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Tumor Cell Programmed Death Ligand 1-Mediated T Cell Suppression Is Overcome by Coexpression of CD80

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Programmed death ligand 1 (PDL1, or B7-H1) is expressed constitutively or is induced by IFN-γ on the cell surface of most human cancer cells and acts as a “molecular shield” by protecting tumor cells from T cell-mediated destruction. Using seven cell lines representing four histologically distinct solid tumors (lung adenocarcinoma, mammary carcinoma, cutaneous melanoma, and uveal melanoma), we demonstrate that transfection of human tumor cells with the gene encoding the costimulatory molecule CD80 prevents PDL1-mediated immune suppression by tumor cells and restores T cell activation. Mechanistically, CD80 mediates its effects through its extracellular domain, which blocks the cell surface expression of PDL1 but does not prevent intracellular expression of PDL1 protein. These studies demonstrate a new role for CD80 in facilitating antitumor immunity and suggest new therapeutic avenues for preventing tumor cell PDL1-induced immune suppression. The Journal of Immunology, 2011, 186: 000–000.

Tumor-induced immune suppression is a major obstacle for therapies aimed at activating an individual’s immune system to eliminate autologous cancer cells. This immune suppression can be mediated by nonmalignant host cells such as T regulatory cells (1) and myeloid-derived suppressor cells (2) that are driven by tumor-secreted factors, as well as by malignant cells that express immune inhibitory molecules. Programmed death ligand 1 (PDL1, also known as B7 homolog 1 [B7-H1] or CD274) is an inhibitory molecule that is either induced or constitutively expressed by many malignant cells (3–6). Tumor cell-expressed PDL1 facilitates tumor progression by at least two mechanisms: (i) tumor-reactive T cells are tolerized by PDL1 binding to its receptor programmed death 1 (PD1; CD279) on T cells (3, 7); and (ii) tumor cells are rendered resistant to T cell-mediated and FasL-mediated lysis by PD1 signaling through tumor cell-expressed PDL1 (8). PD1 may also tolerate tumor-reactive T cells by reverse signaling through CD80 on T cells, as has been shown in vitro T cell activation systems (9) and in vivo in an oral tolerance system (10). Regardless of the mechanism by which tumor cell-expressed PDL1 promotes tumor growth, blocking PDL1–PD1 interactions with anti-PDL1 or PD1 Abs improves activation of tumor-reactive T cells and reduces tumor progression (4, 11–13), confirming that tumor cell-expressed PDL1 is a major obstacle for cancer immunotherapies.

Many cancer immunotherapies are designed to stimulate the production of cytokines and chemokines that mobilize tumor-reactive, cell-mediated effector cells such as T lymphocytes, NK cells, and M1 macrophages (14–17). A key cytokine in this process is IFN-γ, which polarizes macrophages toward an M1 phenotype (18), activates NK cells (19), and facilitates the development of type 1 helper CD4+ T cells and cytotoxic CD8+ T cells (20). However, IFN-γ is also a potent inducer of PDL1 (21). Therefore, cancer immunotherapies that optimally activate desirable effector cells may concomitantly upregulate tumor cell expression of PDL1 and thereby limit their own effectiveness.

We have generated tumor cell-based cancer vaccines that induce high levels of IFN-γ and are potent activators of tumor-reactive T cells. Our “MHC class II” vaccines were designed to activate tumor-reactive type 1 CD4+ Th cells and consist of tumor cells transfected/transduced with genes encoding the costimulatory molecule CD80 and syngeneic HLA-DR molecules. In vitro studies with three human solid tumor cell lines demonstrated that the MHC class II (MHC II) vaccines are potent activators of a diverse repertoire of tumor-reactive and tumor-specific CD4+ T cells (22–25), and in vivo studies with three mouse tumor systems confirmed that the vaccines prolong survival of mice with established primary and/or metastatic disease (26). In contrast to accepted dogma (27, 28), T cell activation and tumor rejection required vaccine cell expression of CD80 in the boost stage, suggesting that CD80 was playing a role other than as a costimulatory molecule to prime naïve T cells. We now report that CD80 also prevents constitutive and IFN-γ–induced tumor cell expression of PDL1 and thereby facilitates tumor immunity by inhibiting PDL1-mediated immune suppression.
Materials and Methods

