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B7-H1–Dependent Sex-Related Differences in Tumor Immunity and Immunotherapy Responses

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CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs) are immunopathogenic in cancers by impeding tumor-specific immunity. B7-homologue 1 (B7-H1) (CD274) is a cosignaling molecule with pleiotropic effects, including hindering antitumor immunity. In this study, we demonstrate sex-dependent, B7-H1–dependent differences in tumor immunity and response to immunotherapy in a hormone-independent cancer, murine B16 melanoma. Antitumor immunity was better in B7-H1−/− females versus males as a result of reduced regulatory T cell function in the B7-H1−/− females, and clinical response following B7-H1 blockade as tumor immunotherapy was significantly better in wild-type females than in males, owing to greater B7-H1 blockade-mediated reduction of Treg function in females. Wild-type female Tregs expressed significantly lower B7-H1 versus males but were insensitive to estrogen in vitro. Female B7-H1−/− Tregs were exquisitely sensitive to estrogen-mediated functional reduction in vitro, suggesting that B7-H1 effects occur before terminal Treg differentiation. Immune differences were independent of known B7-H1 ligands. Sex-dependent immune differences are seldom considered in designing immune therapy or interpreting immunotherapy treatment results. Our data demonstrate that sex is an important variable in tumor immunopathogenesis and immunotherapy responses through differential Treg function and B7-H1 signaling. The Journal of Immunology, 2010, 185: 2747–2753.

Women generally exhibit more robust immunity than men postinfection (1) and increased allograft rejection (2) and experience a generally greater risk for autoimmunity (3). Perhaps because estrogens are anti-inflammatory (3), studies of male–female immune differences tend to focus on inflammatory pathways, such as through TLRs (4, 5).

B7-homologue 1 (B7-H1) is a cosignaling molecule abundantly expressed on APCs and other immune cells (6). It contributes to tumor immune evasion (7–9) and to induced T regulatory cell (Treg) function (10, 11). We found that B7-H1–mediated Treg function is modulated in an estrogen-dependent manner; therefore, we examined sex-dependent Treg functional differences in cancer given the central role that Tregs play in tumor immunopathology (10, 12–14). We hypothesized that B7-H1 signals would differentially affect female versus male tumor immunity and that response to B7-H1 blockade as cancer immunotherapy (9) would consequently be more effective in females. We tested hypotheses using B16 melanoma, a well-described, transplantable tumor without known hormonal influences on its growth or induced immunity, and which responds favorably to immunotherapy (15). B16 lacks a Y chromosome (16); thus, immunity to it is not influenced by minor sex-related antigenic differences. Premenopausal women have a greater melanoma risk compared with age-matched men, but this trend later reverses, such that men >50 y old have a greater melanoma risk compared with age-matched women. Many factors aside from immunity, including hormonally controlled genetic repair mechanisms, could play roles in these sex-associated disparities (17).

We showed that B7-H1−/− females resisted syngeneic B16 melanoma tumor better than males as a result of reduced Treg function, which allowed the development of superior antitumor immunity. Strikingly, anti–B7-H1 blockade was significantly more clinically effective in wild-type WT females than in WT males as a result of greater female B7-H1 blockade-mediated reduction in Treg function. B7-H1 expression on naive WT female Tregs was significantly lower than in naive males, but it did not alter Treg suppression in the presence of estrogen in vitro. By contrast, female B7-H1−/− Tregs were exquisitely sensitive to estrogen-mediated reduction in suppression. Effects are not dependent on programmed death-1 (PD-1) or CD80, the known ligands of B7-H1 (18), suggesting a novel B7-H1 signaling pathway. These data demonstrate an unexpected B7-H1–dependent, sex-related difference in Treg function that causes sex-dependent, B7-H1–mediated differences in tumor immunity and immunotherapy responses.

