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Maternal PD-1 Regulates Accumulation of Fetal Antigen-Specific CD8⁺ T cells in Pregnancy

Elizabeth S. Taglauer^{1,2}, Thomas M. Yankee³, and Margaret G. Petroff^{1,2,*}

²Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA

³Department of Microbiology, Molecular Genetics and Immunology, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA

Abstract

The failure to reject the semi-allogeneic fetus suggests that maternal T lymphocytes are regulated by potent mechanisms in pregnancy. The T cell immunoinhibitory receptor, Programmed Death-1 (PD-1), and its ligand, B7-H1, maintain peripheral tolerance by inhibiting activation of self-reactive lymphocytes. Here, we investigated the role of the PD-1/B7-H1 pathway in maternal tolerance of the fetus. Antigen-specific maternal T cells both proliferate and upregulate PD-1 *in vivo* at mid-gestation in response to paternally inherited fetal antigen. In addition, when these cells carry a null deletion of PD-1, they accumulate excessively in the uterus-draining lymph nodes ($P < 0.001$) without a concomitant increase in proliferation. *In vitro* assays showed that apoptosis of antigen-specific CD8⁺PD-1^{-/-} cells was reduced following peptide stimulation, suggesting that the accumulation of these cells in maternal lymph nodes is due to decreased cell death. However, the absence of neither maternal PD-1 nor B7-H1 had detectable effects on gestation length, litter size, or pup weight at birth in either syngeneic or allogeneic pregnancies. These results suggest that PD-1 plays a previously unrecognized role in maternal-fetal tolerance by inducing apoptosis of paternal antigen-specific T cells during pregnancy, thereby controlling their abundance.

1. Introduction

Hemochorial placentation is a highly efficient anatomical arrangement for the transport of maternal nutrients to the developing fetus *in utero*. However, this type of placentation also creates a particularly intimate association between the maternal immune system and the immunologically disparate fetus. Continuous bathing of trophoblast cells in maternal blood allows hematogenous access of fetal antigens to maternal secondary lymphoid organs, possibly by the shedding of trophoblast debris and apoptotic bodies into the maternal circulation (Erlebacher et al., 2007; Taglauer et al, 2008a). Indeed, concurrent with the onset of a maternal blood supply to the placenta, fetal antigen can be detected in the maternal spleen and lymph nodes, and in response, antigen-specific peripheral T lymphocytes proliferate (Erlebacher, et

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*Correspondence: Margaret G. Petroff, Department of Anatomy and Cell Biology, University of Kansas Medical Center, 3901 Rainbow Blvd., Mail Stop 3038, Kansas City, KS 66160-7400. Tel: 913 588 2781; Fax: 913 588 7180; email: mpetroff@kumc.edu .

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al., 2007). This maternal immunological recognition of the fetus would seem to pose the risk of an immune response against the fetus. However, the co-evolution of these two systems has ensured accommodation of this apparent paradox, allowing the fetus to thrive.

Numerous overlapping immunomodulatory mechanisms appear to be instrumental in controlling maternal leukocytes during pregnancy, including the B7 family of immune cell costimulatory molecules (Petroff, 2005). B7 molecules are cell surface proteins belonging to the immunoglobulin superfamily, and they bind to CD28 family receptors on leukocytes to influence their activation, either negatively or positively. One B7 family member, B7-H1, inhibits T lymphocyte activation through engagement of its receptor, PD-1 (Freeman, et al., 2000). PD-1 is expressed on activated lymphocytes in blood, peripheral lymphoid organs, and tissues undergoing an immune response (Ishida, et al., 1992; Agata, et al., 1996). Consequences of B7-H1/PD-1 interactions include suppression of proliferation, alteration of cytokines, and induction of apoptosis (Freeman, et al., 2000; Hori, et al., 2006; Keir, et al., 2007).

The suppressive action of PD-1 is also demonstrated through *in vivo* studies in which targeted mutation or blockade of PD-1 results in spontaneous tissue-specific autoimmune disease (Nishimura, et al., 1999, 2001; Martin-Orozco, et al., 2006; Keir, et al., 2007). This inhibitory receptor is also involved in preventing allograft rejection (Tanaka, et al., 2007; Wang, et al., 2007; Yang, et al., 2008). Like PD-1-deficient animals, B7-H1^{-/-} mice are susceptible to experimentally induced autoimmune diseases (Dong, et al., 2004; Latchman, et al., 2004; Keir, et al., 2006). PD-1 expressing T cells in the decidua are susceptible to modulation of cytokine production by B7-H1 (Taglauer et al., 2008). Finally, the absence of B7-H1 in pregnant dams was shown to reduce the survival of semi-allogeneic fetuses in one study (Guleria, et al., 2005). The B7-H1/PD-1 pathway may thus have a central function in maintaining immunological tolerance to self tissues and foreign grafts, including the fetal allograft.