Cell lines
Primary uveal melanoma cell lines MEL202 and MEL270, metastatic uveal melanoma line OMM2.3 (29), and cutaneous melanoma cell lines MEL1011 (30), C8161 (31), and 624MEL (32) were obtained from the cited sources except for 624MEL and C8161, which were obtained from F. Marincola (National Cancer Institute, National Institutes of Health) and E. Seltor (Children’s Memorial Research Center, Chicago, IL), respectively. These lines were cultured in RPMI 1640 (BioSource, Rockville, MD) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT), 10 mM HEPES (Invitrogen, Grand Island, NY), 1% penicillin/streptomycin (BioSource, Rockville, MD), 1% 2-mercaptoethanol (VWR, West Chester, PA), 2 mM Glutamax (BRL/Life Sciences, Grand Island, NY), 0.1% gentamicin (BioSource), and 5 μg/ml Prophylactic Plasmocin (InvivoGen, San Diego, CA). MCF10CA1 (hereafter called MCF10) (33) mammary carcinoma and bronchioloalveolar adenocarcinoma H358 (34) cell lines were obtained from the American Type Culture Collection and cultured as described (25, 35) except 5 μg/ml Prophylactic Plasmocin was included in the media. Human mature dendritic cells (mDCs) were generated as described (36) from PBMCs derived from apheresis of healthy donors, except media was supplemented with 10% human AB serum instead of autologous serum. Cell lines and procedures with human materials were approved by the institutional review boards of the participating institutions.

IFN-γ treatment
Cells (3 x 10^5/ml/T25 flask) were incubated at 37°C for 48 h in their culture medium supplemented with 50–100 U/ml recombinant human IFN-γ (Pierce Biotechnology, Rockford, IL) and then washed with excess culture medium.

Plasmids, transfections, and transfectants
The human CD80 molecule (accession no. P35881; www.uniprot.org/uniprot/P35881) contains 288 aa of which the C-terminal ~21 aa constitute the cytoplasmic domain (37–39) (structure prediction programs at http://ca.expasy.org/tools/). The CD80tr construct was generated from the pLHCX/CD80 plasmid (22) construct by PCR using the primers 5’-GATCCTATGGGCCACACACGGAGG-3’ and 3’-GGGGCAAAGCAGTA-5’. PCR products were analyzed on a 1.0% agarose gel. The resulting PCR product was cloned into the NotI site of the pCI-neo vector. The final construct was confirmed by DNA sequencing analysis. To facilitate construction of additional C-terminal variants of CD80, the CD80tr construct was modified to include a BsmI site over-lapping the terminal proline codon. The HLA-DR sequence was amplified by PCR using the primers 5’-AGAGTGGCACAGGAGGAG-3’ and 5’-AGGCAAGCTTATTATGGGCGAAAGGAGG-3’, which amplified the whole but all the 19 aa of the C-terminal cytoplasmic domain. The final construct was confirmed by DNA sequence analysis. To facilitate construction of additional C-terminal variants of CD80, the CD80tr construct was modified to include a BsmI site over-lapping the terminal proline codon. The HLADR–chain cytoplasmic region was added to this modified construct by ligation of the double-stranded oligonucleotide adapter 5’-GGAGTGCGCAAAAGCAATGCAGCAGAACGACGAGGACCGGGGCTCTGTGTAAC-3’ and 5’-AGACTTACAGAGGCCATGCAGGAGGGGCTGGTGCTTCTGCATTGGGCTTACCTGGCCG-3’ to a BsmI-digested CD80tr construct. The resulting fusion molecule (CD80tr) contains 288 aa of which the C-terminal 21 aa constitute the cytoplasmic domain (37–39) (structure prediction programs at http://ca.expasy.org/tools/).

