OBESITY

Innate PD-L1 limits T cell–mediated adipose tissue inflammation and ameliorates diet-induced obesity

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Obesity has become a major health problem in the industrialized world. Immune regulation plays an important role in adipose tissue homeostasis; however, the initial events that shift the balance from a noninflammatory homeostatic environment toward inflammation leading to obesity are poorly understood. Here, we report a role for the costimulatory molecule programmed death-ligand 1 (PD-L1) in the limitation of diet-induced obesity. Functional ablation of PD-L1 on dendritic cells (DCs) using conditional knockout mice increased weight gain and metabolic syndrome during diet-induced obesity, whereas PD-L1 expression on type 2 innate lymphoid cells (ILC2s), T cells, and macrophages was dispensable for obesity control. Using in vitro cocultures, DCs interacted with T cells and ILC2s via the PD-L1:PD-1 axis to inhibit T helper type 1 proliferation and promote type 2 polarization, respectively. A role for PD-L1 in adipose tissue regulation was also shown in humans, with a positive correlation between PD-L1 expression in visceral fat of people with obesity and elevated body weight. Thus, we define a mechanism of adipose tissue homeostasis controlled by the expression of PD-L1 by DCs, which may be a clinically relevant finding with regard to immune-related adverse events during immune checkpoint inhibitor therapy.

INTRODUCTION

The increasing global prevalence of obesity has become one of the greatest challenges for health care systems. Obesity is a frequent comorbidity affecting treatment of patients undergoing surgery or immunotherapy. Increasing evidence demonstrates profound dysregulation of the immune system in people with obesity (PWO) leading to a state of low-grade inflammation (*1*).

Under homeostatic conditions in a lean state, group 2 innate lymphoid cells (ILC2s), eosinophils, anti-inflammatory macrophages, regulatory T cells (T_{regs}), and $CD4^+$ T helper 2 (T_H2) cells populate adipose tissue (*2*–*6*). During progression to obesity, the composition of inflammatory cells in adipose tissue changes and is characterized by the recruitment and accumulation of M1 macrophages, type 1 innate lymphoid cells (ILC1s), neutrophils, $CD8^+$ T cells, T_H1 cells, and T_H17 cells (*7*–*10*). However, the immunological events that dysregulate tissue homeostasis and promote the development of obesity are still incompletely understood.

In particular, the interface of innate and adaptive immunity plays an important role in shaping the T cell response. Dendritic cells (DCs), macrophages, and type 2 innate lymphoid cells (ILC2s) interact with $CD4^+$ T cells through major histocompatibility complex class II (MHCII)–T cell receptor interaction and regulate T cell activation and polarization through costimulatory molecules.

DCs account for about 1 to 2% of the stromal vascular fraction in the adipose tissue and their percentage increases during obesity (*11*). As prototypic antigen-presenting cells (APCs), DCs are critical regulators of T cell polarization. In adipose tissue, two distinct subsets of conventional DCs (cDCs) were shown to contribute to an anti-inflammatory milieu and to counteract obesity-induced inflammation and insulin resistance (*12*). Moreover, adipose tissue DCs from obese mice preferentially promote T_H1 and T_H17 responses (*13*).

In recent years, ILC2s have emerged as important regulators of adipose tissue homeostasis. ILC2s have been shown to promote the accumulation of eosinophils in adipose tissue and to support M2 macrophage and T_H2 polarization (*3*, *14*). Indeed, dysregulated ILC2 responses correlated with obesity in mice and humans (*2*, *15*). In addition to cytokine-mediated regulation of T cells, direct interaction between ILC2s and $CD4^+$ T cells promoting T_H2-polarized responses has been clearly established in a number of disease models (*16*).

The imbalance of anti-inflammatory T_{regs} and T_H2 cells with proinflammatory T_H1 and T_H17 cells has severe consequences for pathogen clearance, tumor rejection, and metabolic disease. T_{reg} accumulation in lean adipose tissue was shown to be dependent on the transcription factors peroxisome proliferator–activated receptor gamma, interferon (IFN) regulatory factor (IRF) 4, basic leucine zipper ATF-like transcription factor (BATF), and interleukin-33 (IL-33) signaling (*17*). Human adipose tissue T_H2 cell frequency negatively correlates with inflammation and insulin resistance, indicating a protective function in metabolic disease (*18*). Indeed, T cell–derived IL-4 and IL-13 contributed to weight loss and reversal of metabolic dysfunction in an adoptive transfer model (*19*). Mechanistically, type 2 cytokines can promote M2 macrophage polarization and eosinophil recruitment, whereas IFNγ promotes M1 polarization. Obesity increases MHCII and costimulatory molecules

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on APCs and promotes T_H1 polarization (*20*–*23*). The increase in IFNγ stimulates M1 macrophage polarization and interferes with insulin signaling (*24*). Similarly, IL-17 and T_H17 cells are increased in obese adipose tissue, which may directly contribute to inflammation (*25*).

To date, it is unclear how these T cell responses in obese tissue are controlled. Programmed death-ligand 1 (PD-L1) is one of the key molecules involved in the regulation of adaptive immunity (*26*), and we have previously shown that PD-L1 is a potent regulator of T cell polarization (*27*). Macrophages, DCs, and ILC2s were found to express PD-L1 and regulate PD-1–expressing T cells and ILC2s (*27*–*30*). Antibody-mediated inhibition of PD-L1 releases the brake from T_H1 cells in antitumor and autoimmune responses (*31*, *32*). Thus, we hypothesized that PD-L1 plays an important role in maintenance of adipose tissue homeostasis and investigated the cell-specific function of PD-L1 during obesity using transgenic mice in models of diet-induced obesity and samples from patients with obesity.

RESULTS

PD-L1 delays onset of obesity

First, we addressed the question whether the expression of PD-L1 directly affects the development of obesity. To this end, we compared the course of obesity in wild-type (WT) and *PD-L1*$^{-/-}$ mice in the well-established model of high-fat diet (HFD)–induced obesity. Macroscopically, we observed that despite a comparable starting weight (23.69 ± 1.00 g and 23.86 ± 0.88 g), *PD-L1*$^{-/-}$ mice appeared more obese after the same period on HFD (Fig. 1A). Indeed, PD-L1–deficient mice gained significantly ($P = 0.0034$) more weight after 12 weeks on HFD (Fig. 1B), which was also reflected by significantly ($P = 0.0095$) larger deposits of epididymal white adipose tissue (WAT) (Fig. 1C). *PD-L1*$^{-/-}$ mice showed a lower rate of glucose uptake from the blood during a glucose tolerance test (Fig. 1D) and had increased insulin resistance (Fig. 1E) compared to control animals. Thus, the metabolism of *PD-L1*$^{-/-}$ mice was more severely affected by obesity than the metabolism of WT mice. Histologic analysis of adipose tissue revealed significantly ($P = 0.0113$) enhanced hypertrophy of adipocytes (Fig. 1, F and G). These results clearly demonstrate a critical role of the immunoregulator PD-L1 for the control of obesity.

PD-L1 deficiency impairs adipose tissue T cell polarization

We observed increased expression of PD-L1 on DCs, macrophages, $CD4^+$ T cells, and ILC2s isolated from adipose tissue of obese animals (Fig. 1H). In line with previous findings (*28*, *33*), PD-1 expression was up-regulated on ILC2 and $CD4^+$ T cells, whereas expression was unaltered on DCs and lowered on macrophages (Fig. 1I). The analysis of *Rag1*$^{-/-}$ mice (lacking T- and B-lymphocytes) and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice [deficient for lymphocytes, natural killer (NK) cells, and ILC] revealed that weight gain during diet-induced obesity was reduced when adaptive immune cells were absent, which was even more pronounced in the combined absence of innate and adaptive immunity (fig. S1A).

Next, we investigated the impact of PD-L1 deficiency on the cellularity of adipose tissue immune cells. As expected, the loss of PD-L1 that led to increased weight gain also impaired type 2 immune cells (Fig. 1, J and K). The frequencies of ILC2s ($P = 0.0025$), eosinophils ($P = 0.0472$), and M2 macrophages ($P = 0.049$) were significantly reduced (Fig. 1J). The analysis of T_H subsets demonstrated impaired T_H2 and T_{reg} responses, whereas T_H17 polarization was unaffected (Fig. 1K). In contrast, T_H1 polarization in adipose tissue was significantly ($P = 0.0009$) increased (Fig. 1K), which is in line with the proinflammatory state observed during obesity. Increased PD-1 expression was detected on $CD4^+$ T cells (Fig. 1L) and on DCs and macrophages, which has been observed previously in constitutive *PD-L1*$^{-/-}$ mice (*34*). These data demonstrate that PD-L1 plays an important role in the regulation of adaptive immune responses within adipose tissues during diet-induced obesity.

PD-L1 expression on macrophages, T cells, and ILCs is redundant during obesity

We have previously reported that PD-L1 contributes to T_H2 polarization during type 2 immune responses (*27*). Earlier studies have also shown that PD-L1 is involved in the initiation and maintenance of T_{regs} (*35*). Therefore, we analyzed the cell-specific function of PD-L1 in the course of HFD-induced obesity. Thus, we generated conditional knockout animals in which we restricted PD-L1 deficiency to ILCs, T cells, macrophages, or DCs. *PD-L1*$^{fl/fl}$*Il7r*$^{Cre/+}$ mice, which lack expression of PD-L1 on ILCs (identified as $CD45^+lin^-ST2^+KLRG1^+$) and T cells (Fig. 2A), and Cre-negative littermates were fed an HFD and monitored for weight changes. Contrary to our hypothesis that ILC2s controlled T cell polarization within the adipose tissue via PD-L1, the loss of PD-L1 on ILC2s did not affect weight gain (Fig. 2B) or the metabolic status of mice (Fig. 2C). As the Cre-recombinase in *Il7r*^{Cre} mice also affects T cells, we generated *PD-L1*$^{fl/fl}$*CD4*^{Cre} mice to exclude T cell–mediated regulation of PD-1–expressing ILC2s (Fig. 2D). $CD3^+CD4^+$ T cell–specific deficiency of PD-L1 did not alter the course of obesity and metabolic disease (Fig. 2, E and F). Adipose tissue macrophages, in particular proinflammatory M1 macrophages, express high amounts of PD-L1 (Fig. 1H). Therefore, we generated conditional LysMCre mice and confirmed robust deletion of PD-L1 on $CD45^+F4/80^+CD11b^+$ MHC^+CD301^+ macrophages (Fig. 2G). However, we did not observe any disease aggravation (Fig. 2, H and I). Adipose tissue DCs in *PD-L1*$^{fl/fl}$*Lyz2*$^{Cre/+}$ mice were not affected by PD-L1 deletion (fig. S1, B and C). Thus, we conclude that PD-L1 expression by ILCs, T cells, and macrophages is nonessential for adipose tissue homeostasis or inflammation.