Materials and Methods

Mice

All mice were on the C57/BL6 (BL6) background. WT mice were purchased from the National Cancer Institute (Bethesda, MD), CD80−/− and MHC class I-restricted OVA-specific TCR transgenic (OT-I) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B7-H1−/−, PD-1−/−, and Foxp3–internal ribosome entry site-monomeric red fluorescent protein (FIR)
mice were gifts from Lieping Chen (The Johns Hopkins University, Baltimore, MD), Tatsuki Honjo (Kyoto University, Sakyoku-ku, Kyoto, Japan), and Richard A. Flavell (Yale University, New Haven, CT), respectively. All mice were housed under specific pathogen-free conditions and used at 6–10 wk of age.

**Abs**

Anti-CD45RB (16A), anti-CTLA-4 (UC10-4F10-11), anti–glucocorticoid-induced TNFR (DTA-1), anti–IFN-γ (XMG12), anti-CD25 (PC61), anti-CD4 (GK1.5), anti-CD3 (500A2), anti-CD11c (HL-3), anti–B7-H1 (MH5), and matched isotype control Abs were from BD PharMingen (San Diego, CA). Anti-Foxp3 (FJK-16a), anti-CD62L (MEL14), anti–IL-10 (JES5-16E3), and anti–granelyme B (16G6), and respective matched isotype control Abs were from eBioscience (San Diego, CA). Anti-CD80 (SH10) and control isotype Abs were from CalTag Laboratories (Burlingame, CA). Polyclonal anti-neuropilin-1 and monoclonal anti–TGF-β Abs were purchased from R&D Systems (Minneapolis, MN). PE-conjugated OVA-specific pentamers were purchased from ProImmune (Oxford, U.K.). Intracellular staining was performed according to the manufacturer’s instructions. Data were acquired on an LSR II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR).

**Cell lines and tumor induction**

B16F10 melanoma, an X chromosome monoyzogotic tumor (16), was purchased from the American Type Culture Collection (Manassas, VA). ID8 epithelial ovarian carcinoma was the gift of George Coukos (University of Pennsylvania, Philadelphia, PA). B16F10 was transfected with a plasmid encoding GFP-OVA. OVA expression was confirmed by ELISA (Supplemental Fig. 1). A total of 1.25 × 107 ID8 cells were injected into sterilized flanks intradermally with a 27-gauge needle. Intradermal tumor size was measured using Vernier calipers and confirmed by histologic analysis. Tumor volume was calculated as width × (length)2 × 0.5. A total of 1 × 105 ID8 cells were injected into the peritoneum. Ascites was harvested aseptically, and dendritic cells (DCs) were isolated as described (20). Briefly, 100 μg/ml Liberase (research grade, Roche, San Francisco, CA) and 0.2 mg/ml DNAse (Roche) mild digestion was applied to release DCs from murine TDLN fragments at 37˚C for 30 min. CD11c+ cells were then positively selected (>90% purity by FACS) using EasySep mouse CD11c positive selection kits (StemCell Technologies, Vancouver, British Columbia, Canada). A total of 25,000 CD11c+ DCs were pulsed with SIINFEKL (10 ng/ml) or nothing for 1 h and incubated with 50,000 CFSE-labeled, sex-matched OT-I cells for 72 h. CFSE dilution and IFN-γ expression were assessed by flow cytometry, gating on CD3+ cells.

**Adoptive cell transfers**

One third of the control OT-I cells in OT-I mouse spleens were labeled with CFSE, as described above, and injected i.v. into mice. The next day, B16 tumor was given, as described above, and the mice were sacrificed 4 d later to assess OT-I cell proliferation by flow cytometry. Cells from FIR mice were sorted as CD4+RFP+ or CD4+RFP- in the CD3 gate, and 2 × 106 cells per mouse were given i.v. in PBS. B16 tumor was given the next day. At sacrifice, spleen cells were analyzed by flow cytometry or were sorted for ex vivo functional tests.

**In vivo treatments**

Endotoxin-free anti–B7-H1 Ab (10F9G2) or isotype control IgG2b Ab (both from BioLegend, San Diego, CA) was administered i.p. at 200 μg/mouse every 3 d starting 1 d before tumor challenge and until sacrifice. Denileukin difitox (DT; Eisai, Research Triangle Park, NC) was given at 5 μg/mouse twice weekly, starting 4 d after tumor challenge until sacrifice.

**Statistical analysis**

Data are expressed as mean ± SEM. The Student t test or ANOVA was performed, as appropriate, with two-tailed p < 0.05 considered significant.