Western blots
Western blots were performed as described (22) with the following modifi-cations. Cell lysates were resuspended in sample buffer without 2-mercaptoethanol. After electrophoresis on 10% SDS-PAGE gels, proteins were transferred to polyvinylidene difluoride membranes (Amersham, Piscataway, NJ) using a Bio-Rad PowerPac HC (100 V for 70 min) and blocked with 4% nonfat dry milk in TBST. PBMC and cell lines were detected using 1 μg/ml CD80 mAb (clone MIH1; eBioscience) and 0.05 μg/ml β-actin mAb, respectively, followed by 1:5000 dilution of goat anti-mouse HRP (BD Biosciences).

PBMC activation
PBMCs were obtained and then primed and boosted with vaccine cells as described (22, 23) except they were expanded for 7 d with IL-15 (20 ng/ml; PeproTech, Rocky Hill, NJ). Alternatively, PBMCs (1 x 10^7/200 μl) were cocultured with 5 μg/ml PHA (Sigma-Aldrich, St. Louis, MO) and irradiated tumor cells (C8161 and C8161/CD80, or MEL202 and MEL202/CD80, 5000 and 10,000 rads, respectively) at 37°C. 5% CO2 for 72 h. Re-combined human and mouse PDL1–Fc fusion proteins (R&D Systems) were added to some coculture experiments. IFN-γ production was measured by ELISA (23).

Microscopy
Tumor cells were cultured in 6-well plates for 48 h after transfection, then washed with excess PBS containing 2% FCS (2% PBS), labeled with CD80 PE-Cy7 (clone 2D10) for 30 min, and again washed with excess 2% PBS. Live fluorescence images were captured with an Olympus IX-81 (Olympus, Center Valley, PA) microscope using Slidebook software (Intelligent Imaging Innovations, Denver, CO). A minimum of 150 cells/sample was counted.

Statistical analysis
SD and Student t test were calculated using Microsoft Excel version 2008. Mann–Whitney U test was performed using http://faculty.vassar.edu/lowry/VassarStats.html.

Results

Tumor cell expression of CD80 facilitates the boosting of primed T cells
Priming of naive T cells by professional APCs requires two signals, an Ag-specific signal delivered by a peptide–MHC complex and a costimulatory signal. Boosting of primed T cells by professional APCs requires an Ag-specific signal but does not require a costimulatory signal (40). To determine if costimulation is optional in the boosting phase of CD4+ T cells when tumor cells are the APCs, we used uveal melanoma (MEL202) and lung adenocarci-noma (H358) tumor cells transfected with genes encoding HLA-DR and the costimulatory molecule CD80 (B7.1; MEL202/DR1/CD80 and H358/DR7/CD80, respectively). Because of their ability to activate tumor-reactive CD4+ T cells that do not react with nonmalignant tissue, we have called these genetically
modified tumor cells “MHC II vaccines” (22, 25, 41). Priming and boosting HLA-DR” or HLA-DR1” PBMCs with HLA-DR syngeneic MHC II lung adenocarcinoma (Fig. 1A) or uveal melanoma vaccine cells (Fig. 1B), respectively, yielded high levels of IFN-γ. In contrast, priming with syngeneic HLA-DR”CD80” tumor cells and boosting with HLA-DR” tumor cells lacking CD80 induced significantly less IFN-γ. Therefore, if APCs are tumor cells, then costimulation in the boosting phase is essential for optimal T cell activation.