PD-L1 expression by DCs regulates T cell responses during obesity

As DCs in adipose tissue had elevated expression of PD-L1, we deleted PD-L1 on DCs by crossing *PD-L1*$^{fl/fl}$ to *Zbtb46*^{Cre} mice (termed *PD-L1*$^{fl/fl}$*DC*^{Cre} mice). Analysis of PD-L1 expression on adipose tissue DCs (identified as $CD45^+Ly6G^-F4/80^-CD11b^-CD11c^+$ $MHCII^+CD301^-$) showed robust deletion of PD-L1 on DCs in *PD-L1*$^{fl/fl}$*DC*^{Cre} mice comparable to constitutive *PD-L1*$^{-/-}$ mice (Fig. 3, A and B). PD-L1 expression remained intact in other cell populations including ILC2s, T cells, and macrophages. *PD-L1*$^{fl/fl}$*DC*^{Cre} mice kept on HFD gained significantly ($P = 0.0233$) more weight than their Cre-negative littermates (Fig. 3C). Furthermore, the proportion of epididymal WAT was also increased (Fig. 3D) with an associated deteriorated metabolic status (Fig. 3E), analogous to changes seen in constitutive *PD-L1*$^{-/-}$ mice. To exclude that conditional or constitutive PD-L1 ablation led to type 1 diabetes–like autoimmune inflammation in the pancreas, we analyzed pancreatic islets by immunofluorescence microscopy and histology (fig. S2, A and B).

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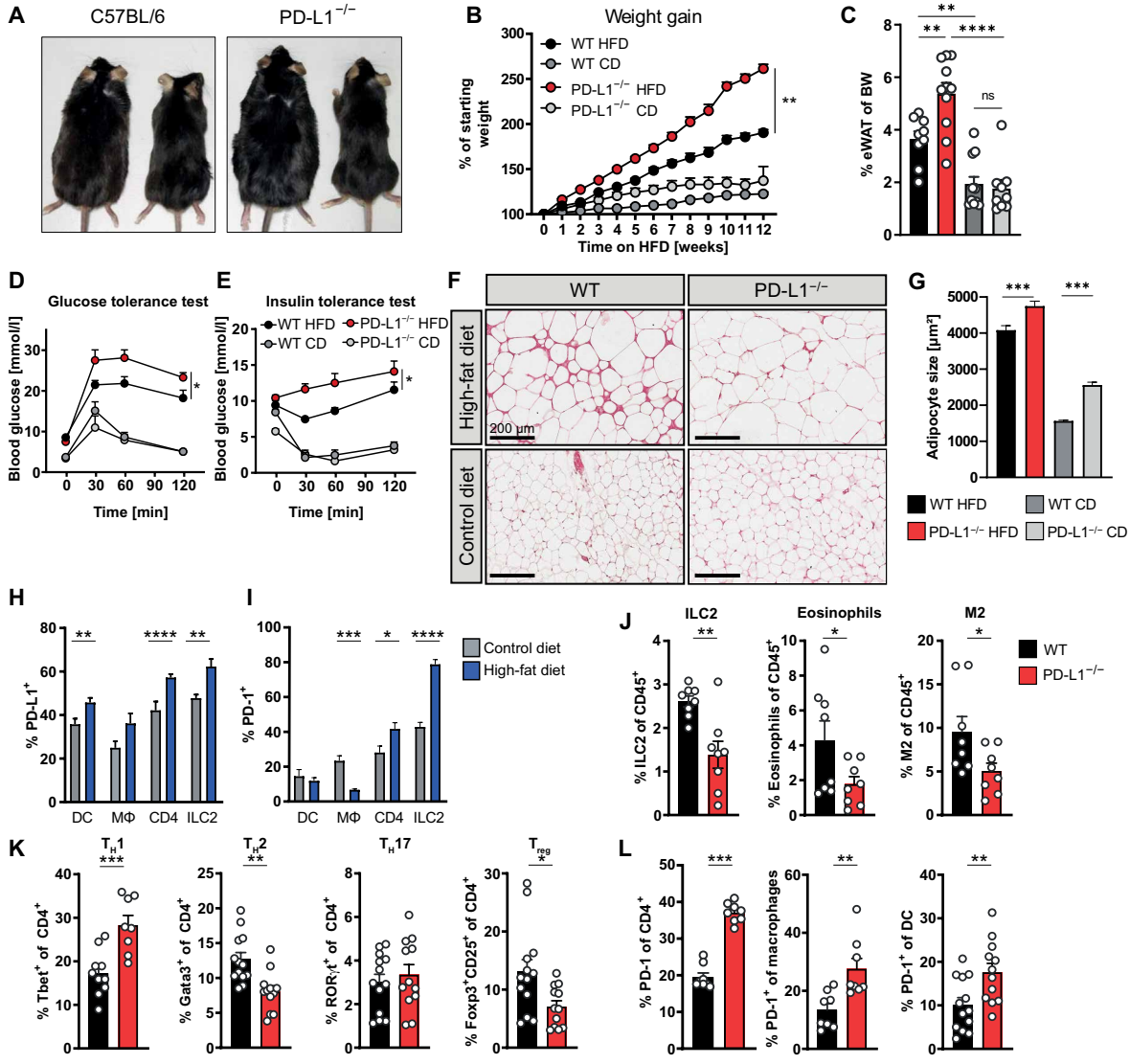

Fig. 1. PD-L1 is a checkpoint for obesity and regulates adipose tissue immune cell composition. (**A**) Representative photographs of wild-type (WT; C57BL/6; left) and PD-L1$^{-/-}$ mice (right) on control diet (CD) or high-fat diet (HFD). (**B**) Relative weight change of WT (black) and PD-L1$^{-/-}$ (red circles) mice kept on HFD or CD (gray). Weight was measured weekly and calculated as a percentage of the starting weight. Graphs show the means + SEM (8 to 12 mice per group) from three independent experiments. $^{**}P < 0.01$, one-way ANOVA with Tukey's multiple comparisons of area under curve (AUC). (**C**) Weight of epididymal white adipose tissue (eWAT) as a percentage of total body weight (BW) in WT (black) and PD-L1$^{-/-}$ (red) mice on HFD and WT mice on CD (gray). Bar graphs show the means + SEM (8 to 12 mice per group) from three independent experiments. $^{**}P < 0.01$, $^{****}P < 0.0001$, ANOVA with Tukey's multiple comparisons. (**D** and **E**) WT (black) and PD-L1$^{-/-}$ (red) were injected with glucose intraperitoneally after 8 weeks (D) or insulin intraperitoneally after 10 weeks (E) on HFD or CD. Blood glucose levels were measured at indicated time points after injection. Graph shows the means + SEM of four mice per group from two independent experiments. $^{*}P < 0.05$, ANOVA with Tukey's multiple comparisons. (**F**) Hematoxylin and eosin–stained sections of eWAT from WT (left) and PD-L1$^{-/-}$ (right) mice kept on HFD (top) or CD (bottom). Scale bars, 200 µm. (**G**) Quantification of adipocyte size from sections in (F). ImageJ was used for automated measurements of at least 1000 cells from four mice per group. (**H** and **I**) Frequency of DCs, macrophages (MΦ), CD4$^+$ T cells, and ILC2s in adipose tissue from WT mice on CD (gray) or HFD (blue) expressing PD-L1 (H) and PD-1 (I). Bar graphs show the means + SEM of at least eight mice per group of three independent experiments. (**J**) Frequency of ILC2s, eosinophils, and M2-polarized macrophages in adipose tissue of WT (black) and PD-L1$^{-/-}$ (red) mice kept on HFD for 12 weeks. Bars show the means + SEM of eight mice per group from two independent experiments. (**K**) Frequency of T_H1, T_H17, T_H2, and T_{reg} cells in adipose tissue of WT (black) and PD-L1$^{-/-}$ (red) mice kept on HFD for 12 weeks. Bars show the means + SEM of at least eight mice per group from three independent experiments. (**L**) Frequency of PD-1 expression on CD4$^+$ T cells, macrophages, and DCs in adipose tissue of WT (black) and PD-L1$^{-/-}$ (red) mice kept on HFD for 12 weeks. Bars show the means + SEM of eight mice per group from two independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, Student's *t* test or Mann-Whitney *U* test.

The size and cellular composition of islet cells were comparable between HFD-diet treated WT, *PD-L1*$^{-/-}$, and *PD-L1*$^{fl/fl}$*DC*^{Cre} mice (fig. S2C). Furthermore, postprandial insulin release after a 5-hour fasting period is not impaired in the absence of PD-L1 on DCs (fig. S2D). Whereas PD-L1 expression on DCs was ablated, the frequency of DCs within adipose tissue was not affected by the conditional knockout (Fig. 3F). Similarly, macrophage recruitment to adipose tissue was not changed. However, significantly ($P = 0.0234$) more CD4$^+$ T cells were present in the adipose tissue, whereas ILC2 numbers declined (Fig. 3F). Within the T_H compartment,