In this study we investigated the physiological function of maternal PD-1 in pregnancy using murine T cell receptor transgenic and knockout models. Based on the published evidence of the central role of PD-1 in tolerance to self and foreign tissues, we hypothesized that this receptor has an important function in maternal tolerance of the fetal allograft.

2. Materials and Methods

2.1 Mice

PD-1^{-/-} and B7-H1^{-/-} mice on the C57BL/6 (B6) background were gifts from the laboratories of T. Honjo (Kyoto University) and L. Chen (Johns Hopkins University) respectively (Nishimura, et al., 1998; Dong, et al., 2004). Wild type (WT) B6, BALB/c, CBA/J, B6-Tg (Tcr α Tcr β)l 100Mjb/J (OT-I), and B6-Tg(ACTB-OVA)916Jen/J (OVA-Tg) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). All mice were housed under pathogen-free conditions. For adoptive transfer experiments, PD-1^{+/-} OT-I and PD-1^{-/-} OT-I mice were generated by crossing OT-I with PD-1^{-/-} mice. PD-1^{-/-} and B7-H1^{-/-} mice were genotyped using primers specific for PD-1 (Fwd: ACC AGG AAC TCC CCG TTA GT; Rev: TAT TTA GGG TGC AGC CTC GT), B7-H1, and neomycin (Dong, et al., 2004). OT-I and OVA-Tg mice were genotyped using protocols from Jackson Laboratories. Surface protein phenotypes of PD-1^{-/-} and B7-H1^{-/-} mice were confirmed by stimulation of splenocytes with 3 μ g/mL concanavalin A (Sigma-Aldrich, St. Louis, MO, USA) for 72 h followed by flow cytometry. All experimental protocols were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

2.2 Breeding experiments and assessment of fecundity

Virgin WT or PD-1^{-/-} females were bred either syngeneically with B6 (H2b) males or allogeneically to BALB/c (H2d) males. Similarly, virgin WT or B7-H1^{-/-} female mice were mated syngeneically with B6 or allogeneically to CB A/J (H2k) males to examine the role of maternal B7-H1 in pregnancy. The day on which a vaginal plug was noted was designated as gestation day (gd) 0.5. Evaluation of reproductive success included enumeration of healthy and resorbed implantation sites on gd 13.5 by examination of gravid uteri after extraction from the maternal abdominal cavity. Resorbed sites were identified by their small size and associated blood clots. Fecundity was further evaluated by examining gestation length, litter size, pup weight at birth as well as pup weight and male:female ratios at weaning (postnatal day 21).

2.3 Adoptive transfer experiments

For adoptive transfer experiments, virgin B6 female mice were bred with either B6 WT (WT-bred) or OVA-Tg (OVA-bred) males. To obtain OT-I cells for adoptive transfer, splenocytes were isolated from virgin female WT OT-I or PD-1^{-/-} OT-I mice. OT-I surface phenotype was verified by flow cytometry in all experiments. Prior to adoptive transfer, 80–100 × 10⁶ splenocytes were labeled with 5 µg/mL carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions so that their proliferation could be tracked *in vivo* (Lyons and Parish, 1994). 10 × 10⁶ CFSE-labeled splenocytes were transferred into WT- or OVA-bred recipient female mice on gd 10.5 via injection into the tail vein. Injection consistency was monitored by calculating the percentage of undivided (CFSE^{bright}) non-antigen-specific cells/total para-aortic (PALN) lymphocytes, and did not differ significantly between experimental groups (*P* = 0.569, data not shown). On gd 13.5, PALN and spleen cells from recipient female mice were isolated and subjected to flow cytometric analysis for quantification of OT-I cells and evaluation of proliferation.