**Results**

B16 cells engineered to express OVA (OVA+B16 is hereinafter “B16”; see Supplemental Fig. 1 and Materials and Methods) grew equally in WT males and females, but in support of our hypothesis,

![FIGURE 1. B7-H1−/− females resist B16 better than males through superior antitumor immunity independent of PD-1 or CD80. A, Groups of five mice were challenged with 125,000 B16 cells/flank. Tumor growth was measured with Vernier calipers. B, Tumor-specific immunity was assessed as proliferation of CFSE-labeled, adoptively transferred OT-I cells using flow cytometry 4 d after transfer. C, Groups of five mice each were challenged with B16, as above. D, Treg (CD4+CD25hi T cell) function was tested in groups of five naive mice each in vitro. DLN, draining lymph node; SPL, spleen.](http://www.jimmunol.org/)

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they grew significantly slower in B7-H1−/− females versus B7-H1−/− males (p = 0.037) and versus WT females or males (Fig. 1A). Minor male histocompatibility Ags cannot explain these results because B16 is X chromosome monochromatic and lacks a Y chromosome (16). Tumor (ova)-specific T cells were not detected in untreated B16-bearing WT mice of either gender (data not shown). Supporting an immune-based mechanism for differential tumor growth, we detected CD8+ tumor-specific T cells in vivo in three of three B7-H1−/− females versus zero of three B7-H1−/− males (Fig. 1B, Supplemental Fig. 2). Supporting microenvironment-specific effects, tumor-specific CD8+ T cells were generated in TDLNs but not spleen (Fig. 1B). PD-1 and CD80 are the two known B7-H1 ligands (18). However, there was no significant difference in tumor growth (Fig. 1C) or Treg function (Fig. 1D, Supplemental Fig. 3) in WT versus PD-1−/− or CD80−/− mice of either sex, suggesting a novel B7-H1 ligand mediating Treg effects.

Naive B7-H1−/− males and females have comparable numbers of phenotypic CD4+CD25+Foxp3+ Tregs, although Treg function is significantly reduced in vitro and in vivo in naive B7-H1−/− females (P.-Y. Lin, L. Sun, V. Hurez, S. Thibodeaux, R. Vadlamudi, M. Kious, C. Livi, R. Bahar, M.E. Wierman, R.R. Tekmal, A. Pierce, B.J. Daniel, and T.J. Curiel, submitted for publication). To test reduced Treg function as a mechanism for improved immunity in B7-H1−/− females, we depleted Tregs in tumor-bearing mice using the IL-2–diphtheria fusion toxin DT (21). DT equally depleted phenotypic CD4+CD25+Foxp3+ Tregs, although Treg function is significantly reduced in vitro and in vivo in naive B7-H1−/− males, with no effect on low baseline Treg function in B7-H1−/− females (Fig. 2B). Treg functional depletion was accompanied by significantly reduced tumor growth (Fig. 2C) and improved tumor-specific immunity (Fig. 2D, Supplemental Fig. 5) in B7-H1−/− males, but not females, supporting reduced Treg function as the basis for female B7-H1−/− tumor resistance and improved antitumor immunity. To test possible differential susceptibility to Treg-mediated regulation of female B7-H1−/− effector T cells in the tumor microenvironment, we sorted Tregs from tumor-bearing B7-H1−/− females and tested their capacity to suppress B7-H1−/−CD4+CD25− effector T cells from naive or tumor-bearing B7-H1−/− females. B7-H1−/−CD4+CD25− effector T cells from naive or tumor-bearing mice were equally susceptible to regulation by tumor-associated B7-H1−/− Tregs (Fig. 2E). These data support the concept that reduced susceptibility of tumor effector cells to Treg-mediated suppression did not contribute to differential sex-based immune and clinical responses to tumor, as well as demonstrate that effector cell B7-H1 is dispensable for their regulation by Tregs.