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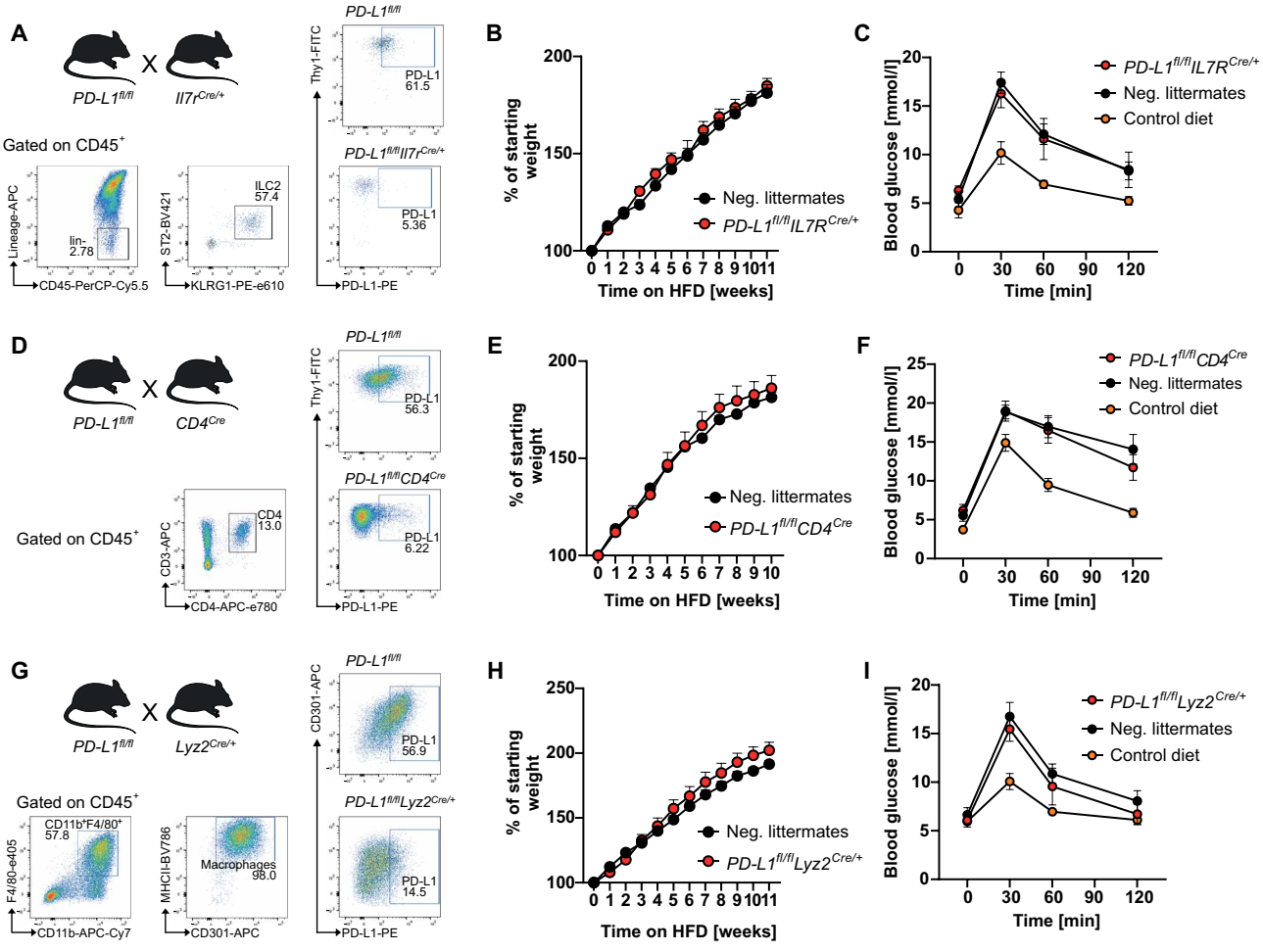

Fig. 2. Conditional knockout of PD-L1 in ILCs, T cells, and macrophages does not affect obesity. (**A**) Conditional knockout of PD-L1 in ILCs. ILC2s were identified as $CD45^+lin^-KLRG1^+ST2^+$ in *PD-L1*$^{fl/fl}$ (top) and *PD-L1*$^{fl/fl}$*IL-7R*$^{Cre/+}$ (bottom) mice. (**B** and **C**) Weight change (B) and glucose tolerance test (C) in *PD-L1*$^{fl/fl}$*IL-7R*$^{Cre/+}$ (red) mice and negative littermates (black) kept on HFD. Graph shows means + SEM of seven to eight mice per group from three independent experiments. (**D**) Conditional knockout of PD-L1 in T cells. $CD4^+$ T cells were identified as $CD45^+CD3^+CD4^+$ in *PD-L1*$^{fl/fl}$ (top) and *PD-L1*$^{fl/fl}$*CD4*^{Cre} (bottom) mice. (**E** and **F**) Weight change (E) and glucose tolerance test (F) in *PD-L1*$^{fl/fl}$*CD4*^{Cre} (red) mice and negative littermates (black) kept on HFD. Graph shows means + SEM of at least four mice per group from two independent experiments. (**G**) Conditional knockout of PD-L1 in macrophages. Macrophages were identified as $CD45^+F4/80^+CD11b^+MHCII^+CD301^+$ in *PD-L1*$^{fl/fl}$ (top) and *PD-L1*$^{fl/fl}$*Lyz2*$^{Cre/+}$ (bottom) mice. (**H** and **I**) Weight change (H) and glucose tolerance test (I) in *PD-L1*$^{fl/fl}$*Lyz2*$^{Cre/+}$ (red) mice and negative littermates (black) kept on HFD. Graph shows means + SEM of at least eight mice from three independent experiments for weight change (H) and one representative result with four mice per group for glucose tolerance (I).

predominantly T_H1 cells expanded, whereas T_H2 and T_H17 cells were unchanged and the number of T_{reg} cells was reduced (Fig. 3G). T_H1 cells also displayed the highest increase of PD-1 expression in the absence of PD-L1 on DC (Fig. 3H). Thus, we hypothesized that PD-L1 expression on DCs limits adipose tissue inflammation and development of obesity by keeping T cells in check through PD-L1:PD-1 interaction.

DC-mediated regulation of T cells and ILC2s via PD-L1:PD-1 interaction

We speculated that the low-level chronic inflammation that develops in adipose tissue contributes to the sustained up-regulation of PD-L1 on DCs. Indeed, bone marrow–derived DCs (BMDCs) activated with different stimuli showed significant up-regulation of PD-L1 in response to IL-4, IFNγ/lipopolysaccharide (LPS), and leptin (Fig. 4A). We further explored whether DC–T cell cross-talk via PD-L1:PD-1 interaction affects T cell polarization. Cocultures of BMDCs with freshly isolated naive splenic CellTrace-labeled $CD4^+$ T cells were analyzed after 4 days. As expected from checkpoint inhibitor treatments (*36*), in vitro blockade of PD-1 or PD-L1 increased the proliferation of T cells (Fig. 4B). However, polarization toward T_H2 was significantly impaired in the absence of PD-L1:PD-1 signaling ($P < 0.05$; Fig. 4C), supporting our earlier findings on preferential activating functions of PD-L1 on type 2 immunity (*27*). Indeed, when we included polarizing cytokines in the DC-T cell cocultures, only the addition of T_H1-polarizing IL-12, but not T_H2-polarizing IL-4, increased T cell proliferation during simultaneous PD-L1 or PD-1 blockade (Fig. 4D). As expected from the PD-L1–mediated inhibition of T_H2 polarization, $CD4^+$ T cells from cocultures with naïve or IL-4–pretreated BMDCs produced less IL-13 in the presence of PD-L1–blocking antibodies (Fig. 4E). In contrast, PD-L1 blockade led to the expansion of T_H1 cells, which was also reflected by a significant ($P = 0.022$) increase of IFNγ production when BMDCs were pretreated with IL-12 (Fig. 4F). Next,

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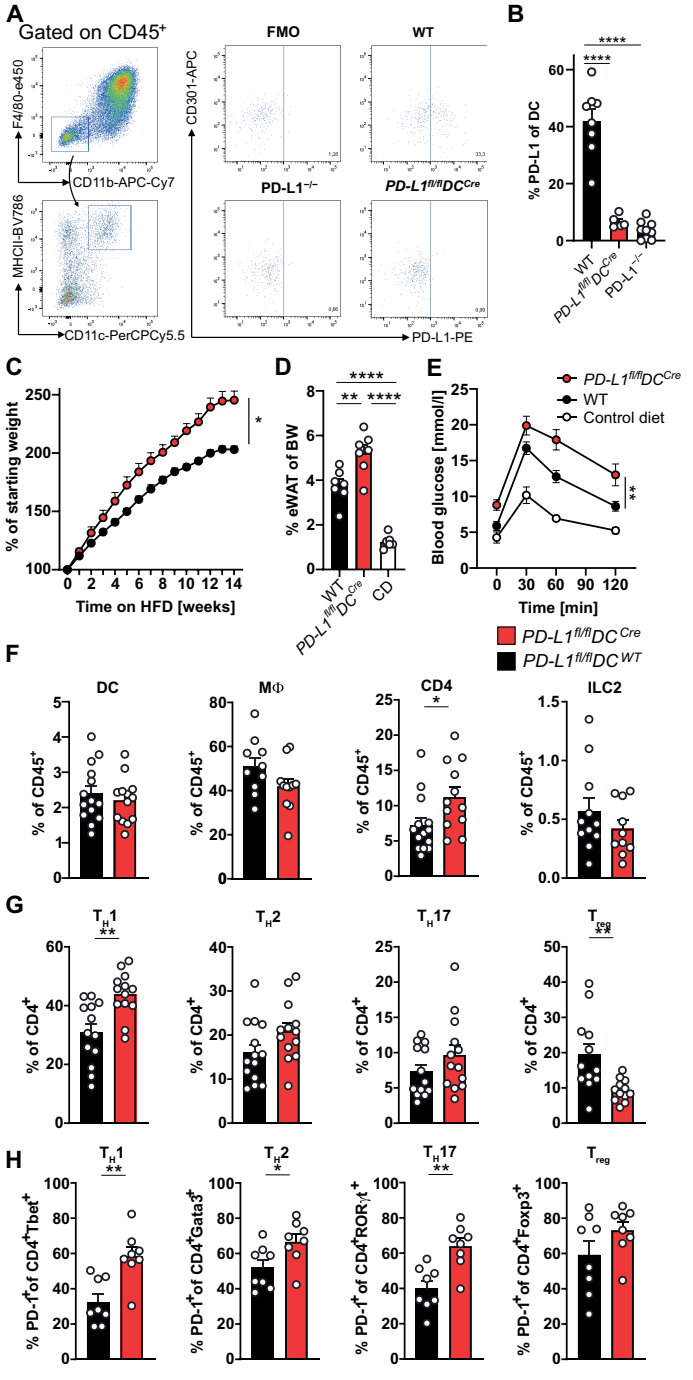