Expression of PDL1 on the cell surface of tumor cells is reduced by transfection of HLA-DR and CD80

Many tumor cells constitutively express or are induced by IFN-γ to express PDL1 on their cell surface (21), raising the possibility that MHC II vaccines may induce T cell apoptosis due to PDL1 expression. To assess this possibility, H358/DR7/CD80 and a mammary carcinoma MHC II vaccine (MCF10/DR7/CD80) (24, 35) and their parental cells were stained for CD80 and PDL1 (mAb 29E.2A3) and analyzed by flow cytometry (Fig. 2A). Parental H358 and MCF10 cells constitutively express PDL1; however, H358/DR7/CD80 and MCF10/DR7/CD80 vaccine cells did not have detectable PDL1. To confirm that the lack of PDL1 on the vaccine cells was not a peculiarity of the 29E.2A3 mAb, H358 and H358/DR7/CD80 cells were stained with two other PDL1 mAbs (mAbs MIH1 and 27A2) (Supplemental Fig. 1A). As for the MIH1 and 27A2 mAbs, most vaccine cells did not express PDL1, whereas most parental cells constitutively expressed PDL1.

To determine if MHC II vaccine cells were refractory to IFN-γ induction of PDL1, uveal melanoma MEL202 and its corresponding vaccine (MEL202/DR1/CD80) were cultured in IFN-γ induction of PDL1, whereas MEL202/DR1/CD80 cells had detectable PDL1. To confirm that the lack of PDL1 on the vaccine cells was not a peculiarity of the 29E.2A3 mAb, parental and vaccine cells were also stained with the MIH1 and 27A2 mAbs (Supplemental Fig. 1A). As for the MIH1 and 27A2 mAbs, most vaccine cells did not express PDL1, whereas most parental cells constitutively expressed PDL1.

To determine if MHC II vaccine cells were refractory to IFN-γ induction of PDL1, uveal melanoma MEL202 and its corresponding vaccine (MEL202/DR1/CD80) were cultured in IFN-γ, and subsequently stained for CD80 and PDL1 (mAb 29E.2A3) and analyzed by flow cytometry. Data are representative of four independent experiments.

**FIGURE 1.** Tumor cell expression of CD80 facilitates the boosting of primed T cells. A. PBMCs from an HLA-DR7” healthy donor were primed in vitro with irradiated H358 lung adenocarcinoma cells transduced with HLA-DR7 and CD80 (H358/DR7/CD80) and boosted with either H358/DR7/CD80, single gene transductants (H358/DR7 or H358/CD80), or parental cells. B. PBMCs from an HLA-DR1” healthy donor were primed with irradiated uveal melanoma cells transduced with HLA-DR1 and CD80 (MEL202/DR1/CD80) and boosted with either MEL202/DR1/CD80, single gene transductants (MEL202/DR1 or MEL202/CD80), or parental cells. IFN-γ production was measured by ELISA. Data are representative of three independent experiments.

**FIGURE 2.** Expression of PDL1 at the cell surface of tumor cells is reduced by transfection of HLA-DR and CD80. A. Expression of HLA-DR and CD80 blocks constitutive expression of PDL1. Parental H358 (adenocarcinoma) and MCF10 (mammary adenocarcinoma) cells and their HLA-DR7 and CD80 transductants were stained for cell surface CD80 and PDL1 (mAb 29E.2A3) and analyzed by flow cytometry. B. Tumor cells expressing HLA-DR and CD80 are not induced by IFN-γ to express PDL1. Parental MEL202 (uveal melanoma) cells and their HLA-DR1 and CD80 transductants were either untreated or cocultured in IFN-γ for 48 h, stained for cell surface CD80 and PDL1 (mAb 29E.2A3), and analyzed by flow cytometry. Data are representative of three independent experiments.

(IFig. 2B). IFN-γ treatment induced cell surface expression of PDL1 on MEL202 cells, whereas MEL202/DR1/CD80 cells did not have detectable cell surface PDL1. To ascertain the absence of cell surface PDL1 on IFN-γ-treated MEL202/DR1/CD80 cells was not a peculiarity of the 29E.2A3 mAb, parental vaccine cells were also stained with the MIH1 and 27A2 mAbs (Supplemental Fig. 1B). In agreement with Fig. 2B, cell surface PDL1 was not detected. PDL1 was similarly induced on the cell surface of uveal melanoma MEL270 and its metastatic derivative OMM2.3 but not detected by the MIH1 mAb if the tumor cells were transfected with CD80 and HLA-DR (data not shown). Therefore, human tumor cells transfected with CD80 and HLA-DR do not constitutively express, nor are they induced by IFN-γ to express, cell surface PDL1.

