with appropriately matched isotype controls, were purchased from BD Pharmingen, eBioscience, and Biologic, and are as follows: PD-L1 (M1H5), CD11c (N418), CD8a (Ly-2), PD-1 (RMP1-30), LFA-1 (M17/4), CD4 (RM4-5), ICOS (7E.17G9), Ki-67 (B56), ICOS ligand (ICOS-L; HMK1), TCR-β (H2Kd), CD43 (B11), and foxp3 (150D). PE-labeled H-2Kd HA pentamers were purchased from ProImmune. CFSE was purchased from Molecular Probes. Flow cytometry was performed using a BD FACSCalibur or a FACSCanto II instrument and analyzed using Flowjo software (Tree Star).

Animals

BALB/c (H-2b) wild-type mice were purchased from the Animal Resources Centre and maintained under specific pathogen-free conditions. All experiments used female mice between 6 and 8 wk of age. Clone 4 TCR transgenic mice, which express a TCR specific for the H-2d-restricted peptide IYSTVASSL (residues 518–526) of A/PR8/8/34 (H1N1) influenza virus HA, were generated and screened, as previously described (18). Animal experiments were conducted according to University of Western Australia Animal Ethics Committee approvals.

Tumor cell culture and inoculation

Generation of the BALB/c-derived mouse mesothelioma cell line AB1 and transfection with the gene encoding influenza HA (AB1-HA) have been previously described (18). Cell lines were maintained in RPMI 1640 (Invitrogen Life Technologies) supplemented with 20 mM HEPEs, 0.05 mM 2-ME, 60 µg/ml penicillin, 1 mg/ml collagenase, 100 µg/ml gentamicin (West), and 10% FCS. For digestion of solid tumors, 100 µg/ml of RPMI 1640 containing 2% FCS, HEPEs, 1 mg/ml collagenase, and 100 µg/ml DNase was injected into the tumor tissue before mincing. Tumor fragments were resuspended in the same solution and digested with mixing for 1 h at room temperature. A single-cell suspension was then generated using the protocol described above.

In vivo CTL assay

Detection of HA-specific in vivo CTL was conducted, as previously described (18, 22). Briefly, naive BALB/c spleens were homogenized into cell suspensions, and erythrocytes were lysed by incubation of cells in RBC lysis buffer for 3 min, followed by two washes in RPMI 1640 with 10% FCS. Cells were resuspended at 5 × 10⁶ cells/ml, and then divided into two populations, one of which was pulsed with 1 µg/ml clone 4 peptide for 90 min at 37°C. Cells were washed and then labeled with CFSE for 10 min at room temperature in serum-free conditions with a final concentration of 0.5 µM for peptide-pulsed cells (CFSE<sup>high</sup>) and 0.2 µM for unpulsed cells (CFSE<sup>low</sup>). Both populations were washed in FCS three times, followed by sterile saline before being pooled in equal proportions, and 2 × 10⁶ cells were injected i.v. into recipients. After 18 h, draining and nondraining lymph nodes, tumors, and spleens were harvested and processed, as described above, and analyzed by FACS for the recovery of CFSE<sup>high</sup> and CFSE<sup>low</sup> cells. Percentage of killing was calculated using the following formula: (1 − (CFSE<sup>high</sup>/CFSE<sup>low</sup> events/CFSE<sup>events</sup>)) × 100.

Statistics

Data were statistically evaluated using Prism software (GraphPad), with growth curves compared using two-tailed paired Student’s t test, with pairs defined by time point and survival responses analyzed by Kaplan-Meier using a log-rank test. Significance was defined as p < 0.05.