To test possible functional differences between female and male B7-H1−/− Tregs, we assessed granzyme B, IL-10, and TGF-β expression but found them comparable in mean fluorescence intensity and expressing other molecules associated with Treg function (12, 14), including CTLA-4, glucocorticoid-induced TNFR, and neuropilin-1, in both sexes in spleen and TDLNs (Fig. 2A, Supplemental Fig. 4). Despite equal DT-mediated phenotypic Treg depletion, DT reduced Treg function in B7-H1−/− males, with no effect on low baseline Treg function in B7-H1−/− females (Fig. 2B). Treg functional depletion was accompanied by significantly reduced tumor growth (Fig. 2C) and improved tumor-specific immunity (Fig. 2D, Supplemental Fig. 5) in B7-H1−/− males, but not females, supporting reduced Treg function as the basis for female B7-H1−/− tumor resistance and improved antitumor immunity. To test possible differential susceptibility to Treg-mediated regulation of female B7-H1−/− effector T cells in the tumor microenvironment, we sorted Tregs from tumor-bearing B7-H1−/− females and tested their capacity to suppress B7-H1−/−CD4+CD25− effector T cells from naive or tumor-bearing B7-H1−/− females. B7-H1−/−CD4+CD25− effector T cells from naive or tumor-bearing mice were equally susceptible to regulation by tumor-associated B7-H1−/− Tregs (Fig. 2E). These data support the concept that reduced susceptibility of tumor effector cells to Treg-mediated suppression did not contribute to differential sex-based immune and clinical responses to tumor, as well as demonstrate that effector cell B7-H1 is dispensable for their regulation by Tregs.

FIGURE 2. Defective Treg function in B7-H1−/− females contributes to increased tumor resistance and superior antitumor immunity. Groups of five mice were challenged with 125,000 B16 cells/flank, treated with DT 5 μg or PBS twice a week beginning 4 d later, and sacrificed 17 d after tumor challenge. Effects of DT on phenotypic Treg depletion in spl and D LN (A), Treg (CD4+CD25+ T cell) function (B), and tumor growth (C) were determined. D, Effects of DT on tumor-specific immunity was assessed by OT-I cell proliferation using flow cytometry as for Fig. 1B. E, Tregs were obtained from spleens of B16-bearing B7-H1−/− females and tested for regulation of B7-H1−/−CD4+CD25− Eff from naive or tumor-bearing females. F, Granzyme B, IL-10, and TGF-β expression gated on CD3+CD4+CD25+Foxp3+ cells in B16-bearing mice. n = 6/group. G, OT-I cell proliferation and IFN-γ expression induced by CD11c+ DCs obtained from spleens and D LNs of B16-bearing mice 7 d after tumor challenge. n = 3/group. Control mice received DCs with no peptide. DLN, draining lymph node; Eff, effector T cell; OVAp, DC loaded with SIINFEKL peptide; SPL, spleen.
in the percentage of positive cells among CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells (Fig. 2F). To test the possibility that altered APC function could explain sex-based immune differences, we obtained CD11c<sup>+</sup> DCs from B16-bearing mice 7 d after tumor challenge and assessed their ability to activate OT-I cell proliferation and IFN-γ expression (Fig. 2G). Non-tumor microenvironmental (spleen) CD11c<sup>+</sup> DCs from tumor-bearing B7-H1<sup>−/−</sup> males and females activated OT-I cell proliferation similarly. DCs from female TDLNs activated OT-I cells statistically significantly better than male DCs, although the magnitude of the difference was probably not biologically relevant. Female B7-H1<sup>−/−</sup> spleen DCs activated OT-I cell IFN-γ significantly better than did male spleen APCs, whereas IFN-γ activation from TDLNs using DCs from either sex was indistinguishable. Together, these data suggest that female B7-H1<sup>−/−</sup> DCs may function slightly better versus male B7-H1<sup>−/−</sup> DCs, but the net effect on tumor immunity may not be very significant.