Fig. 3. PD-L1 on conventional DCs limits adipose tissue inflammation during obesity. (**A**) Conditional knockout of PD-L1 in DCs. Representative dot plots of PD-L1 expression in $CD45^+F4/80^-CD11b^-CD11c^+MHCII^+$ DCs in WT, *PD-L1*$^{-/-}$, and *PD-L1*$^{fl/fl}$*DC*^{Cre} mice. (**B**) Frequency of PD-L1$^+$ DC in WT (black), *PD-L1*$^{fl/fl}$*DC*^{Cre} (red), and *PD-L1*$^{-/-}$ (gray) mice. Bars show the means + SEM from five to nine mice from two independent experiments. ****$P < 0.0001$, ANOVA and Tukey's multiple comparisons test. (**C**) Weight change in WT (black) and *PD-L1*$^{fl/fl}$*DC*^{Cre} (red) mice kept on HFD. Graph shows the means + SEM of 11 mice from four independent experiments. ***$P < 0.001$, Student's *t* test of AUC. (**D**) Weight of eWAT as a percentage of total body weight in WT (black) and *PD-L1*$^{fl/fl}$*DC*^{Cre} (red) mice on HFD and WT mice on CD (white). Bars show the means + SEM of seven mice per group from three independent experiments. **$P < 0.01$, ****$P < 0.0001$, ANOVA. (**E**) WT (black) and *PD-L1*$^{fl/fl}$*DC*^{Cre} (red) were injected with glucose intraperitoneally after 8 weeks on HFD or CD. Blood glucose concentration was measured at indicated time points after injection. Graph shows the means + SEM of at least eight mice on HFD per group from three independent experiments. **$P < 0.01$, Student's *t* test of AUC. (**F**) Frequency of DC, MΦ, $CD4^+$ T cells, and ILC2 in adipose tissue of *PD-L1*$^{fl/fl}$*DC*^{Cre} (red) and negative littermates (black). Bars show the means + SEM of at least 10 mice per group from four independent experiments. (**G**) Frequency of T_H1 ($CD3^+CD4^+Tbet^+$), T_H2 ($CD3^+CD4^+Gata3^+$), T_H17 ($CD3^+CD4^+ROR\gamma t^+$), and T_{reg} ($CD3^+CD4^+CD25^+Foxp3^+$) in adipose tissue of *PD-L1*$^{fl/fl}$*DC*^{Cre} (red) and negative littermates (black). (**H**) Expression of PD-1 on T cell subsets identified in (G). Bars show the means + SEM of at least eight mice per group from four independent experiments. (F to H) *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; Student's *t* test or Mann-Whitney *U* test.

we investigated whether DCs also interacted with ILC2s through PD-1 and PD-L1. A previous study found that DCs can directly activate ILC2s and ILC2-derived IL-13 led to an up-regulation of IRF4 in DCs that was required for T_H2 polarization in a model of airway inflammation (*37*). Indeed, DCs directly activated ILC2s as measured by *Il13* transcript abundance and protein production (Fig. 4, G and H). Antibody-mediated blockade of PD-L1 interfered with DC-ILC2 cross-talk and led to a decrease of *Il13* transcripts, as well as protein abundance of IL-13 and IL-5 (Fig. 4H). Consequently, *Irf4* up-regulation was abolished in cocultured cells and impaired the transcription of *Ccl17*, an important T_H2 cell–attracting chemokine (Fig. 4G). The expression of *Irf4* was also decreased in DCs isolated from the adipose tissue of obese *PD-L1*$^{-/-}$ and *PD-L1*$^{fl/fl}$*DC*^{Cre} mice (Fig. 4I). Using cells from PD-L1$^{-/-}$ and PD-1$^{-/-}$ donors, we further confirmed that PD-L1 on DCs and PD-1 on ILC2s were required for *Irf4* up-regulation in BMDCs (Fig. 4J). These results indicate that PD-L1:PD-1 interaction occurs between DCs and T cells as well as DCs and ILC2s, promoting type 2 responses while inhibiting T_H1 proliferation.

PD-L1 expression is increased in adipose tissue of PWO

Having established that PD-L1 is an important factor in regulating adipose tissue in the mouse model of obesity, we were interested whether PD-L1 is relevant to human obesity. Therefore, we analyzed PD-L1 expression in the adipose tissue of people with a lean body mass index (BMI; 20 to 25 kg/m^2) and PWO (BMI > 30 kg/m^2) undergoing surgery. We found significantly ($P < 0.001$) increased expression of PD-L1 (*CD274*) in the adipose tissue of patients with grade III (BMI > 40 kg/m^2) obesity. We also observed significantly ($P < 0.001$) higher mRNA expression of the proinflammatory cytokine *TNF* in the obese cohort, whereas *IL13* transcripts were decreased compared to lean individuals (Fig. 5A). There was a positive correlation ($R^e = 0.50$, $P = 0.002$) between relative PD-L1 transcript expression in visceral, but not subcutaneous, adipose tissue and the BMI of patients (Fig. 5B). Next, we analyzed which cell populations expressed PD-L1 in WAT of PWO. In general, PD-L1 was

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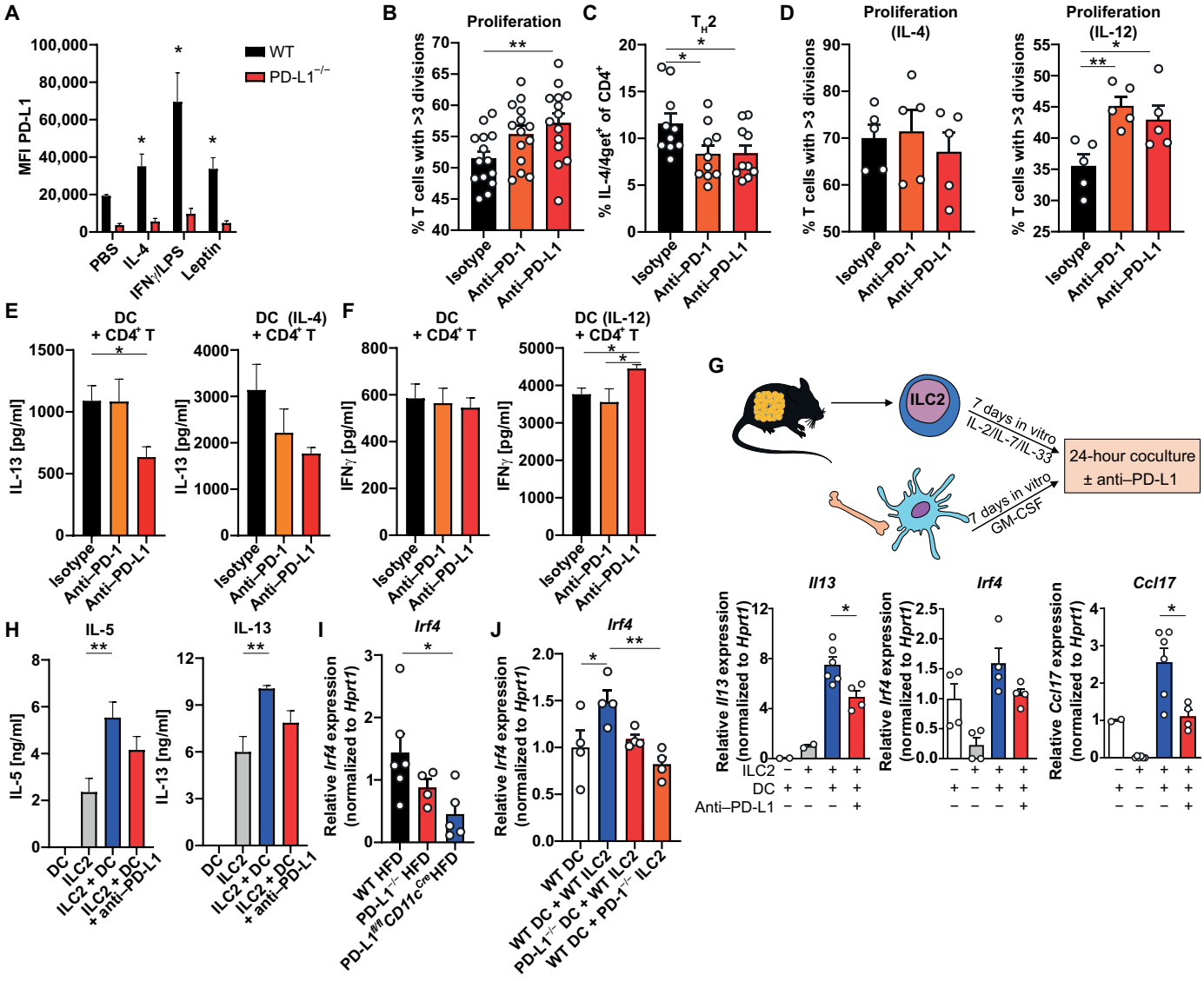

Fig. 4. DCs interact with CD4$^+$ T cells and ILC2s through PD-L1:PD-1 interactions in vitro. (**A**) Mean fluorescence intensity (MFI) of PD-L1 on bone marrow–derived DCs (BMDCs) stimulated with PBS, IL-4, IFNγ/LPS, or Leptin. Bar graphs show the means + SEM from five independent experiments. (**B** and **C**) Proliferation (B) and T_H2 (C) polarization of naïve CellTrace-labeled T cells cultured together with BMDCs and anti–PD-1 (orange), anti–PD-L1 (red), or isotype control (black) antibody (5 μg/ml each). Cells were analyzed after 4 days in culture by flow cytometry. Bars show the means + SEM of 14 (B) and 10 (C) samples per group from four independent experiments. (**D**) Proliferation of naive CellTrace-labeled T cells cultured together with IL-4–pretreated (left) or IL-12–pretreated (right) BMDCs and anti–PD-1 (orange), anti–PD-L1 (red), or isotype control (black) antibody (5 μg/ml each). Cells were analyzed after 4 days in culture by flow cytometry. Bars show the means + SEM of five samples per group from two independent experiments. (**E**) IL-13 concentration in the supernatants of BMDC-CD4$^+$ T cell cocultures treated with isotype control (black), anti–PD-1 (orange), or anti–PD-L1 (red) antibodies was analyzed by ELISA. BMDCs were either left unstimulated (left) or pretreated overnight with IL-4 (right). *$P < 0.05$, ANOVA. (**F**) IFNγ concentration was analyzed in the supernatants of BMDC-CD4$^+$ T cell cocultures treated with isotype control (black), anti–PD-1 (orange), or anti–PD-L1 (red) antibodies. BMDCs were either left unstimulated (left) or pretreated overnight with IL-12 (right). **$P < 0.01$, ANOVA. (**G**) Schematic representation of ILC2-DC–coculture setup. ILC2s and BMDCs were cultured alone (white: BMDC; gray: ILC2) or in a ratio of 1:3 and stimulated for 24 hours with IL-33 in the presence (red) or absence (blue) of PD-L1–blocking mAb. Expression of *Il13*, *Irf4*, and *Ccl17* transcript was analyzed by qPCR and normalized to *Hprt1*. Bars show the means + SEM of four to six samples from two independent experiments. (**H**) Supernatants of BMDCs (white, not detected), ILC2s (gray), untreated (blue), and anti–PD-L1–treated (red) BMDC-ILC2 cocultures were analyzed by ELISA for IL-5 (left) or IL-13 (right) production. *$P < 0.05$, ANOVA. (**I**) Relative *Irf4* expression was determined by qPCR of sort-purified DC from WT (black), PD-L1$^{-/-}$ (red), or *PD-L1$^{fl/fl}$CD11c^{Cre}* (blue) mice kept on HFD. Bars show the means + SEM from DC sorted from four mice per group from two independent experiments. (**J**) WT or PD-L1$^{-/-}$ (red) BMDCs were cultured alone (white) or in the presence of WT (blue) or PD-1$^{-/-}$ (orange) ILC2 as outlined in (G). Relative *Irf4* expression was analyzed by qPCR after 24 hours of coculture. Bars show the means + SD from two independent experiments. *$P < 0.05$ and **$P < 0.01$; Student's *t* test or ANOVA.