2.4 In vitro apoptosis and proliferation assays

Splenocytes from virgin female WT OT-I or PD-1^{-/-} OT-I mice were isolated and placed into 24-well tissue culture plates at a density of 1 × 10⁶ cells/well. Cells were treated with either medium alone or 100 ng/mL of the peptide SIINFEKL (Ser-He-He-Asn-Phe-Glu-Lys-Leu), which is the eight amino acid epitope of ovalbumin (amino acids 257–264) that, when presented by the class I MHC molecule H-2K^b, is the cognate antigen of OT-I T cells (ProImmune, Bradenton, FL, USA). After 48 or 72 h, the cells were collected and analyzed by flow cytometry. For analysis of proliferation, splenocytes were labeled with 5 µg/mL of CFSE (Invitrogen) prior to culture (Lyons and Parish 1994). Apoptosis was evaluated in separate experiments by combined cell labeling with a live/dead stain and anti-annexin V antibody, as previously described (Vermes et al., 1995), following the culture period.

2.5 Flow cytometry

Isolated cells from spleen and/or lymph nodes were stained with the following antimouse antibodies or reagents: CD8 phycoerythrin (PE)-Cy5 (clone H35-17.2, eBioscience, San Diego, CA, USA), Vα2-PE (clone B20.1; BD Pharmingen, San Jose, CA, USA), Vβ5-fluorescein isothiocyanate (FITC) (clone MH3-2; BD Pharmingen), PD-1-PE (clone J43, eBioscience), B7-H1-PE (clone MIH5, eBioscience), and Annexin V-PE (BD Pharmingen). In all experiments, cells were labeled with LIVE/DEAD® Fixable Violet Dead Cell Stain Kit (Invitrogen). A minimum of 500,000 lymphocytes for adoptive transfer experiments and 20,000 lymphocytes for *in vitro* apoptosis/proliferation assays were collected in a gate based on forward and side scatter characteristics. All samples were processed on a BD LSRII instrument and analyzed using BD FACSDiva™ software (BD Pharmingen).

2.6 Statistics

Statistical significance was determined using Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks for *in vitro* analysis of apoptosis, and one-way ANOVA for all other experiments. All pairwise multiple comparisons were performed with a Student-Newman-Keuls post-test. Differences were considered significant at $\alpha = 0.05$.

3. Results

3.1 PD-1 controls accumulation of fetal antigen-specific T cells during pregnancy

To determine whether PD-1 influences the fate of paternal antigen-specific T cells, we adopted an OVA-OT-I model system (Erlebacher et al., 2007), in which OT-I T cell receptor (TCR)-transgenic T cells recognize the ovalbumin-derived SIFNFEKL peptide. B6 WT females were bred to either B6 WT or OVA-Tg males. At gestation day 10.5, CFSE-labeled OT-I splenocytes were adoptively transferred intravenously into pregnant females. After three days (gd 13.5), cells from uterus-draining lymph nodes and spleens were collected and stained for OT-I T cell receptor markers and PD-1 and analyzed by flow cytometry (Fig. 1). In the PALN of females mated with WT males, OT-I cells were rare and did not proliferate (Fig. 1a). In contrast, in the PALN of OVA-Tg-mated females, OT-I cells were readily detected and proliferated strongly in response to fetal antigen (OVA-Tg bred vs WT-bred, $P < 0.01$), while non-antigen-specific spleen cells did not proliferate (Fig. 1b). In addition, proliferating paternal antigen-specific CD8⁺ T cells expressed the PD-1 receptor (Fig. 1c). Similar proliferation and PD-1 expression on OT-I cells was observed within the spleens of OVA-Tg-mated females (data not shown).

We next examined the effect of PD-1 deficiency during paternal antigen-specific T cell responses by transferring either WT OT-I or PD-1^{-/-} OT-I CFSE-labeled cells into virgin, WT-bred, or OVA-bred female recipients (Fig. 2). In the PALN of OVA-Tg-bred females, paternal-antigen-specific T cells lacking PD-1 were more abundant than WT OT-I cells, relative to both total lymphocytes ($P < 0.001$, Fig. 2a, c) and CD8⁺ T cells ($P < 0.001$, Fig. 2b, c). This difference was not observed in WT-bred recipients, as the abundance of both WT-OT-I and PD-1^{-/-} OT-I cells in these mice was similar to that of virgin females (Fig. 2 a, b). Conversely, in the spleens of OVA-Tg-bred females, OT-I T cells accumulated regardless of PD-1 expression, particularly relative to endogenous splenic CD8⁺ T cells (Fig. 2 e–g). However, no significant differences in the number of fetal resorption sites in females receiving OT-I WT or OT-I PD-1^{-/-} cells were observed (data not shown).