To investigate further whether defective Tregs contributed to improved antitumor immunity in B7-H1<sup>−/−</sup> females, we adaptively transferred RFP<sup>+</sup>CD4<sup>+</sup> T cells from female homozygous FIR mice into B7-H1<sup>−/−</sup> females and challenged them with B16. RFP<sup>+</sup>CD4<sup>+</sup> T cell recipients exhibited significantly faster tumor growth (Fig. 3A), together with impaired tumor-specific immunity and CD8<sup>+</sup> T cell IFN-γ production (Fig. 3B), compared with tumor-bearing littermates receiving CD4<sup>+</sup>RFP<sup>+</sup> (non-Treg) T cells. CD4<sup>+</sup>RFP<sup>+</sup> cell recipients had greater percentages of CD4<sup>+</sup>Foxp3<sup>+</sup> T cells compared with CD4<sup>+</sup>RFP<sup>−</sup> cell recipients (Fig. 3C), consistent with increased Treg content. CD4<sup>+</sup>RFP<sup>+</sup> cells recovered from tumor-bearing CD4<sup>+</sup>RFP<sup>+</sup> cell recipients (transferred Tregs) were significantly more suppressive compared with CD4<sup>+</sup>CD25<sup>+</sup>RFP<sup>+</sup> T cells (endogenous Tregs; Fig. 3D), demonstrating that transferred (WT) Treg function was superior to endogenous (B7-H1<sup>−/−</sup>) Treg function and was maintained in the female B7-H1<sup>−/−</sup> tumor environment. By comparing Treg function in tumor-bearing mice with or without RFP<sup>+</sup> cell transfer, we further demonstrated that adoptively transferred functional Tregs did not boost Treg function in endogenous Tregs (compare RFP<sup>−</sup> [endogenous] Treg function in RFP<sup>+</sup> recipients in Fig. 3D with endogenous Treg function in tumor-bearing B7-H1<sup>−/−</sup> females not receiving any cell transfers in Fig. 3E), suggesting that female B7-H1<sup>−/−</sup> Tregs resist infectious tolerance (22). Some transferred CD4<sup>+</sup>RFP<sup>+</sup> cells converted into functionally suppressive CD4<sup>+</sup>RFP<sup>+</sup> Tregs in vivo in tumor-bearing B7-H1<sup>−/−</sup> female recipients (Fig. 3D), demonstrating that inducible Treg (23) conversion occurred in the absence of host B7-H1 but to an extent ineffective in significantly altering tumor growth or antitumor immunity in these studies. Together, these data are consistent with a causal role for Tregs in mediating the immune and clinical effects observed. B16 cells also express B7-H1 (Supplemental Fig. 6), which could affect Treg differentiation or function (10). However, Treg function in tumor-bearing B7-H1<sup>−/−</sup> females remained significantly lower than in tumor-bearing B7-H1<sup>−/−</sup> males (Fig. 3E), which was also comparable to the relative differences in Tregs from naive B7-H1<sup>−/−</sup> males and females (P.-Y. Lin et al., submitted for publication). Altogether, these data establish defective Treg function as a basis for sex-dependent differential tumor immunity in a B7-H1−/− system.

**FIGURE 3.** Adoptively transferred, functional female WT Tregs reverse immune and clinical differences in B7-H1<sup>−/−</sup> females. RFP<sup>+</sup> or RFP<sup>−</sup> CD4<sup>+</sup> T cells were sorted from female FIR mice and transferred into B7-H1<sup>−/−</sup> females. A, Mice were challenged with B16 the day after transfer. At sacrifice 14 d after tumor challenge, flow cytometry was used to detect CD8<sup>+</sup> T cell IFN-γ production (β) and tumor-specific CD8<sup>+</sup> T cells (OVA pentamer stain) or (C) Foxp3 expression in CD4<sup>+</sup>CD25<sup>+</sup> spleen cells. The percentage positive gated events is shown. D, Functional status of endogenous, transferred, or converted Tregs in B16-bearing mice was tested 15 d after adoptive transfer. E, Endogenous Treg (CD4<sup>+</sup>CD25<sup>+</sup> T cell) function was tested 14 d after B16 tumor challenge in mice not receiving adoptive cell transfers. F, Treg function 28 d after i.p. ID8 tumor challenge with 10 × 10<sup>6</sup> ID8 cells. G, Ascites development (>30% weight increase) in mice challenged with ID8 tumor.
dependent manner. We next challenged WT or B7-H1−/− females (n = 12) with syngeneic ID8 epithelial carcinoma by i.p. injection. Consistent with results using B16, Treg function remained defective in B7-H1−/− females (Fig. 3F, Supplemental Fig. 7) following ID8 tumor challenge. Also consistent with reduced Treg function in B7-H1−/− females, tumor ascites, a clinical surrogate for tumor burden, following ID8 challenge developed more slowly than in WT females (Fig. 3G), confirming that sex-based differences in tumor Treg function and tumor survival are not specific to B16 melanoma nor to the intradermal anatomic compartment.