Results

Expression of PD-L1 by tumor cells and in the tumor milieu

Several studies have demonstrated that the efficacy of antitumor immunotherapy or adoptive T cell transfer is limited by negative PD-1/PD-L1 interactions (12, 23, 24). We used a mouse model of mesothelioma (15, 17) to assess the role of PD-1-mediated T cell inhibition in natural tumor-induced immune responses (i.e., without additional therapy). First, we evaluated the expression patterns of PD-L1 on AB1-HA mesothelioma cells. Under in vitro culturing conditions, we found that AB1-HA cells expressed low levels of PD-L1 (Fig. 1A). PD-L1 expression was rapidly induced after exposure to IFN-γ (Fig. 1B). This is similar to findings in other tumor models (1), suggesting that IFN-γ-dependent PD-L1 expression may be a general feature of tumor cells. To analyze in vivo PD-L1 expression patterns, i.e., in the tumor milieu, we prepared single-cell suspensions from growing tumors and analyzed these for PD-L1 expression. Although the majority of cells from the tumor were PD-L1<sup>low</sup>, we observed subpopulations of PD-L1<sup>int</sup> and PD-L1<sup>high</sup> cells. Under in vitro culturing conditions with a final concentration of 100 µg/ml of RPMI 1640 containing 2% FCS, HEPEs, 1 mg/ml collagenase, and 100 µg/ml DNase was injected into the tumor tissue before mincing. Tumor fragments were resuspended in the same solution and digested with mixing for 1 h at room temperature. A single-cell suspension was then generated using the protocol described above.

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Statistics

Data were statistically evaluated using Prism software (GraphPad), with growth curves compared using two-tailed paired Student’s t test, with pairs defined by time point and survival responses analyzed by Kaplan-Meier using a log-rank test. Significance was defined as p < 0.05.
represent tumor stroma cells, such as DCs. To test this, tumor-resident CD11c<sup>+</sup> DCs were analyzed for PD-L1 expression. Indeed, we observed high-level PD-L1 expression on CD11c<sup>high</sup> cells in the tumor, whereas CD11c<sup>low</sup> cells expressed low levels of PD-L1 (Fig. 1E). PD-L1 analysis on DCs from tumor-draining and nondraining lymph nodes revealed systemic, high-level PD-L1 expression (Fig. 1F). Thus, PD-L1 is ubiquitously expressed both within the tumor milieu and in the lymphatic system in tumor-bearing mice. Importantly, total PD-L1 expression in the tumor is sensitive to IFN-γ, suggesting that potential IFN-γ-secreting antitumor T cells may induce a counterregulatory response in the tumor, which could block their efficacy.

**T cells in tumor-draining lymph nodes express PD-1**

PD-1 expression on CD8 T cells is usually associated with persistent antigenic stimulation and may predispose these cells to functional inactivation (4, 6). To test whether this was the case in AB1-HA tumor-bearing mice, CD8 T cells in tumor-draining and nondraining lymph nodes were analyzed for PD-1 expression. Because AB1-HA tumor Ags are exclusively cross-presented in tumor-draining lymph nodes (25), we reasoned that a comparison between PD-1 expression levels on T cells in tumor-draining and nondraining lymph nodes would provide us with a surrogate for PD-1 expression on the tumor-specific T cell pool. As shown in
PD-1 expression on CD8 T cells in the tumor and the local lymph nodes. A, PD-1 expression on CD8<sup>+</sup> T cells from the tumor-draining lymph nodes (DLN) was significantly increased compared with non-DLN (NDLN); unpaired Student’s t test. Representative dot plots gated on lymphocytes show PD-1 expression on CD8<sup>+</sup> T cells from the non-DLN (left panel) and DLN (right panel). Percentage of PD-1<sup>high</sup> CD8<sup>+</sup> T cells is indicated. B, TCR-transgenic HA-specific CD8<sup>+</sup> T cells from the NDLN (left panel), DLN (center panel), and tumor (right panel) were analyzed for expression of PD-1 and LFA-1. Percentages of PD-1<sup>+</sup>CD8<sup>+</sup> T cells are indicated. Cells shown are gated on CD8<sup>+</sup> cells. C, PD-1 expression on CD4<sup>+</sup> T cells from the tumor nondraining lymph nodes (left panel) and draining lymph nodes (right panel). Percentages of PD-1<sup>+</sup>CD4<sup>+</sup> T cells are indicated.