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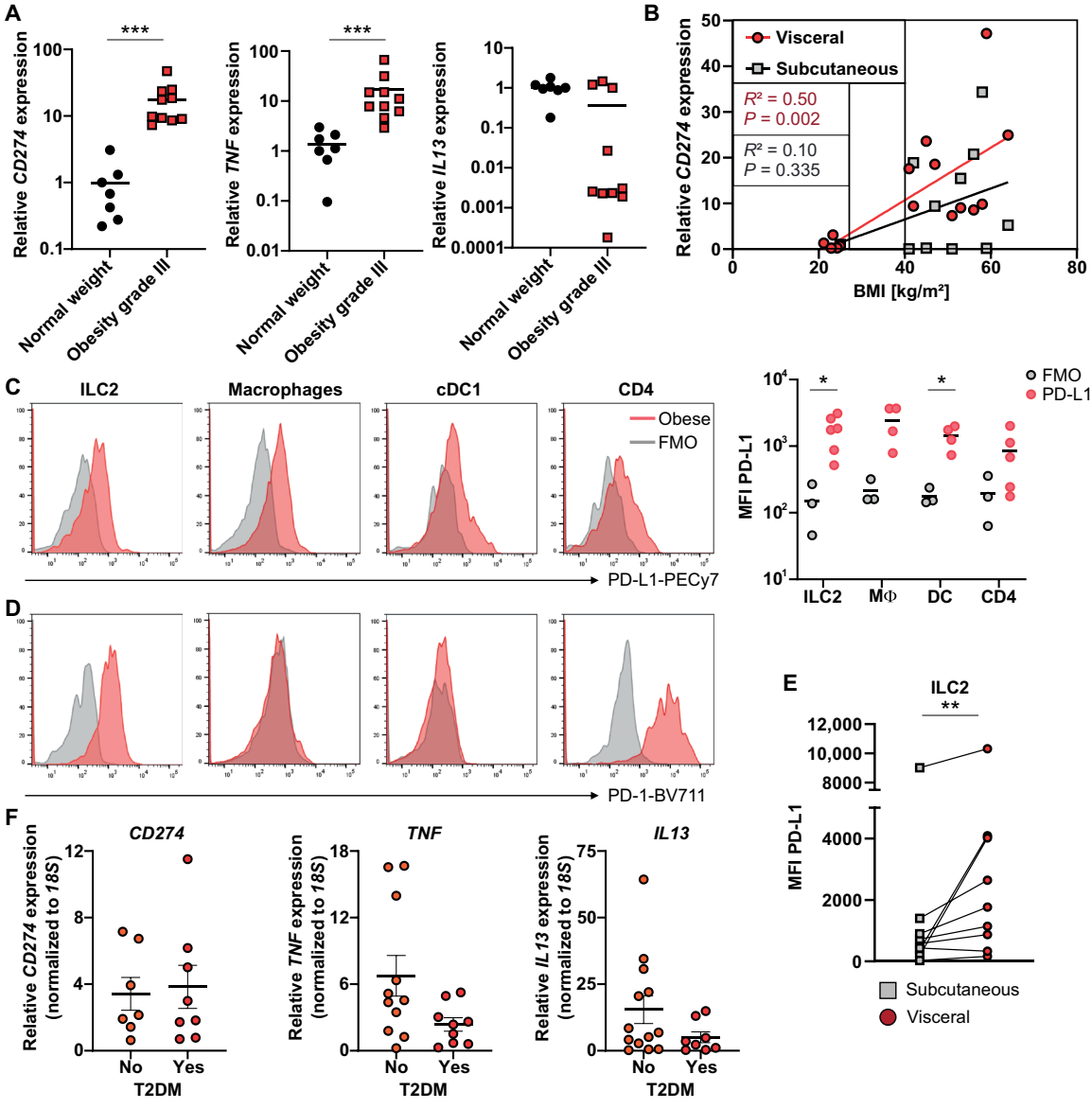

Fig. 5. PD-L1 is up-regulated in human adipose tissue. (**A**) Relative quantification of *CD274* (left), *TNF* (middle), and *IL13* (right) transcript in visceral adipose tissue of patients with normal weight (black circles; $n = 7$) and obesity grade III (BMI > 40 kg/m^2; red squares; $n = 10$). *18S* rRNA was used for normalization. Displayed are the mean and individual values. ***$P < 0.001$; Mann-Whitney *U* test. (**B**) Correlation of CD274 expression in visceral (red circles) and subcutaneous (gray squares) adipose tissue with the BMI of individual patients. (**C** and **D**) Representative histogram overlays of PD-L1 and bar graph of PD-L1 MFI (C) and PD-1 (D) expression [red; fluorescence-minus-one (FMO) gray] on indicated cell populations in the adipose tissue of patients with obesity (grade III). *$P < 0.05$; Student's *t* test. (**E**) MFI of PD-L1 on ILC2s in subcutaneous (gray) and visceral (red) adipose tissue within the same individuals. **$P < 0.01$; Wilcoxon matched-pairs signed rank test. (**F**) Obese patients were stratified according to type 2 diabetes mellitus (T2DM) status and expression of PD-L1 (*CD274*), *TNF*, and *IL13* in WAT analyzed by qPCR.

up-regulated on innate and adaptive cell populations in WAT of PWO (Fig. 5C). Whereas adipose tissue CD4$^+$ T cells ($P = 0.203$) and macrophages ($P = 0.057$) only showed a modest elevation, the surface expression of PD-L1 was significantly increased in cDC1s ($P = 0.013$) and ILC2s ($P = 0.028$; Fig. 5C). Because ILC2s and DCs are capable to regulate T cell responses via the PD-L1:PD-1 pathway, we also studied the expression of PD-1 on adipose tissue–resident immune cells of PWO. As previously reported (*28*, *33*), adipose tissue CD4$^+$ T cells and ILC2s expressed high amounts of PD-1 (Fig. 5D). PD-L1 expression on ILC2s was consistently higher in visceral adipose tissue than in subcutaneous adipose tissue (Fig. 5E). To investigate whether adipose tissue PD-L1 expression in PWO was associated with metabolic disease, we stratified patients according to type 2 diabetes (T2DM) status. There were no differences in the transcript abundance of *CD274* (PD-L1), *TNF*, or *IL13* in adipose tissue from obese individuals with or without T2DM (Fig. 5F). Regarding the therapeutic use of immune checkpoint inhibitors and possible immune-related adverse events, the increased expression of PD-L1 and PD-1 on innate and adaptive immune cells within human adipose tissue warrants further investigation.

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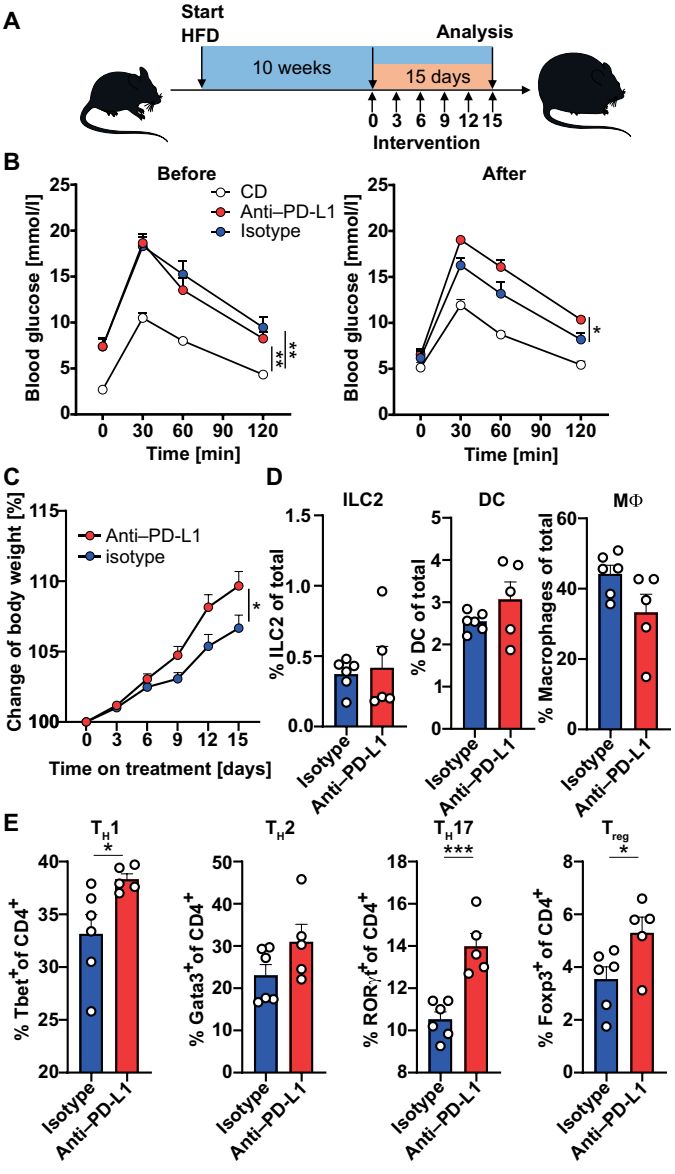

Fig. 6. Checkpoint inhibition during obesity aggravates disease. (**A**) Schematic of checkpoint inhibition. Mice were kept on HFD for 10 weeks before mAb treatment. Mice on HFD were then treated with 5 mg/kg body weight anti–PD-L1 (red) or isotype control (blue) mAb every third day for a total of five injections. Mice kept on CD throughout the experiment served as controls (gray). (**B**) Glucose tolerance test before and on day 15 after the start of intervention. Graphs show the means + SEM of four mice (before intervention) or 10 mice (after intervention) per group from two independent experiments. *$P < 0.05$, Student's *t* test of AUC. (**C**) Percentage of weight change relative to the weight at the start of therapeutic intervention. (**D**) Frequency of adipose tissue ILC2s, DCs, and macrophages (MΦ) in mice after treatment. (**E**) Polarization of $CD4^+$ T cells into T_H1 ($Tbet^+$), T_H2 ($Gata3^+$), T_H17 ($ROR\gamma t^+$), and T_{reg} ($Foxp3^+CD25^+$) cells in adipose tissue after treatment. Bar graphs show the means + SD of five to six mice per group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; Student's *t* test.