Based on the preceding data, we hypothesized that WT females would benefit more than males from B7-H1 blockade as tumor immunotherapy. We tested this hypothesis by treating B16 tumor-bearing WT males or females with anti–B7-H1 Ab. Strikingly, but consistent with data from tumor-bearing B7-H1−/− mice (Fig. 1), anti–B7-H1 Ab reduced tumor growth to a greater degree in WT females compared with males (Fig. 4A). Supporting our proposed mechanism, anti–B7-H1 Ab treatment reduced Treg function significantly greater in tumor-bearing WT females compared with WT males (Fig. 4B). Anti–B7-H1 treatment augmented antitumor immunity in WT males and females, consistent with the treatment effects seen (Fig. 4A), but the relative increase in tumor-specific T cell prevalence was greater in anti–B7-H1–treated females, as were their absolute numbers and relative increase following B7-H1 blockade (Fig. 4C), consistent with the enhanced treatment effect in females. Because B7-H1 blockade affected males and females differently, we next tested for sex-dependent B7-H1 expression differences. WT naive female CD4+CD25hi Tregs expressed significantly lower B7-H1 versus WT males, although this difference was unaffected by 10−8 M (physiologic concentration) estrogen (Fig. 4D). We then tested B7-H1 expression in tumor-bearing WT males and females (Fig. 4E). Surprisingly, WT male and female Treg B7-H1 expression in tumor was equivalent (and also equivalent to naive male WT). Thus, tumor factors likely augment female B7-H1 Treg expression as we previously reported for tumor DC B7-H1 expression (7). Finally, to help understand why female B7-H1−/− Tregs were functionally defective, we incubated them with 10−8 M E2, which completely abolished their function. The estrogen receptor antagonist ICI182,780 rescued estrogen-mediated reduction of Treg suppression (Fig. 4F), demonstrating that estrogen receptor signaling is involved. By contrast, female WT Treg function was not significantly affected by 10−8 M (Fig. 4F) or supraphysiologic 10−7 M (data not shown) E2.

**Discussion**

Interest in immune therapy for cancer is resurging as the result of a better understanding of the underlying immune dysfunction that must be corrected for improved clinical efficacy (12). Nonetheless, advances in some areas have lagged. Notably, there is little published regarding potential sex-based differences in antitumor immunity or potential sex-based differential responses to tumor immunotherapy. Such differences are likely to exist based on the well-known sexual dimorphisms in immunity in males and females (3).

We studied potential sexual dimorphisms using B16 melanoma because it has no known hormonal influences on its growth or induced immunity (15). We studied anti–B7-H1 Ab treatment as
a known antitumor immunotherapeutic agent (9) and established that B7-H1 blockade was more effective in treating B16 melanoma in WT females versus males, which was due, in part, to the greater ability of anti-B7-H1 Ab to reduce Treg function in WT females. We recently showed that B7-H1 seems to desensitize Tregs to estrogen-mediated functional reduction through altering Treg mammalian target of rapamycin (mTOR) and phosphatase and tensin homolog signals (P.-Y. Lin et al., submitted for publication). However, because males derived some benefit from B7-H1 blockade, factors in addition to inhibited Treg function are likely also involved in treatment effects.