Fig. 2A, PD-1 expression levels on CD8 T cells in the draining lymph nodes were variable, but overall significantly (p < 0.001) higher than in nondraining nodes. PD-1 expression patterns on tumor-specific CD8 T cells were further evaluated by analyzing T cell responses in TCR-transgenic clone 4 mice. TCR-transgenic CD8 T cells from these mice are specific for a K<sub>d</sub>-restricted epitope in the influenza virus HA protein (HA<sub>518-526</sub>: IYS-TVASSL), which is expressed as a neo-Ag in AB1-HA tumor cells (17). Thus, TCR-transgenic mice were inoculated with AB1-HA tumor cells, and PD-1 expression on tumor-specific CD8 T cells was measured in draining and nondraining lymph nodes, as well as in the tumor. Again, we found higher PD-1 expression levels in draining compared with nondraining lymph nodes. PD-1<sup>high</sup> TCR-transgenic CD8 T cells were LFA-1<sup>high</sup> and CD62L<sup>low</sup>, consistent with an activated phenotype (Fig. 2B and data not shown). High PD-1 expression levels were also observed on tumor-infiltrating transgenic CD8 T cells (Fig. 2B). Note that PD-1 expression levels on CD8<sup>+</sup><sup>negative</sup> cells were higher than on CD8<sup>+</sup><sup>positive</sup> cells (Fig. 2A).
It seemed likely that these CD8<sup>negative</sup> PD-1<sup>high</sup> cells were CD4 T cells. Indeed, we found that CD4 T cells expressed PD-1 (Fig. 2C) and that PD-1 expression was largely confined to the tumor-draining lymph nodes (Fig. 2C).

Thus, the data show that PD-1 is expressed on both CD4 and CD8 T cells in the tumor-draining lymph nodes, and suggest that immunosuppression in this tumor model could be driven by PD-1/PD-L1 interactions.

**PD-L1 blockade slows tumor growth and activates lymph node CD4 T cells**

To test whether AB1-HA tumor growth depended on PD-1/PD-L1-mediated inhibition of antitumor T cell responses, we treated tumor-bearing mice with PD-L1 blocking Abs. Treatment was started when tumors were just palpable (day 8 after inoculation), using a four doses every third day schedule (200 μg of Ab, i.p.). PD-L1 blockade had a weak, but significant (  < 0.01) effect on tumor growth (Fig. 3A). We then assessed the impact of PD-L1 blockade on T cells by analyzing expression of PD-1 and ICOS on CD4 and CD8 T cells. ICOS (CD278) is a B7 family member that is expressed on activated T cells after TCR ligation (26). Interestingly, we observed that PD-L1 blockade induced a population of activated PD-1<sup>high</sup> ICOS<sup>high</sup> CD4 T cells in tumor-draining lymph nodes (Fig. 3B, lower right panel). CD8 T cells in the draining lymph nodes were not activated to a similar extent, in particular in terms of the expression levels of PD-1 and ICOS (as judged on the basis of mean fluorescence intensity; Fig. 3C, lower right panel).

Given the key role of CD8 T cells in AB1-HA tumor resolution (22), it is possible that there is a correlation between the relatively weak antitumor effects of PD-L1 blockade and the relatively stronger CD4 T cell activation (compared with CD8 T cell activation, in terms of frequency and mean fluorescence intensity).