Immune checkpoint inhibitor therapy affects adipose tissue homeostasis and metabolic health

To gain insight into how therapeutic intervention with blocking antibodies may interfere with adipose tissue immune cells during obesity, we treated obese mice with monoclonal antibodies (mAbs) against PD-1 or PD-L1. Obese WT mice that had been on HFD for 10 weeks and received blocking mAb against PD-L1 every 3 days (six doses in total; Fig. 6A) did not show any signs of overt systemic inflammation. Before mAb intervention, both groups of mice (isotype control– and anti–PD-L1–treated mAb) had similar responses during the glucose tolerance test. However, anti–PD-L1 mAb treatment led to a significant ($P < 0.05$) decrease in glucose tolerance relative to the isotype control–treated mice, suggesting that blockade of PD-L1 led to a worse metabolic state (Fig. 6B). We also observed increasing weight gain (Fig. 6C), similar to our results from *PD-L1*$^{-/-}$ animals, in anti–PD-L1 mAb-treated obese mice. Anti–PD-L1 mAb treatment did not significantly alter the frequency of adipose tissue ILC2s, DCs, or macrophages (Fig. 6D). However, PD-L1 blockade of obese mice led to an enhanced differentiation of T_Hs, in particular toward T_H1 and T_H17 subsets within the adipose tissue (Fig. 6E).

We then were interested whether PD-L1 played a role during the early phase of diet-induced obesity and, if so, whether it affected the subsequent T cell response. Thus, we injected anti–PD-L1 mAb in the first 3 weeks of diet-induced obesity, before mice were obese (fig. S3, A and B). However, we did not observe a significantly unchecked T_H1 response ($P = 0.114$) when we analyzed T cell polarization 9 weeks after mAb intervention. Conversely, when we administered recombinant PD-L1 to mice during the early phase of diet-induced obesity, T cell differentiation was generally impaired—spanning proinflammatory T_H1 and T_H17 as well as anti-inflammatory T_H2 and T_{reg} cells—and ILC2 expansion was limited during obesity (fig. S3, C and D). As recombinant PD-L1–treated mice showed increased weight gain, these results suggest that PD-L1 acts in a cell-specific manner during the onset of obesity. Therefore, we injected WT or *PD-L1*$^{-/-}$ BMDCs into *PD-L1*$^{-/-}$ mice during the first weeks of diet-induced obesity. Indeed, the expression of PD-L1 on transferred DCs into PD-L1–deficient mice was sufficient to significantly ameliorate HFD-induced weight gain after 6 weeks on HFD ($P < 0.05$; fig. S3E).

Because patients receiving cancer treatment usually change their diet or lose weight due to the disease, we decided to switch obese mice back on a conventional control diet (CD; Fig. 7A). Interventions targeting the PD-L1:PD-1 axis in mice on HFD did not alter food intake or water consumption. Whereas untreated obese mice that remained on HFD further increased their body weight, obese mice placed on CD (HFD-CD) and treated with an isotype control mAb started to lose weight (Fig. 7B). Antibody-mediated blockade of PD-1 ($P < 0.001$) and PD-L1 ($P = 0.013$) of HFD-CD groups of mice both significantly accelerated weight loss in mice relative to the isotype control group (Fig. 7B). Concurrently, the proportion of epididymal WAT decreased (Fig. 7C), although we did not see a significant effect of mAb treatment after 7 days. In HFD-CD mice on CD for 1 week, the cellularity of adipose tissue started to change. ILC2 and eosinophil numbers increased (Fig. 7D), indicative of the generation of type 2 response in adipose tissue. The fact that ILC2 numbers were not restored in response to anti–PD-L1 treatment supports our finding that PD-L1 on ILC2s is redundant and suggests that PD-1 may be more important for their function in adipose tissue. The expression of PD-1 on T cells decreased in response to anti–PD-1 mAb treatment (Fig. 7E). The frequency of adipose tissue DCs did not change after mAb treatment (Fig. 7F). Whereas PD-1 blockade also decreased surface PD-L1 on DCs, PD-L1 blockade did not significantly alter expression of PD-1 on

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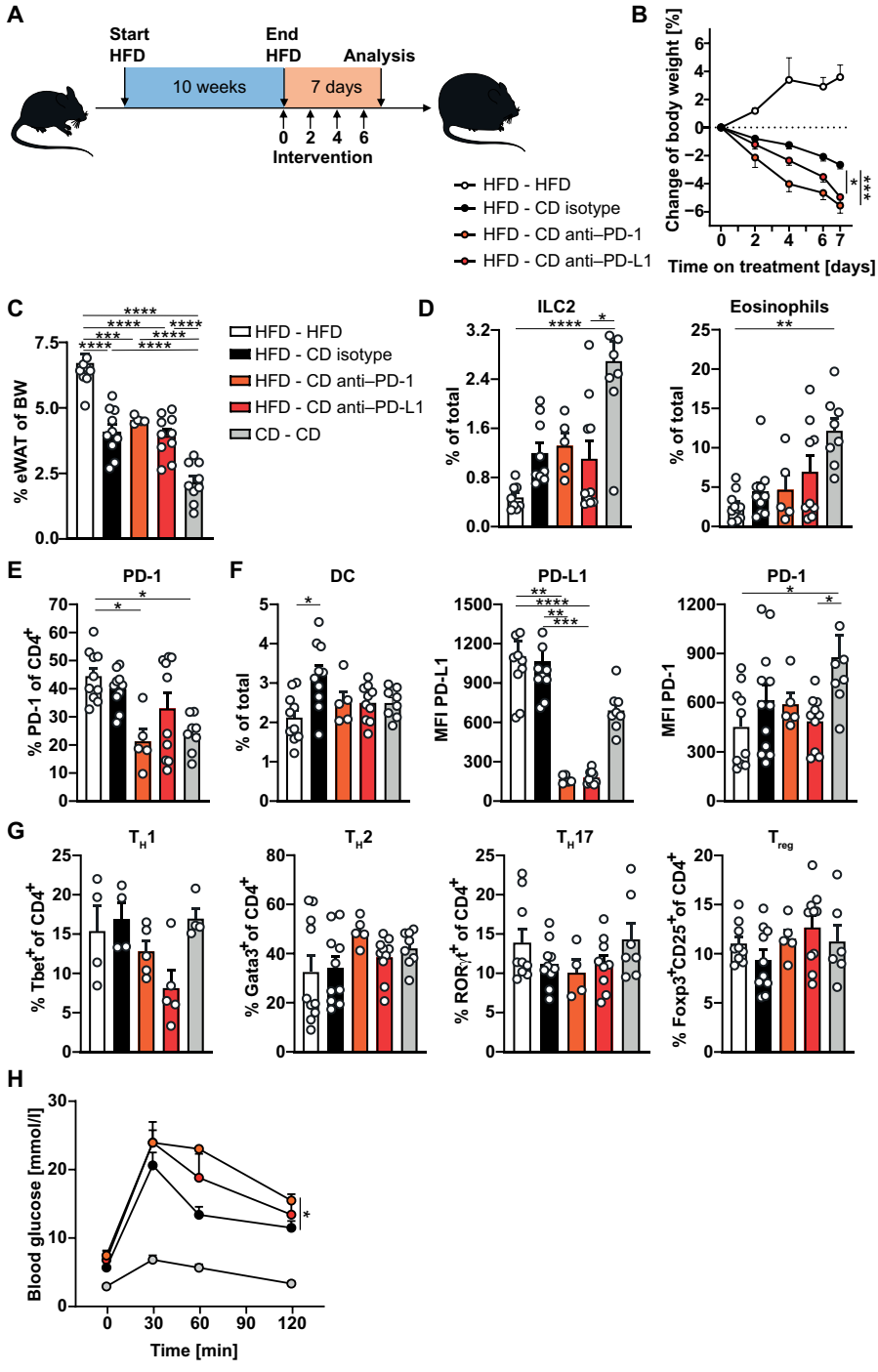

Fig. 7. Therapeutic checkpoint inhibition accelerates weight loss of obese mice. (**A**) Schematic of therapeutic intervention. Mice were kept on HFD for 10 weeks before mAb treatment. Mice were either kept on HFD (white) or changed back to CD and treated with 5 mg/kg body weight anti–PD-1 (orange), anti–PD-1 (red), or isotype control (black) mAb every other day for a total of four injections. Mice kept on CD throughout the experiment served as controls (gray). (**B**) Percentage of weight change relative to the weight at the start of therapeutic intervention. (**C**) Weight of eWAT relative to body weight following therapeutic intervention. (**D**) Frequency of ILC2s and eosinophils in mice after treatment. (**E**) PD-1 expression on adipose tissue $CD4^+$ T cells after treatment. (**F**) Frequency of DCs among total adipose tissue cells and MFI of PD-L1 and PD-1 on DCs following treatment. (**G**) Polarization of $CD4^+$ T cells into T_H1 ($Tbet^+$), T_H2 ($Gata3^+$), T_H17 ($ROR\gamma t^+$), and T_{reg} ($Foxp3^+CD25^+$) cells in adipose tissue after treatment. Bar graphs show the means + SEM from two independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$; ANOVA with post hoc Tukey test. (**H**) Glucose tolerance test on day 8 after the start of therapeutic intervention.