Based on the sexually dimorphic response to B7-H1 blockade, including significantly reduced Treg function in B7-H1−/− females, we hypothesized that female B7-H1−/− mice would resist a transplantable tumor challenge better than males, by virtue of improved antitumor immunity. Our data support this concept by showing that B16 grew more slowly in B7-H1−/− females because they had superior antitumor immunity. This resistance to tumor growth was lost upon transfer of functional Tregs into B7-H1−/− females, reducing tumor-specific immunity. These data establish reduced Treg function as a basis for improved antitumor immunity in females in the presence of deficient B7-H1 signals. Female B7-H1−/− DCs in tumor functioned slightly better compared with male B7-H1−/− DCs in activating tumor-specific immunity, but the net effect on tumor immunity may not be very significant based on the relatively small magnitudes of differences. Nonetheless, we cannot exclude improved DC performance as contributing to improved female antitumor immunity in B7-H1−/− deficiency. However, because functional Treg transfer into B7-H1−/− females recapitulated the reduced antitumor immunity observed in B7-H1−/− and WT males, improved DC performance in B7-H1−/− females could be due to their reduced Treg function, with reduced capacity, thereby, to degrade DC function in tumors (14).

Anti–B7-H1 Ab significantly reduced tumor growth in WT males and WT females bearing B16 melanoma, consistent with its ability to reduce Treg function in males and females and consistent with known beneficial anti–B7-H1 effects on other elements of antitumor immunity (9). Nonetheless, anti–B7-H1 Ab reduced Treg function significantly greater in WT females versus WT males in vivo, consistent with sex-dependent B7-H1 signaling. Strikingly, E2 essentially eliminated female B7-H1−/− Treg function in vitro, whereas E2 had no discernible effect on WT Treg function. These data suggest that E2 effects can be directly on Tregs and that effects likely occur prior to terminal Treg differentiation. We recently demonstrated that Tregs differentiating in the presence of E2 in a B7-H1−/−-deficient environment are highly sensitive to E2-mediated functional inhibition (P.-Y. Lin et al., submitted for publication), which accounts for these observations. Prior work demonstrated that PD-1 blockade is beneficial to antitumor immunity (24). Nonetheless, PD-1−/− mice did not phenocopy the sexually dimorphic responses we now demonstrate. Thus, PD-1 effects may not exhibit sexual dimorphism as does B7-H1, or not, in this setting; thus, PD-1 blockade could benefit a different subset of tumors or clinical scenarios. We previously reported that B7-H1 signals in the tumor environment induce T cell IL-10 and reduce T cell IFN-γ, inhibiting antitumor immunity (7). The effects were not through known B7-H1 receptors. Thus, B7-H1 was reported to exert detrimental effects in tumor that are not through known receptors, as we extend in the present work. Our data also suggest that known beneficial effects of anti–PD-1 in tumors may not always involve ligation with B7-H1. Additional factors aside from differential Treg effects could also help to explain the sexually dimorphic differences, but they remain to be discovered.

A sex-dependent difference in response to tumor immunotherapy based on a sex-dependent difference in Treg function predicts that males and females will respond differently to certain immunotherapies, a fact not incorporated into most current trial designs (3). For example, because depleted Tregs in tumor regenerate rapidly (25), our data suggest that combining Treg depletion with B7-H1 blockade could be more beneficial as antitumor immunotherapy in females versus males, or when B7-H1 blockade plus Treg depletion are combined with an E2 agonist. Our use of transplantable tumors demonstrated important proofs of principle regarding antitumor immunity and tumor immunotherapy. Studies using autochthonous tumor will yield additional information regarding potential sexual dimorphism in antitumor immune surveillance. For example, antiestrogens reduce male risk for hepatocarcinoma in a mouse model for immune tumor surveillance, attributed to anti-inflammatory effects (4).

Our data finally suggest a rethinking of the modes of action of certain agents. For example, antiestrogens, long used to treat hormone-sensitive tumors, also affect Treg function (26, data presented in this study). Thus, Treg effects as an additional mechanism of action of antiestrogens should be explored further. The B7-H1−/−-associated Treg defect we report depends on estrogen-dependently sexual dimorphic differential mTOR and phosphatase and tensin homolog Treg signaling (P.-Y. Lin et al., submitted for publication). Thus, mTOR inhibitors, now undergoing significant clinical trials as anticancer agents, in addition to uses as immune modulators in autoimmunity, graft rejection, and other indications (27, 28), likewise warrant a reanalysis for potential sexually dimorphic effects.

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Disclosures
The authors have no financial conflicts of interest.

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