**CD8 T cells are activated by PD-L1 blockade after CD4 T cell depletion**

To further investigate the effect of PD-L1 blockade on the activation of CD8 T cells, we performed PD-L1 blockade experiments in the absence of CD4 T cells. CD4 T cells were depleted using GK1.5 mAbs (three doses every third day), and depletion was verified by staining PBMC 3 days after Ab injection. Ab treatment routinely led to >99% CD4<sup>+</sup> T cell depletion at 3 days after injection (Fig. 4A), and high levels of depletion were maintained throughout the experiment (day 16 lymph node data shown; Fig. 4B). All treatments were started when tumors were just palpable (day 7–8 after tumor cell inoculation). To study T cell activation in response to PD-L1 blockade, we analyzed the phenotypes of CD4 and CD8 T cells in tumor-draining lymph nodes using PD-1 and ICOS as activation markers. These experiments revealed that PD-L1 blockade in CD4-depleted mice induced high frequencies of activated PD-1<sup>+</sup> ICOS<sup>+</sup> CD8 T cells (Fig. 4Civ). PD-L1 blockade alone did not activate CD8 T cells (Fig. 4Cii), but did induce a population of activated CD4 T cells (Fig. 4Cvi). CD4 depletion without PD-L1 blockade resulted in modest levels of activated CD8 T cells, as judged by ICOS and PD-1 expression (Fig. 4Ciii).

By analyzing CD8 T cell numbers using BD Biosciences TruCount tubes, we found that CD8 T cells did not have a homeostatic response to CD4 depletion (data not shown), excluding the possibility that PD-1 was up-regulated on CD8 T cells as a response to homeostatic proliferation (27). Thus, PD-L1 blockade leads to CD8<sup>T</sup> cell activation only when CD4 T cells are absent. Interestingly, low frequencies of remaining or recovering CD4 T cells (~5%; Fig. 4B) can be detected in CD4-depleted mice (Fig. 4C, vii and viii), and these CD4 T cells display an activated phenotype.

PD-L1 blockade in CD4-depleted mice induced further activation of the recovering CD4 T cells (Fig. 4Cviii).

We then assessed the proliferative status of anti-PD-L1-activated CD4 and CD8 T cells by intracellular Ki-67 staining (Fig. 4D). Ki-67 is a nuclear protein that is only expressed in cycling cells (28) and that can be used to identify activated T cells (29). Activated PD-1<sup>+</sup> ICOS<sup>+</sup> CD4 T cells, induced by PD-L1 blockade, were Ki-67<sup>high</sup>, indicating that they did not proliferate (Fig. 4Dvi). In contrast, we did observe Ki-67<sup>high</sup> proliferating cells among the PD-1<sup>+</sup> ICOS<sup>+</sup> CD8 T cells that were induced by PD-L1 blockade in CD4-depleted mice (Fig. 4Dvi). The small, but activated CD4 T cell populations in GK1.5-treated mice harbored Ki-67<sup>high</sup> cells (Fig. 4Dvii). Importantly, PD-L1 blockade in CD4-depleted mice resulted in even stronger activation and Ki-67 expression of the remaining CD4 T cells (Fig. 4Dviii).

To confirm that PD-L1 blockade activated a specific antitumor CD8 T cell response and that this response was augmented by CD4 T cell depletion, we performed an in vivo CTL assay and we used a MHC I pentamer specific for the K<sup>A</sup>-restricted HA epitope. In vivo CTL activity was measured by injecting CSFE-labeled HA peptide-coated and uncoated targets into tumor-bearing mice and measuring their recovery 18 h later. We compared the effects of PD-L1 blockade in CD4-depleted and control mice. The results (Fig. 5A) revealed that there was little to no in vivo CTL activity in untreated tumor-bearing mice, consistent with a strong suppression of antitumor immune responses. The CTL response was somewhat increased in mice receiving PD-L1 blocking Abs, consistent with the modest antitumor effect of this treatment. CD4 depletion resulted in higher in vivo CTL responses, but the variability in this treatment group was very high, indicating that not all animals responded (Fig. 5A). Finally, PD-L1 blockade in CD4-depleted mice resulted in high in vivo CTL responses in all mice (Fig. 5A). Responses were detected in draining and nondraining lymph nodes, as well as in the spleen and in the tumor. These results were confirmed by pentamer staining (Fig. 5B). Few pentamer<sup>+</sup> CD8 T cells were detected in untreated tumor-bearing mice, and these cells were ICOS<sup>low</sup> and CD43<sup>low</sup> (Fig. 5B and data not shown). PD-L1 blockade or CD4 depletion alone led to a modest increase in the frequency of pentamer<sup>+</sup> CD8 T cells (Fig. 5B); however, PD-L1 blockade in CD4-depleted mice triggered a strong increase in pentamer<sup>+</sup> cells (Fig. 5B). These cells were CD43<sup>low</sup> (Fig. 5B) and expressed high levels of ICOS (Fig. 5C), consistent with an activated phenotype.