DCs ($P \geq 0.05$; Fig. 7F). Treatment of mice with anti–PD-1 or anti–PD-L1 mAb did not significantly change T cell differentiation ($P \geq 0.05$; Fig. 7G). However, in contrast to the beneficial effect on weight loss, interference with PD-L1 led to a deterioration of metabolic health (Fig. 7H), suggesting a systemic inflammatory effect of mAb treatment in a state of low-grade inflammation during obesity. These data show that mAb-mediated inhibition of PD-L1:PD-1 interaction directly affects immune cells within the adipose tissue during obesity and can lead to the deterioration of metabolic health.

DISCUSSION

Immune cells play an important role in the homeostasis of adipose tissue and dysregulation of the balance between pro- and anti-inflammatory cells promotes progression of obesity. The central objective of this work was to investigate the functional role of the immunoregulatory molecule PD-L1 during adipose tissue homeostasis. The present study demonstrates in lean and obese individuals that there is increased expression of PD-L1 on cells, including DCs, in visceral fat, with elevated expression of *CD274* mRNA correlative with BMI. In mouse models, a major role for PD-L1 expressed on DCs as a potent regulator of adipose tissue immunity was shown; first, adipose tissue DCs express high amounts of PD-L1 in mice, as well as in humans; second, constitutive and DC-specific PD-L1 deficiency enhances weight gain, impairs metabolic function, and shifts T cell polarization toward T_H1; and last, PD-L1 blockade in vitro affects T_H2 polarization and ILC2 function. Together with our results on checkpoint inhibition in vivo that show a direct effect on T cell polarization and weight loss, these data suggest that PD-L1 on DCs maintains a homeostatic adipose tissue environment and counteracts inflammation.

In mice, increased PD-L1 expression in adipose tissue was observed during HFD, and here in particular during early time points, and in mice on short-term HFD (*38*, *39*). We also observed that up-regulation of PD-L1 (*CD274*) mRNA in human adipose tissue had a positive correlation with BMI but not type 2 diabetes, which is in line with previous findings, when adipose tissue was analyzed in children or adults with an obese BMI (*40*, *41*). In contrast to our data, another study reported a negative correlation between BMI and PD-L1 expression (*42*), which warrants further investigation especially regarding

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patient stratification and cell-specific expression of PD-L1. In a recent study, single-cell RNA sequencing was performed on human WAT of persons with or without obesity (*43*). Indeed, analysis of the published dataset (GSE156110) (*43*) confirmed the human data presented here with up-regulation of PD-L1 on adipose tissue DCs.

We observed elevated expression of PD-L1 protein on adipose tissue DCs, macrophages, ILC2s, and T cells in mice and humans, which has been reported previously in other tissues (*27*, *28*, *30*). In this study, we now have deciphered the cell-specific function of PD-L1 during obesity. Expression of PD-L1 on DCs is an important factor in the inhibition of proinflammatory T_H1 and T_H17, as well as cytotoxic T cells during autoimmune and antitumor responses (*29*, *30*, *44*). In line with these findings, deletion of PD-L1 on DCs unleashed T_H1 cells during obesity. We have previously reported an activating function of ILC2-expressed PD-L1 on T_H2 cells in the context of type 2 inflammation (*27*). Furthermore, PD-L1 was shown to be required for the development and maintenance of T_{regs} (*35*). With the current study, we now show that PD-L1 expressed on DCs can promote adipose tissue T_{reg} responses in vivo, while inhibiting effector T cell responses. It appears that PD-L1 on DCs plays a distinct role in limiting T_H1 differentiation, because therapeutic intervention during established obesity led to the expansion of adipose tissue $CD4^+$ T cells in general. Similarly, recombinant PD-L1 blocked T cell differentiation toward all examined subsets. A similar inhibitory effect of recombinant PD-L1 on both effector and T_{regs} was observed in a model of dextran sulfate sodium–induced colitis with reduced IFNγ, IL-17A, and IL-10 produced from T cells (*45*).

In recent years, ILC2s emerged as critical regulators of adipose tissue homeostasis (*3*, *15*). Although we previously observed an important role of PD-L1 expression on ILC2s for T_H2 polarization during type 2 lung inflammation (*27*), PD-L1 on ILC2s turned out to be dispensable for T cell responses during obesity. Instead, the elevated expression of PD-1 on ILC2s we observed during obesity has previously been implicated in adipose tissue homeostasis. One study has suggested that tumor necrosis factor (TNF)–mediated release of IL-33 activated ILC2s and promoted their PD-1 expression, as well as PD-L1 expression on macrophages (*28*). PD-1 blockade partially restored tissue homeostasis (*28*). Based on our observation that ILC2 can interact with DCs via PD-L1 in vitro, it is likely that ILC2s interact in vivo with PD-L1–expressing DCs in addition to macrophages. Additional experiments using genetic ablation of PD-1 on ILC2s will be required for assessment. Recently, it was shown that PD-1 signaling on pulmonary ILC2s regulates their metabolism and limits viability and inflammation (*46*). Furthermore, in a model of pulmonary inflammation, ILC2s were shown to be necessary to elicit $IRF4^+CD11b^+CD103^-$ DCs to produce the T_H2-cell attracting cytokine CCL17 (*37*). In line with these findings, we also observed increased *Irf4* and *Ccl17* expression in BMDC-ILC2 cocultures. This up-regulation was dependent on intact PD-1:PD-L1 interaction in vitro and in vivo. Further studies are required to assess to which extent PD-L1–expressing DCs control function of pulmonary and adipose tissue ILC2s.

We did not observe a regulatory role for PD-L1–expressing macrophages, which has been previously suggested (*28*). An earlier study found—in addition to macrophages—significant deletion of MHCII on a majority of adipose tissue DCs in Lyz2Cre mice that led to reduced $CD4^+$ T cell accumulation in adipose tissue (*22*). In our experiments, we did not observe reduced expression of PD-L1 in $PD\text{-}L1^{fl/fl}Lyz2^{Cre/+}$ mice, which may be due to the use of heterozygous Lyz2Cre mice for our experiments as we observed robust deletion of PD-L1 on adipose tissue macrophages in vivo. In our hands, deletion of PD-L1 on macrophages did not lead to hyperactivity of $PD\text{-}1^+$ WAT ILC2s, which was expected to limit obesity based on previous results (*28*). The discrepancy may be due to the use of monoclonal anti–PD-1 antibodies in this earlier study, which possibly affected not only ILC2s and their interaction with PD-L1–expressing macrophages but also the interaction with adipose tissue DCs, and different $CD4^+$ effector and Tfh cells, and cytotoxic $CD8^+$ T cells (*29*, *44*).

Interaction of PD-L1 with its receptor PD-1 generally leads to the inhibition of T cell survival and cytokine production, and to T cell exhaustion (*26*). Furthermore, PD-1 expression is up-regulated on T cells of PWO (*47*), which we and others also observed in obese animals (*33*). PD-1 is expressed on activated $CD4^+$ and $CD8^+$ T cells (*48*) and Tfh cells and is not only indicative of exhausted T cells (*49*), especially under chronic inflammatory conditions (*50*). Moreover, we and others previously observed an activating effect of PD-L1 on PD-1–expressing T_H2 and T_{reg} cells (*27*, *35*, *51*). Adipose tissue–resident T_{regs} play an important role in limiting adipose tissue inflammation (*52*). Increased IL-33 in male mice led to the recruitment of T_{regs} in a Blimp1-dependent manner (*52*). Previously, it was shown that the function of adipose tissue T_{regs} was dependent on IRF4 (*17*) and that PD-L1:PD-1 interactions promoted T_{reg} maintenance and function (*35*, *53*). We observed that *Irf4* expression in adipose tissue DCs is altered in a PD-L1–dependent manner and a decrease in Tregs in $PD\text{-}L1^{fl/fl}DC^{Cre}$ mice. Thus, DC-IRF4 may also contribute to the decrease in adipose tissue T_{regs} in IRF4-deficient mice (*17*). Checkpoint inhibition with anti–PD-L1 mAb or anti–PD-1 mAb also targets interaction between PD-1–expressing immune cells and adipocytes, which were shown to express PD-L1 (*42*, *54*). PD-L1 was reported as an activation-independent marker of brown adipose tissue (*54*), and others also reported high expression in WAT (*42*). Indeed, adipocyte-specific deletion of PD-L1 on WAT contributed to exacerbated weight gain during diet-induced obesity, while the underlying mechanism was not investigated except for the presence of increased numbers of inflammatory macrophages (*42*). Further studies are necessary to investigate adipocyte-PD-L1–mediated regulation of other PD-1–expressing cells, including ILC2s and T cells.

Recent studies have revealed increased efficacy of PD-1/PD-L1 blockade in patients with cancer and tumor-bearing mice. Progression-free survival and overall survival of patients with melanoma and obesity treated with anti–PD-(L)1 inhibitors were improved compared to patients with normal BMI (*47*). The same study suggested that obesity-mediated T cell aging leading to higher PD-1 expression rendered T cells more responsive to checkpoint inhibitor therapy. The increased expression of PD-L1 we and others observed in adipose tissue therefore is an important and possibly overlooked factor in cancer therapy. Our results on checkpoint blockade indicate a direct negative effect of mAb-mediated inhibition on adipose tissue effector T cells, which may be important for tumors embedded in an adipose tissue-rich environment. Indeed, adipocyte-PD-L1 expression attenuated anti–PD-L1 effects on $CD8^+$ T cells in vitro, and pharmacologic inhibition of adipocyte-PD-L1 improved checkpoint inhibition in a mouse model of breast cancer in vivo (*55*). Our results, together with data showing a critical role for PD-L1 expressed on DCs during PD-L1 blockade (*29*), highlight the need for further studies investigating the cell-specific contribution of PD-L1

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to immune responses and immune-related adverse events of checkpoint inhibitor therapy - in particular, taking into consideration that autoimmune insulin-dependent diabetes can occur in up to 1% of patients treated with anti–PD-L1 or –PD-1 checkpoint inhibitors (*56*).