To determine a potential mechanism for paternal antigen-specific T cell accumulation in the absence of PD-1, we evaluated the proliferation of adoptively transferred cells at gd 13.5. There was no alteration in the percentage of dividing WT OT-I or PD-1^{-/-} OT-I cells in the PALN or spleen of OVA-Tg-bred females (Fig. 2 d, h), suggesting that accumulation was not due to increased proliferation by PD-1-deficient cells.

We next examined the possibility that PD-1 limits the accumulation of paternal antigen-specific T cells through induction of apoptosis. WT OT-I or PD-1^{-/-} OT-I splenocytes were stimulated *in vitro* with SIINFEKL peptide followed by examination of proliferation and apoptosis. CFSE-labeled OT-I cells stimulated with SIINFEKL peptide showed no significant difference in proliferation between WT and PD-1^{-/-} cells (Fig. 3 a, b), consistent with our observations *in vivo*. In parallel experiments, decreased percentages of peptide-stimulated PD-1^{-/-} OT-I cells were positive for Annexin V compared with WT OT-I cells (Fig. 3 c, d), suggesting that PD-1-deficient cells are less susceptible to activation-induced cell death.

3.2 PD-1 is not required for fetal survival during allogeneic pregnancy

Because PD-1 controls the accumulation of paternal antigen-specific T cells, we next examined whether maternal PD-1 is required for immunological protection of the semiallogeneic fetus. C57BL/6 WT and PD-1^{-/-} females were mated with either B6WT or BALB/c males followed by evaluation of various parameters of fecundity (Fig. 4). The absence of PD-1 in PD-1^{-/-} mice was confirmed through flow cytometry (Fig. 4a). No significant alterations in gestational or neonatal offspring parameters between syngeneic and allogeneic full-term pregnancies in WT and PD-1^{-/-} mice were observed (Fig. 4 b–d). There were also no consistent trends in the ratios of male and female offspring among the breeding groups examined (data not shown). On the other hand, the weaning weight was increased in offspring born from PD-1^{-/-} mothers, an effect seen in both syngeneic and semi-allogeneic litters (Fig. 4e).

These results were unexpected, given a previous report that maternal tolerance of semiallogeneic fetuses is dependent on the PD-1 ligand, B7-H1 (Guleria, et al., 2005). We therefore re-examined whether B7-H1 was critical to the success of allogeneic pregnancy by evaluating the fecundity of female B7-H1^{-/-} mice during syngeneic and allogeneic pregnancy (Fig. 5). Our results from the B7-H1^{-/-} female syngeneic or allogeneic crosses were consistent with those observed for PD-1^{-/-} mice, with no obvious changes in the gestation rate or offspring viability (Fig. 5 b–e). Overall, these data strongly suggest that neither maternal PD-1 nor B7-H1 is required for the viability of semi-allogeneic fetuses.

4. Discussion

In this study, we investigated the role of the immunoinhibitory receptor PD-1 on maternal T cells in pregnancy using an approach that enables the analysis of paternal antigen-specific responses. Using this system, we identified a novel pathway by which fetal antigen-specific lymphocytes are controlled in pregnancy. These cells upregulate PD-1 upon recognition of cognate fetal antigen, and in the absence of this receptor, accumulate excessively in the maternal uterus-draining lymph nodes. A previous study using this model showed that adoptively transferred paternal antigen-specific T cells fail to accumulate despite a high level of proliferation, suggesting that the cells were being deleted (Erlebacher, et al., 2007). Our results agree with this study, and further suggest that PD-1 may be responsible for mediating their deletion. On the other hand, we had not observed decreased accumulation of WT OT-I cells in the spleen by three days post-adoptive transfer, suggesting that the kinetics of paternal antigen-specific T cell deletion may differ between the PALN and spleen.

Other reports similarly suggest that paternal antigen-specific T cell deletion is mediated through CD28/B7 and/or Fas/FasL interactions (Vacchio and Jiang, 1999; Vacchio and Hodes, 2003, 2005). While these studies examined endogenous, rather than adoptively transferred, maternal T cells, the current study suggests that PD-1 may be an additional mechanism for controlling accumulation of paternal antigen-specific T cells in lymphoid organs during pregnancy. Continued analysis of these models using mice lacking PD-1 along with Fas/FasL and/or the CD28/B7 proteins would provide insight into the potential cooperative functions of these pathways in preventing propagation of maternal effector T cell activity during pregnancy.