**Impact of PD-L1 blockade on tumor growth with or without CD4 T cells**

PD-L1 blockade potently activated CD8<sup>T</sup> cells and tumor-specific CD8<sup>T</sup> cell responses when CD4 T cells were depleted. Thus, we examined the effect of PD-L1 blockade in CD4-depleted mice. PD-L1 blockade in the absence of CD4 T cells resulted in a complex response pattern (Fig. 6). Clearly, PD-L1 blockade was much more effective when CD4 T cells were depleted (Fig. 6), identifying CD4<sup>T</sup> T cells as a limiting factor. CD4 depletion alone also had a strong antitumor effect, suggesting that CD4<sup>T</sup> cells are overall immunosuppressive (Fig. 6). Detailed analysis of the effects of PD-L1 blockade in CD4-depleted mice revealed a biphasic response pattern. Comparison of CD4-depleted mice with CD4-depleted and PD-L1-blocked mice revealed a highly significant (  < 0.005) benefit of PD-L1 blockade from day 13–28 after tumor cell inoculation (i.e., day 6–21 after Ab treatment; Fig. 6). Importantly, tumor regression was only observed with the PD-L1 blockade/CD4 depletion combination, whereas the effects of CD4 T cell depletion only were limited to tumor growth arrest. From day 28 onward, tumor growth recurred and tumors in PD-L1-blocked and CD4-depleted mice grew faster than tumors in...
mice that had only received CD4-depleting Ab (p < 0.05, day 28–41; Fig. 6). Thus, PD-L1 blockade in CD4-depleted mice initially enhanced antitumor responses, leading to tumor regression, but then resulted in accelerated tumor growth that coincided with CD4 recovery (last GK1.5 injection at day 14 after inoculation).

**PD-L1 blockade increases regulatory T cell numbers**

Our data indicate that a pool of immunosuppressive CD4 T cells exists. CD25<sup>+</sup> foxp3<sup>+</sup> regulatory T cells constitute a well-described population of immunosuppressive CD4 T cells (30). We recently found that CD25 depletion alone did not affect tumor growth, but that it did enhance the efficacy of antitumor chemotherapy, identifying regulatory CD4 T cells as candidates for suppressive cells (16). Thus, we measured foxp3<sup>+</sup> CD4 T cell numbers in tumor-draining lymph nodes from mice receiving different treatments. Interestingly, PD-L1 blockade resulted in an increase in the frequency of foxp3<sup>+</sup> CD4 T cells (p < 0.05; Fig. 7A). To further evaluate regulatory T cell responses, we analyzed ICOS expression on these cells. ICOS was
FIGURE 4. Increased activation of CD8 T cells by PD-L1 blockade after CD4 depletion. Representative depletion data are shown for PBL samples at day 3 after GK1.5 Ab injection (A) and for lymph nodes at day 16 after GK1.5 Ab injection (B). C. Effect of CD4 depletion on the activation of T cells in the tumor-draining lymph nodes. Tumor-bearing mice were left untreated or depleted of CD4+/H11001 T cells (days 8, 11, and 14) with or without PD-L1 blockade (days 8, 11, and 14). Draining lymph node cells were harvested on day 16 and were gated on CD8+/H11001 (upper row) or CD4+/H11001 (lower row) cells and analyzed for PD-1 and ICOS expression. D. As per C, but showing expression of PD-1 and Ki-67.
selected because it may identify cells that respond to PD-L1 blockade and because it has been linked with a more suppressive regulatory T cell phenotype (31). Interestingly, foxp3−/H11001CD4 T cells in anti-PD-L1-treated mice expressed higher levels of ICOS (Fig. 7B). A potential functional role of ICOS on foxp3−/H11001 regulatory T cells is supported by the expression of ICOS-L on CD11c−/H11001 DCs in the tumor (Fig. 7C) (32). Combined, these data provide a possible link between PD-L1 blockade and activation of suppressive immune responses, i.e., foxp3−/H11001 regulatory T cells.