Our study has limitations. First, we did not analyze the cell-specific function of PD-L1 in adipose tissue in female mice because the diet-induced obesity model is predominantly a model applied to male mice. Second, we did not formally address the intracellular mechanisms that underlie PD-L1:PD-1–mediated interaction between DCs and ILC2s, T_H1, or T_H2 cells. Third, we did not directly show that PD-L1 engagement in PWO affects tissue inflammation, weight gain, or adverse events during checkpoint inhibitor therapy. Addressing these limitations in future studies will be critical to extend our knowledge on PD-L1–mediated adipose tissue inflammation.

In summary, our data demonstrated an increased expression of PD-L1 on cells in adipose tissue of obese individuals, irrespective of T2DM status, and this elevation in PD-L1 expression was significantly associated with BMI. The expression of PD-L1 by DCs regulates T cells in the adipose tissue–ameliorated obesity-induced inflammation and associated metabolic alterations (fig. S4). Our findings are relevant not only for our understanding of the pathogenesis of obesity but also for patients with cancer undergoing PD-L1/PD-1-checkpoint inhibitor treatments that might cause adverse effects due to the modulation of immune cells in adipose tissue.

MATERIALS AND METHODS

Study design

The main goal of this study was to investigate the role of PD-L1 in the regulation of adipose tissue inflammation during diet-induced obesity. We designed multiple studies to address the relevance of PD-L1 during obesity. In vivo obesity studies followed by ex vivo analyses were conducted in mice that lacked expression of PD-L1 on specific cell populations to assess cell-specific functions of PD-L1. In vitro studies on DCs, ILC2s, and T cells complemented our in vivo experiments. Furthermore, we analyzed clinical samples of obese patients to assess PD-L1 expression in human adipose tissue. Main techniques used were flow cytometry, quantitative reverse transcriptase polymerase chain reaction (qPCR), enzyme-linked immunosorbent assay (ELISA), histology, and glucose tolerance testing.

Predefined study components

Sample size calculation for in vivo studies was performed using G*Power (*57*) and previous studies of our laboratory (*2*, *27*, *34*) in accordance with Irish and German animal protection law. End point selection criteria were based on published literature and our previous studies. All technically reliable data points were included in the statistical analyses. All data points are reported in data file S3.

Randomization

In case of intervention studies, purchased mice were randomly assigned to treatment groups. For diet-induced obesity studies in mice, conditional knockout mice and negative littermates were housed together. Experimental mice were matched for sex, age, and weight at the start of the experiment. Clinical samples were collected longitudinally.

Blinding

In case of intervention studies, experiments were performed in a blinded manner. Only after data were analyzed were the experimental groups revealed. For genetically modified mice, weighing was performed by experimenters unaware of the genotype of the respective animals.

Replication

For all studies, biological replicates and number of independently performed experiments are indicated in the figure legends. The sum of the data units is the number of biological replicates, which are depicted as individual values in bar graphs.

Clinical samples

Human adipose tissue (visceral and subcutaneous) was obtained from patients undergoing minimally invasive (bariatric) surgery. Tissue from lean individuals was obtained from nondiabetic patients undergoing antireflux or hiatal hernia surgery. Tissue collection and analysis was approved by the Ethics review board (Ethikkommission) at the Universitätsklinikum Erlangen (208_19 B and 21-338_1-B) or St. Vincent's University Hospital, Dublin. Written informed consent was obtained from each patient before commencement of research activities. The BMI for each participant was calculated as mass (kg)/height $(m)^2$ (Table 1). Samples were fixed in 10% neutral-buffered formalin, snap-frozen in liquid nitrogen for RNA extraction, or digested with Collagenase D (1 mg/ml; Roche) in Dulbecco's modified Eagle's medium [supplemented with 10% fetal bovine serum, L-glutamine, Pen/Strep (all from Thermo Fisher Scientific)] for 45-min shaking (200 rpm) at 37°C. Single-cell suspensions were obtained by filtration through a 100-μm cell strainer (BD), followed by red blood cell lysis. Cells were stained with combinations of fluorochrome-conjugated antibodies (all from BioLegend, except where indicated, also refer to data file S2): Phycoerythrin (PE)–Dazzle594–conjugated anti-CRTH2 (clone BM16), Allophycocyanin (APC)–conjugated anti-CD161 (clone HP-3G10), APC-Fire750–conjugated anti-CD127 (clone A019D5), PE-Cyanine (Cy)7–conjugated anti-PD-L1 (clone MIH3), APC-Fire750–conjugated anti-CD11c (clone S-HCL-3), Brilliant Violet (BV)421-conjugated anti-CD141 (clone M80), BV421-conjugated anti-CD25 (clone BC96), lineage cocktail (BD), BV785-conjugated anti–PD-1 (clone NAT105), PE-Cy7–conjugated anti-CD66b (clone G10F5), PE-Dazzle594–conjugated anti-CD16 (clone 3G8), and Peridinin chlorophyll protein (PerCP)Cy5.5-conjugated anti-CD4 (clone OKT4).

Mice

*Cd274*loxP (PD-$L1^{fl/fl}$; MGI:6115470) mice were generated on the C57BL/6 background and described previously (*27*). B7-$H1^{-/-}$ (PD-$L1^{-/-}$;

Table 1. Patient characteristics of lean and obese patients.

	Obese ($n = 29$)	Lean ($n = 7$)	*P* value
Age, years (mean ± SD)	45 ± 8.9	43 ± 9.2	$P = 0.54$
Sex, male	7/29 (24.1%)	2/7 (28.6%)	
BMI, kg/m^2 (mean ± SD)	54 ± 9.9	23.5 ± 1.4	$P < 0.001$
T2DM	8/29 (27.6%)	0/7 (0%)	
Type of bariatric surgery			
Sleeve gastrectomy	16/29 (55.2%)	0/7 (0%)	
Gastric bypass	13/29 (44.8%)	0/7 (0%)	

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MGI:3043876) mice were originally obtained from L. Chen (Yale University, New Haven, CT) and maintained on a C57BL/6 background. Il7rtm1.1(icre)Hrr (IL7RαCre; MGI:4441349) were previously described and crossed to PD-L1$^{fl/fl}$ (*27*, *58*). Pdcd1$^{-/-}$ (MGI:4397682) (*59*), Zbtb46Cre (B6.Cg-*Zbtb46*$^{tm3.1(cre)Mnz}$/J; MGI:5749231) (*60*), and CD4Cre [Tg(Cd4-cre)1Cwi/BfluJ; MGI:2386448] (*61*) mice were purchased from the Jackson Laboratory. CD11cCre (MGI:3763248) mice were provided by D. Voehringer (Universitätsklinikum Erlangen, Germany) and originally obtained from B. Reizis (Columbia University Medical Center, NY) (*62*). LysMCre (B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J; MGI:1934631) were provided by J. Mattner (Universitätsklinikum Erlangen, Germany) (*63*). Animals were housed in a specific pathogen–free facility. Mice had ad libitum access to food and water, and welfare was checked daily. All animal experiments were performed in compliance with Irish Medicines Board/Health Products Regulatory Authority (HPRA) regulations or German animal protection law and approved by the Trinity College Dublin's BioResources ethics review board and the HPRA (AE19136/P077) or the Federal Government of Lower Franconia (RUF-55.2.2-2532-2-1177). All sections of this report adhere to the ARRIVE guidelines for reporting animal research and a completed ARRIVE guidelines checklist is included in data file S2.

Diet-induced obesity

Age-matched mice were kept on an HFD (60% kcal fat, D12492; Research Diets, Inc., New Brunswick, NJ; or ssniff Spezialdiäten, Soest, Germany) or control diet (10% kcal fat) for 8 to 16 weeks as indicated (*64*). Body weight was recorded weekly. In chronic HFD studies, food intake and water consumption were recorded weekly and measured daily during intervention studies. For glucose and insulin tolerance testing, mice were starved overnight or for 5 hours, respectively. Mice were challenged with 2 g/kg body weight glucose intraperitoneally or 0.75 mU/g human insulin intraperitoneally to test metabolic status. Blood glucose was measured before and 30, 60, and 120 min after challenge using a glucometer (Abbott Laboratories, Abbott Park, IL, USA).

Therapeutic intervention

mAbs directed against PD-1 (clone 29F.1A12; BioXCell, Lebanon, NH), PD-L1 (clone 10F.9G2; BioXCell) or anti-KLH (clone LTF2; isotype control; BioXCell), or recombinant mouse PD-L1 (R&D Systems) were administered every other day intraperitoneally (5 mg/kg body weight) into mice. In one set of experiments, mice were kept on an HFD for 10 weeks before intervention, weight changes were monitored at indicated times, and mice were analyzed 7 days after the start of treatment. In another set of experiments, mice were injected weekly with anti–PD-L1 mAb, anti-KLH mAb, or recombinant PD-L1 or control protein (5 mg/kg body weight) during the first weeks of diet-induced obesity. Weight changes were monitored weekly and mice were analyzed 8 weeks later.

Statistical analysis

Flow cytometry data were analyzed using FlowJo software (v10.6; Tree Star). GraphPad Prism (v8) was used to generate graphs and for statistical analysis. Details on sample size (biological replicates), number of repetitions, and statistical test are listed in figure legends. Shapiro-Wilk test was used to determine normal distribution. Accordingly, Student's *t* test or Mann-Whitney *U* test was used to determine statistical significance between two groups. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used to determine significant differences between multiple groups. P values < 0.05 were considered statistically significant. Only relevant statistics are displayed in the figures. Raw data used to generate graphs and statistical analysis are provided in data file S3.

SUPPLEMENTARY MATERIALS

www.science.org/doi/10.1126/scitranslmed.abj6879
Materials and Methods
Figs. S1 to S4
Data files S1 to S3
MDAR Checklist

View/request a protocol for this paper from *Bio-protocol*.

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Innate PD-L1 limits T cell–mediated adipose tissue inflammation and ameliorates diet-induced obesity

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PD-L1 gives adipose inflammation a brake

Obesity-associated inflammation is known to contribute to comorbidities, but the mechanisms that regulate this inflammation are not entirely clear. Schwartz *et al.* show that programmed death-ligand 1 (PD-L1) on dendritic cells controls adaptive immune responses within adipose tissues to limit diet-induced obesity. In mice on a high-fat diet (HFD), PD-L1 deficiency on dendritic cells promoted increased obesity and impaired adipose tissue T cell polarization. Of note, anti–PD-L1 mAb treatment decreased glucose tolerance in HFD mice. PD-L1 expression was also increased in the adipose tissue of people with obesity, suggesting that PD-L1 on DCs may also counteract adipose inflammation in humans.

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