CD80 inhibits the constitutive and IFN-γ-induced expression of cell surface PDL1 on tumor cells

The absence of detectable PDL1 on the surface of DR”CD80” vaccine cells could be mediated by either HLA-DR or CD80 or could be the result of the transfection, transduction, or drug selection. To distinguish these possibilities, MEL202 cells expressing HLA-DR1 or CD80 were treated with IFN-γ and subsequently labeled with mAbs to CD80 and PDL1 (29E.2A3 mAb) (Fig. 3A). MEL202/DR1 cells had detectable PDL1, whereas MEL202/CD80 cells did not; indicating that IFN-γ-induced cell surface expression of PDL1 was inhibited by coexpression of CD80 and...
FIGURE 3. CD80 blocks PDL1 expression and function

CD80 blocks PDL1 suppressive activity and restores T cell activation

The preceding experiments demonstrate that CD80 prevents detection of PDL1 but do not establish if tumor cell expression of CD80 overcomes PDL1-mediated immune suppression. To test functionality, we compared IFN-γ production by PHA-activated PBMCs with and without various numbers of MEL202, MEL202/CD80, C8161, or C8161/CD80 cells (Fig. 4A). PBMCs cocultured with CD80 transfected tumor cells produced more IFN-γ, consistent with the concept that CD80 prevented PDL1-mediated suppression. To confirm that CD80 increased PBMC activation by inhibiting PDL1-mediated suppression, we compared IFN-γ production by PHA-activated PBMCs cocultured with C8161 or C8161/CD80 in the presence of increasing quantities of recombinant human PD1–Fc fusion protein (Fig. 4B). Recombinant mouse PD1–Fc (mPD1–Fc) was used as a negative control. Inclusion of hPD1–Fc, but not mPD1–Fc, significantly increased IFN-γ production in cultures with C8161 cells. In contrast, there was no significant difference in IFN-γ production in cultures containing C8161/CD80 cells with hPD1–Fc versus mPD1–Fc. Therefore, blocking PDL1 has the same effect as CD80 expression, consistent with the concept that CD80 facilitates T cell activation by inhibiting PDL1–PD1 interactions.

CD80 does not inhibit intracellular expression of PDL1

To determine if CD80 inhibits detection and function of PDL1 by blocking PDL1 transcription, we performed RT-PCR and qPCR on tumor cells that constitutively express PDL1 (C8161) or are induced by IFN-γ to express PDL1 (MEL202). MEL1011 cells, which neither constitutively express nor are induced to express PDL1, and mDCs, which constitutively express PDL1, served as negative and positive controls, respectively (Fig. 5A). PDL1 mRNA was present in constitutive expressers and after IFN-γ induction regardless of CD80 expression, indicating that CD80 does not prevent transcription of PDL1.