Discussion
Inhibition of CD8 T cell responses via PD-1 and PD-L1 interactions has received considerable attention during recent years (3, 6, 9). In several studies, it has been shown that blockade of PD-1/ PD-L1 interactions rescues T cell functionality, translating into improved antiviral (6) or antitumor responses (24). In the current study, we have investigated the effect of PD-L1 blockade on T cell activation and tumor growth. The data show that PD-L1 blockade indeed has the capacity to activate antitumor CD8 T cell responses, and that this effect was much stronger in the absence of CD4 T cells. These CD8 T cell responses are substantial: up to 15% of all CD8 T cells in the tumor-draining lymph nodes is activated in the absence of CD4 T cell-mediated suppression. Importantly, when CD4 T cells are not depleted, PD-L1 blockade activates CD4 T cells instead of CD8 T cells. This suggests that the CD4 T cells, possibly including those activated through PD-L1 blockade, may
prevent CD8 T cell activation. The effect of PD-L1 blockade on tumor growth was weak, but, again, stronger when combined with CD4 depletion. Importantly, CD4 depletion alone already led to tumor growth arrest, pointing toward a role for immunosuppressive CD4 T cells. This phenotype of immunosuppressive CD4 T cells is similar to recently reported work by Melief and coworkers (33). Our data do not rule out the possibility that such suppressive CD4 T cells are also regulated through PD-L1 blockade. Indeed, our observation that PD-L1 blockade induces expansion of and ICOS up-regulation in foxp3+ regulatory T cells could support this view.

The concept that the effects of PD-L1 blockade may not be limited to IFN-γ-producing T cells has first emerged from work published by Freeman et al. (34). These authors showed that, depending on the polarization of the cells, PD-L1 blockade could rescue either IFN-γ or IL-10 production in CD4 T cells. These experiments were done in vitro, but suggested that PD-L1 blockade could also rescue different types of immune responses in vivo. The data presented in our current study could be consistent with this. We show in this study that PD-L1 blockade has the potential to expand and activate foxp3 regulatory CD4 T cells. Control of regulatory T cell responses through PD-1 is supported by a recent study showing that regulatory T cell functionality in chronically infected HCV patients is controlled through PD-1, in a counter-regulatory fashion, i.e., PD-1 ligation shuts down the suppressors (14). Furthermore, PD-1 was up-regulated on CD4 CD25 foxp3 regulatory T cells from nonresponders after hepatitis B surface Ag vaccination, consistent with increased suppressive activity (35). However, as we recently reported, CD25 depletion alone had no effect on tumor growth (16), suggesting that the CD4 suppressive T cell pool that we identified in the current study may be composed of other non-T regulatory suppressive cell types as well. Indeed, the existence of PD-1 CD4 T cells acting as regulatory T cells has recently been reported (36). Our preliminary data show that ICOShi CD4 T cells, purified from

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Effect of CD4 depletion on effect of PD-L1 blockade. Tumor-bearing mice were left untreated or depleted of CD4+ T cells using GK1.5-depleting Ab (days 6, 9, and 12) with or without PD-L1 blockade (days 10, 13, and 16). Data shown are mean (n = 5) from one experiment. **Abbreviations:** anti-PD-L1 vs anti-PD-L1 + anti-CD4, day 13–28; *, p < 0.005, anti-PD-L1 vs anti-PD-L1 + anti-CD4, day 28–41.