The effects of PD-1 on OT-I T cells during pregnancy are congruent with previous models in which exogenous OT-I T cells lacking PD-1 accumulated in lymph nodes draining peripheral ovalbumin-expressing tissues, ultimately leading to tissue destruction (Martin-Orozco, et al., 2006; Keir, et al., 2007). Whereas we did not observe increased fetal resorption mediated by OT-I/PD-1^{-/-} T cells (data not shown), a possible explanation could be the difference in the *in vivo* duration of the cells between the studies. More likely, however, is that these cells would not induce fetal loss, given the results of PD-1^{-/-} female allogeneic pregnancies and the

apparent redundancy of immunosuppressive mechanisms at the maternal–fetal interface (Fig. 4) (Petroff, 2005).

The B7-H1/PD-1 pathway can inhibit T lymphocytes through several mechanisms including control of proliferation, alteration of cytokine production, and induction of apoptosis (Freeman, et al., 2000; Hori, et al., 2006; Keir, et al., 2007). Our results support a role for PD-1 in the apoptosis of paternal antigen-specific T cells rather than the inhibition of proliferation. Indeed, ligation of PD-1 inhibits the PI3K and ERK anti-apoptotic signaling pathways, as well as the prosurvival molecule Bcl-2, culminating in cell death (Ballif and Blenis, 2001; Okazaki, 2001; Yuan, et al., 2005; Keir, et al., 2005). Interestingly, Bcl-2 overexpression causes accumulation and prevents apoptosis of antigen-specific T cells (Redmond, et al., 2008). Our results suggest a role for PD-1 in controlling accumulation of fetal antigen-specific T cells in uterus-draining lymph nodes through the induction of apoptosis, which could be mediated by the downregulation of Bcl-2.

Several studies have shown that PD-1 is involved in maintaining tolerance to both self and allografted tissues (Martin-Orozco, et al., 2006; Keir, et al., 2007; Tanaka, et al., 2007; Wang, et al., 2007). However, the lack of fetal rejection at term pregnancy in PD-1^{-/-} mothers indicates that survival of the fetal allograft might not be dependent on PD-1 alone. This finding was surprising in light of a previous report in which the blockade or genetic deletion of B7-H1 resulted in a loss of maternal tolerance to semi-allogeneic fetuses (Guleria, et al., 2005). Since it was possible that the inhibitory effects of B7-H1 might be propagated through a receptor other than PD-1 (Dong, et al., 2002), we also examined the fecundity of B7-H1-deficient females following syngeneic and allogeneic breeding. Consistent with our results in the PD-1-deficient mice, we found no significant differences in the parameters of pregnancy between B6WT and B7-H1^{-/-} mice (Fig. 5). The discrepant results between the current and previous studies are difficult to reconcile, particularly since both knockout strategies included the deletion of the PD-1 binding site from the B7-H1 gene (Wang, et al., 2003; Dong, et al., 2004; Guleria, et al., 2005). The mice used in our study also lack the leader sequence for B7-H1 (exon 1), precluding the possibility of mutant B7-H1 from being expressed on the cell surface, which we confirmed by flow cytometry (Fig. 5). It remains possible, however, that environmental factors such as noise level or pathogen load vary between the two institutions, resulting in different levels of endogenous stress to the colonies.

While the absence of maternal PD-1 did not affect fetal survival, offspring were significantly heavier at the time of weaning (Fig. 4). This effect was observed in both syngeneic and allogeneic pregnancies, and could be due to an altered immunological environment during pregnancy or lactation. PD-1 is known to inhibit pro-inflammatory cytokine production (Freeman, et al., 2000; Keir, et al., 2007), and thus the absence of PD-1 during pregnancy could potentiate a comparatively inflammatory environment that may influence body composition later in life. A similar effect was also observed in mice lacking the anti-inflammatory cytokine IL-10, i.e., fetuses from IL-10-deficient mothers were heavier at birth (White, et al., 2004). Alternatively, it is possible that PD-1 might play a role in lactation, which would in turn influence the growth of the offspring.

In summary, these results identify a novel pathway for the control of fetal antigen-specific maternal T cells. PD-1 expression on maternal T cells may be important for ensuring that these cells are kept in check. Indeed, the production of cytokines by decidual PD-1-expressing T cells is modulated by B7-H1 (Taglauer et al., 2008b), supporting the idea that the B7-H1/PD-1 pathway, although not indispensable for pregnancy, skews the maternal immune response into supporting a favorable milieu. Furthermore, a functional hierarchy may exist among the network of immunomodulatory pathways present at the maternal–fetal interface. The B7-H1/PD-1 pathway may operate within this network to control the accumulation of paternal antigen-

specific T cells in the uterus-draining lymph nodes through a mechanism involving apoptosis, and operates in conjunction with other immunotolerizing mechanisms for the ultimate goal of maintaining healthy gestation and fetal development.