To determine if CD80 inhibits translation of PDL1 mRNA, C8161, MEL202, and their CD80 transfectants were fixed, permeabilized, and stained for CD80 and PDL1 (mAb 29E.2A3) to visualize intracellular PDL1 protein (Fig. 5B). In contrast to the absence of PDL1 at the cell surface, C8161/CD80 and IFN-γ-
induced MEL202/CD80 contained intracellular PDL1, and there was no difference in the level of intracellular PDL1 in CD80+ versus CD80− cells. Staining with the MIH1 and 27A2 PDL1 mAbs confirmed intracellular PDL1 in C8161/CD80 and IFN-γ–treated MEL202/CD80 cells (Supplemental Fig. 3A, 3B, respectively). To examine further intracellular expression of PDL1 in CD80+ tumor cells, MEL202/DR1/CD80 cells were transiently transfected with a retroviral PDL1–GFP construct and 48 h later stained for cell surface CD80 and analyzed by fluorescence microscopy (Fig. 5C). PDL1 was present intracellularly and on the cell surface of CD80− cells. In contrast, CD80+ cells contained lower levels of intracellular PDL1 and no PDL1 at the cell surface. In agreement with the intracellular staining and fluorescence microscopy, Western blot analysis revealed PDL1 protein in IFN-γ–treated MEL202 and MEL202/CD80 cells (Fig. 5D). These results demonstrate that although CD80+ tumor cells do not have detectable cell surface PDL1, they contain intracellular PDL1 protein.

PDL1 expression is predominately regulated by the extracellular domains of CD80

CD80 signals intracellularly to modulate dendritic cell (43) and T cell (44, 45) function raising the possibility that CD80 may downregulate PDL1 cell surface expression in tumor cells via its cytoplasmic domain. To test this possibility, two retroviral constructs were made (Fig. 6A). One construct encoded CD80 truncated for 19 of the 21 aa of the C-terminal end cytoplasmic domain (CD80truncated, or CD80tr). The second construct

FIGURE 4. CD80 blocks PDL1 suppressive activity and restores T cell activation. A, T cell suppression is reversed by CD80 inhibition of PDL1 expression. PBMCs were activated with PHA in the presence or absence of varying numbers of irradiated MEL202, MEL202/CD80, C8161, or C8161/CD80 cells, and IFN-γ production was measured by ELISA. B, PBMCs were activated with PHA and analyzed for IFN-γ production as in A in the presence or absence of 1.5–6 μg/ml human or mouse recombinant PDL1–Fc fusion protein and irradiated C8161 or C8161/CD80 cells. PBMC/tumor cell ratio is 1:0.5. Data are representative of three and two independent experiments for A and B, respectively. *p < 0.009.

FIGURE 5. CD80 does not inhibit intracellular expression of PDL1. A, RNA from MEL1011 (cutaneous melanoma), C8161, and C8161/CD80 cells was subjected to RT-PCR using PDL1 primers (left). RNA from mDCs, MEL202, MEL202/CD80, and IFN-γ–treated MEL202 and MEL202/CD80 cells was subjected to qPCR using 18S and PDL1 primers (right). B, Fixed and permeabilized C8161, C8161/CD80, MEL202, and MEL202/CD80 cells were treated with IFN-γ for 48 h prior to fixation, permeabilization, and mAb labeling. C, MEL202/DR1/CD80 cells were transiently transfected with a PDL1–GFP plasmid, plated in 6-well plates, stained with CD80–PE–Cy7 (mAb 2D10), and analyzed by fluorescence microscopy. CD80 is false-colored red. Of 167 cells counted, 18% were CD80+PDL1+, 54% were CD80+PDL1−, and 28% were CD80−PDL1+. D, Western blots of untreated (−) or IFN-γ–treated (+) MEL202 and MEL202/CD80 cells probed for PDL1 (mAb MIH1) and β-actin. mDCs and T2 cells served as PDL1+ and PDL1− controls, respectively. Data are representative of three independent experiments.
ralyzed by flow cytometry. Data are representative of two independent
were stained for cell surface CD80 and PDL1 (mAb 29E.2A3) and ana-
C8161 cells were transiently transfected with plasmids encoding wild-type
HLA-DR
chain (CD80DR). Constitutive PDL1-expressing C8161
cells were transiently transfected with these constructs and 48 h
later stained for CD80 and PDL1 (mAb 29E.2A3) (Fig. 6B).
CD80tr transfectants expressed less cell surface CD80 and a lower
percentage of cells were transfected compared with C8161 cells
transfected with the wild-type CD80 construct. This decreased
expression is most likely due to the known role of the cytoplasmic
domain of CD80 in regulating plasma membrane localization of
CD80 (38). However, C8161 cells expressing the CD80tr and
CD80DR proteins were significantly downregulated for cell
surface PDL1, with maximum downregulation observed in the
transfectants expressing the highest quantity of CD80. Similar
results were obtained using the MIH1 mAb (Supplemental Fig. 4).
In contrast, C8161 cells transiently transfected with the same
vector containing a CD80 gene mutated in its N-terminal extra-
cellular domain were not downregulated for PDL1 expression
(data not shown). These results demonstrate that the extracellular
domains and not the cytoplasmic region of CD80 predominantly
regulate cell surface expression of PDL1.