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** PD-L1 blockade increases CD4+ regulatory T cell numbers. A, Percentage of foxp3+ CD4+ T cells present in the draining lymph nodes of untreated mice or mice treated with poly(I:C), anti-PD-L1, or both (days 7, 10, and 13; top panel). Lymph nodes were harvested at day 14, *, p < 0.05; **, p < 0.01; unpaired Student’s t test. Representative plots of the same data (bottom panel). Numbers indicated represent the percentage of CD4+ T cells positive for foxp3. B. ICOS expression on CD4+ foxp3+ T regulatory cells from the draining lymph nodes of mice treated, as for A. C. ICOS-L expression on tumor-infiltrating CD11c+ DCs. CD11c+ DCs were gated (left panel) and analyzed for ICOS-L expression (green graph). Isotype control staining is shown in red.
tumor-draining lymph nodes in anti-PD-L1-treated mice, up-regulate the Th2 cytokines IL-5 and IL-13 (21- and 120-fold, compared with the ICOSlow population: A. J. Currie, A. Prosser, A. L. Cleaver, B. W. S. Robinson, G. J. Freeman, R. G. van der Most, manuscript in preparation). IL-13 could have an important role. For example, type-II CD4+ NKT cells exert their immunosuppressive functions through IL-13 (37, 38). IL-13 activates myeloid suppressor cells, which, in turn, produce the immunosuppressive cytokine TGF-β. Indeed, we have preliminary data showing that a combination of TGF-β neutralization and PD-L1 blockade slows tumor growth (R. G. van der Most, unpublished data). Furthermore, IL-2 produced by activated (ICOS+) CD4 T cells could enhance T regulatory proliferation (39), thereby enhancing immunosuppression.

The role of ICOS is interesting. ICOS is expressed on activated T cells, including CD4 and CD8 T cells (26), as well as regulatory T cells (31). ICOS on CD4 T cells has been linked to a Th2 phenotype (3, 40–42), but also with IL-10 production (31, 32, 40, 41, 43) and with more suppressive regulatory T cells (31, 44). Moreover, ICOS+ CD4 T cells were recently shown to control autoimmune responses (31, 40), and ICOS is expressed on tumor-infiltrating regulatory T cells (31, 44). Thus, there is evidence that is consistent with our conclusion that ICOS+ PD-1+ CD4 T cells may be suppressors, and that such responses can be controlled via PD-1/PD-L1 interactions.

We propose the following model (Fig. 8). When the tumor establishes itself, it induces a CD4 T cell response that is composed of CD25+ regulatory cells and possibly IL-13-producing CD4+ T cells or NKT cells. Combined, these responses inhibit antitumor CD8 responses. The entire response may be under the control of PD-1, but the IFN-γ-dependent up-regulation of PD-L1 on tumor cells guarantees that the balance is tilted toward tumor-specific T cell responses. PD-L1 blockade under these conditions will rescue all PD-1+ cells, including both CD4 and CD8 T cells, but reactivated CD4 T cells will suppress antitumor CD8 T cell responses, resulting in a weak antitumor effect. When CD4 T cells are depleted, PD-L1 blockade will activate CD8 T cells, leading to increased antitumor responses, but this response will be limited by the recovery time for CD4 T cells.

In conclusion, our data show that PD-L1 blockade has the capacity to unleash powerful antitumor CD8 T cell responses, but only when suppressive CD4 T cells are absent. The implication is that PD-L1 blockade may not always optimally activate antitumor CD8 T cell responses.

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Disclosures
G.J.F. has patents and receives patent royalties on the PD-1 pathway.

References


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