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Fig. 1.

Fetal antigen-specific T cells proliferate and express PD-1 during pregnancy. CFSE-labeled spleen cells from OT-I mice were transferred at gd 10.5 into females bred to ova transgenic (OVA) or WT males. On gd 13.5, cells from the peri-aortic lymph nodes were stained for OT-I markers and PD-1 and analyzed by flow cytometry. (a, b) Representative dot plots and histograms of CD8⁺/Va2⁺ OT-I cells in the peri-aortic lymph nodes of either WT- (a) or OVAbred (b) females. (c) Representative dot plot and histogram of PD-1 expression in maternal OT-I cells in the peri-aortic lymph nodes. Empty peak, isotype control. Figure representative of n=4 female recipients.

Fig. 2.

PD-1 controls accumulation of fetal antigen-specific T cells in the uterus-draining lymph nodes. CFSE-labeled splenocytes from WT or PD-1^{-/-} mice were adoptively transferred into virgin, WT-bred or OVA-bred females on gd 10.5. Recipient female peri-aortic lymph nodes and spleens were collected on gd 13.5 and analyzed by flow cytometry. (a, e) Graph represents percentage of OT-I cells out of total peri-aortic (a) or splenic (e) lymphocytes in virgin (n = 3), WT-bred (n = 3) or OVA-bred (n = 4) females. (b, f) Graph represents the percentage of OT-I cells out of peri-aortic (b) or splenic (f) CD8⁺ cells in the same groups as described for a and e. (c, g) Representative dot plots of peri-aortic lymph node (c) and spleen (g) cells stained for CD8 and Vα2. (d, h) Graph represents the percentage of OT-I (CD8⁺Vα2⁺) cells in the peri-

aortic lymph nodes (d) or spleen (h) undergoing more than three cell divisions. Different letters denote groups whose difference was statistically significant and similar letters denote groups whose difference was not statistically significant.

Fig. 3.

PD-1 induces apoptosis without altering proliferation in activated antigen-specific T cells. CFSE-labeled (a, b) or unlabeled (c, d) OT-I WT and PD-1^{-/-} splenocytes were placed into culture with 100 ng/mL of SIINFEKL peptide or medium at 37°C for a total of 72 h followed by flow cytometric analysis for OT-I markers (CD8, Va2) and/or apoptosis markers (Annexin V, Violet Live/Dead Stain). (a) Representative dot plots of CFSE-labeled CD8⁺ Va2⁺ splenocytes analyzed for proliferation following 72-h culture. (b) Graph represents the data summarized from n=4 mice/group. (c) Representative dot plots of CD8⁺ Va2⁺ splenocytes analyzed for apoptosis markers following 48-h culture. Lines were set based on unstained cells for Violet Live/Dead stain and fluorescence minus one controls for Annexin V stain. (d) Graph

represents the data summarized from n=4 mice/group. Different letters denote groups whose difference was statistically significant.

Fig. 4. PD-1 is not required for the maintenance of allogeneic pregnancy

(a) Whole splenocytes were stimulated with concanavalin A for 72 h at 37°C followed by flow cytometric analysis of PD-1. Empty peaks, isotype control. (b–e) Analysis of gestation length (b), litter size (c), and fetal weight at birth (d)/weaning (e) in syngeneic (syn) and allogeneic (allo) pregnancies in WT (syn: n=9, allo: n=7) and PD-1^{-/-} (syn: n=5, allo: n=5) females. Different letters denote groups whose difference was statistically significant.

Fig. 5. B7-H1 is not required for the survival of allogeneic fetuses. (a) Whole splenocytes were stimulated with concanavalin A for 72 h at 37°C followed by flow cytometric analysis of B7-H1. Empty peaks, isotype control. (b–e) Analysis of gestation length (b), litter size (c), and fetal weight at birth (d)/weaning (e) in syngeneic (syn) and allogeneic (allo) pregnancies in WT (syn: n=9, allo: n=4) and B7-H1^{-/-} (syn: n=5, allo: n=5) females. None of the values was significantly different in any of the graphs displayed.