Discussion
PDL1 is expressed constitutively or is induced by IFN-γ on the cell
surface of most human cancer cells and is credited with protecting
tumor cells against T cell-mediated elimination through its de-
delivery of apoptotic signals to tumor-reactive T cells (3). The
results reported in this article using seven cell lines representing
four histologically distinct solid tumors (lung, breast, cutaneous
melanoma, and uveal melanoma) demonstrate that human tumor
cell coexpression of CD80 reverses PDL1-mediated immune
suppression, enables tumor cell boosting of primed tumor-specific
T cells, and restores T cell activation during priming of tumor-
reactive T cells. CD80 mediates this effect by preventing the expression
of PDL1 protein at the cell surface of the tumor cells and
without altering PDL1 transcription or intracellular expression.

Many cells contain PDL1 mRNA but do not contain PDL1 protein (46). Gong and colleagues (47) have shown that PDL1 protein
can be regulated by microRNA-513 (miRNA-513). miRNA-513 inhibits translation of PDL1 mRNA and is down-
regulated by IFN-γ, consistent with the ability of IFN-γ to up-
regulate PDL1 expression. miRNA-513, however, is unlikely to
play a role in CD80-mediated regulation of PDL1 because CD80-
transfected tumor cells that constitutively express PDL1 or are
IFN-γ-treated to express PDL1 contain intracellular PDL1 protein.

Our studies demonstrate that mechanistically, the extracellular
domains of CD80 regulate PDL1 expression. CD80 and PD1 bind
to the same region of PDL1 (9, 42), raising the possibility that
PDL1 is present on the cell surface, but the extracellular domains
of CD80 sterically block binding of anti-PDL1 mAb and PD1. The
dissociation constant (Kₒ) for CD80–PDL1 binding (∼1.4 μM) is
higher than the Kₒ for PD1–PDL1 binding (∼0.77 μM) (42), in-
dicating that PD1 could compete with CD80 for binding to PDL1.
However, PD1–Fc fusion protein did not bind to CD80-expressing
cells, and CD80+/cells facilitated T cell boosting, suggesting that
PDL1 is physically absent from the cell membrane. Furthermore,
if CD80 is mediating its effect by steric interference, then it is
unclear why CD80 does not also obscure PDL1 detection in the
cyttoplasm of CD80+ cells. If CD80 is not sterically blocking
PDL1 at the cell surface, it may mediate its effect by restricting
trafficking of PDL1 to the cell surface or by facilitating the rapid
turnover of cell surface PDL1.

T cell activation is also inhibited by the binding of CD80 to the
negative regulator CTLA-4 (48), CTLA-4 and PDL1 share a par-
tially overlapping binding site on CD80 (42). As a result of this
common binding site, CD80 can bind either CTLA-4 or PDL1, but
not both molecules concurrently. Therefore, if CD80 is bound to
PDL1, then it may not be able to suppress via CTLA-4. Our data
showing MHC II activation of T cells from healthy donors and
cancer patients is consistent with the concept that the vaccines are
potent T cell activators because they deliver activation signals and
do not suppress via either PDL1–PD1 or CD80–CTLA-4 path-
ways.

The cytoplasmic domain of CD80 is required for CD80 co-
stimulation through CD28 (38) and is needed for correct sub-
cellular localization of CD80 and resultant signaling to CD28
(37). It is also needed for PDL1 reverse signaling and resulting
T cell suppression through CD80 (10). However, the CD80 cy-
toplasmic domain is not required for the downregulation of PDL1
on the cell surface of tumor cells. This discrepancy raises the
possibility that CD80-mediated downregulation of PDL1 could be
functionally separated from the costimulatory effects of CD80 and
therefore might be exploited therapeutically to inhibit PDL1-
mediated tumor cell-induced immune suppression.

CD80-mediated blocking of PDL1 at the cell surface appears to
be a tumor-specific effect because nonmalignant cells such as
dendritic cells and macrophages simultaneously express both
molecules (S. Haile, J. Bosch, and S. Ostrand-Rosenberg, unpub-
lished observations, and Refs. 49, 50). PD-L1 downregulation is also proportional to the level of CD80. Quantity of CD80 may also explain why some human leukemias with low-level expression of CD80 have serologically detectable PDL1 (51).

Regardless of how CD80 deters PD-L1 expression, PD-L1 is not serologically detectable and, more importantly, is functionally absent from the cell surface of tumor cells transfected with CD80. This inhibition of PD-L1 is likely responsible for the ability of the MHC II vaccines to efficiently activate and maintain tumor-specific effector T cells and suggests new therapeutic avenues for preventing tumor cell PD-L1-induced immune suppression.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Figure 1. PDL1 expression at the cell surface of tumor cells is reduced by transfection of HLA-DR and CD80.  

(A, B) Expression of HLA-DR and CD80 blocks constitutive expression of PDL1. Parental H358 and their HLA-DR7 and CD80 transductants were cell surface stained for CD80 and PDL1 (mAb MIH1 or mAb 27A2).  

(B) Tumor cells expressing HLA-DR and CD80 are not induced by IFNγ to express PDL1. Parental MEL202 cells and their HLA-DR1 and CD80 transductants were either untreated or cultured in IFNγ for 48 hrs, and stained for CD80 and PDL1 (mAbs MIH1 and 27A2). Data are from 3 independent experiments.
Supplemental Figure 2. CD80 inhibits the constitutive and IFNγ-induced expression of cell surface PDL1 on tumor cells. (A) CD80 is sufficient to prevent cell surface expression of PDL1 on uveal melanoma cells. MEL202 transductants (MEL202/DR1, MEL202/CD80, and MEL202/DR1/CD80) were cultured with IFNγ for 48 hrs and stained for CD80 and PDL1 (mAbs MIH1 and 27A2). (B) CD80 inhibits IFNγ-induced expression of PDL1 on cutaneous melanoma cells. IFNγ-treated 624MEL and 624MEL/CD80 cells were stained for CD80 and PDL1 (mAbs MIH1 and 27A2). (C) Expression of CD80 blocks constitutive expression of PDL1. C8161 and C8161/CD80 cells were stained for CD80 and PDL1 (mAbs MIH1 and 27A2). Data are from 3 independent experiments.
Supplemental Figure 3. CD80 does not inhibit intracellular expression of PDL1. Fixed and permeabilized C8161, C8161/CD80, MEL202, and MEL202/CD80 cells were internally stained for CD80 and PDL1 (mAbs MIH1 and 27A2). MEL202 and MEL202/CD80 cells were cultured with IFNγ for 48 hrs prior to fixation and permeabilization. Data are representative of 2 independent experiments.
Supplemental Figure 4. PDL1 expression is regulated by the extracellular domains of CD80. C8161 cells were transiently transfected with plasmids encoding wild type CD80, CD80 truncated for the 19 amino acids at its carboxyl terminus (CD80tr), or CD80 containing the cytoplasmic region of HLA-DRA (CD80DR). Forty-eight hrs after transfection, the cells were stained for cell surface CD80 and PDL1 (mAb MIH1) and analyzed by flow cytometry. Data are representative of 2 independent experiments.