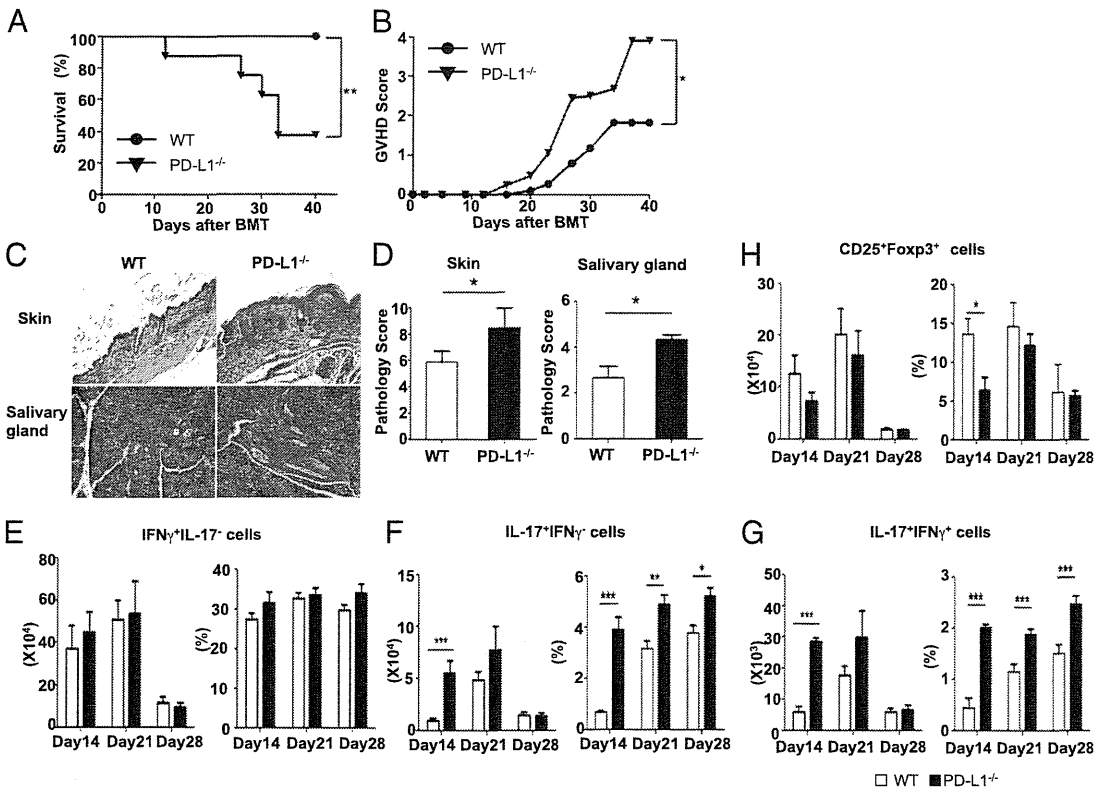

FIGURE 4. PD-L1 deficiency in recipients exacerbates chronic GVHD with Th17/Th1 cell expansion. (**A–D**) Sublethally irradiated WT or PD-L1$^{-/-}$ BALB/c recipients were transplanted from WT B10.D2 donors. Survival (A) and clinical GVHD skin score (B) are shown; data shown are from 1 representative of ≥3 independent experiments (n = 8 in each group). (C and D) Skin and salivary gland from indicated recipients were taken on day 36 after BMT. (C) Representative images with Masson trichrome staining are shown (original magnification ×100). (D) Pathology score of skin and salivary gland on day 36 after BMT is shown. The numbers and percentages of donor-derived CD4$^+$ T cells expressing IFN-γ$^+$IL-17$^-$ (**E**), IL-17$^+$IFN-γ$^-$ (**F**), and IL-17$^+$ IFN-γ$^+$ cells (**G**), and CD25$^+$ Foxp3$^+$ cells (**H**) from pLNs on days 14, 21, and 28 are shown. The means (± SE) of each group are shown. Data shown are from 1 representative of ≥3 independent experiments (n = 6–8 in each group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.

those of the control group ($p < 0.01$, Fig. 3C; $p < 0.05$, Fig. 3D). Pathologic scores of skin and liver were significantly higher in anti–PD-L1–treated mice than in the controls (skin: 7.17 ± 0.17 versus 5.20 ± 0.58, $p < 0.05$; liver: 5.25 ± 0.31 versus 2.75 ± 0.25, $p < 0.05$; salivary gland: 3.67 ± 0.42 versus 2.00 ± 0.32, $p < 0.05$; Fig. 3E, 3F). Clinical and pathogenic scores tended to be worse in anti–PD-L2–treated mice, as compared with those treated with control, although it was not statistically significant (Fig. 3E, 3F). These findings suggest that the PD-1 pathway, especially the PD-1/PD-L1 pathway, plays a critical role in suppressing lethal chronic GVHD.

Lack of PD-L1 expression exacerbated chronic GVHD with IL-17$^+$IFN-γ$^+$ T cell expansion

The anti–PD-L1 mAb neutralized PD-L1 on host cells, as well as on donor cells. To evaluate the contribution of host PD-L1 to chronic GVHD, we used PD-L1$^{-/-}$ mice on BALB/c background as recipients. On transferring WT donor T cells into PD-L1$^{-/-}$ recipients, survival was shortened significantly ($p < 0.01$; Fig. 4A) and skin chronic GVHD scores were enhanced in comparison with WT recipients ($p < 0.05$; Fig. 4B). Histopathologic examination of skin and salivary gland showed that exacerbated GVHD in PD-L1$^{-/-}$ recipients was not simply shifted toward acute GVHD, but rather significantly exacerbated chronic GVHD pathology with decreased fat, dermal fibrosis, epidermal interface changes, diffuse hair loss, and inflammatory cell invasion of skin, fibrosis, and atrophy of salivary gland (skin: 5.88 ± 0.85 versus 8.38 ± 0.38, $p < 0.05$; salivary gland: 2.67 ± 0.49 versus 4.33 ± 0.21, $p < 0.05$; Fig. 4C, 4D).

Our previous study and the current results (Fig. 1D, 1E) showed that Th17/Th1 cell expansion was detected during chronic GVHD and contributed to chronic GVHD progression (27). We next assessed Th subsets from pLNs of WT and PD-L1$^{-/-}$ recipients. Absolute numbers of IFN-γ$^+$IL-17$^-$, IL-17$^+$IFN-γ$^-$, and IL-17$^+$ IFN-γ$^+$ CD4$^+$ T cells from pLNs of PD-L1$^{-/-}$ recipients were modestly increased from days 14 to 21 and declined to the same levels between WT PD-L1$^{-/-}$ recipients because of lymphocytopenia of chronic GVHD (Fig. 4E–G). Intracellular staining showed that no differences were observed in frequency of IFN-γ$^+$ IL-17$^-$ T cells between PD-L1$^{-/-}$ and WT recipients; however, IL-17$^+$IFN-γ$^+$ T cells were detected significantly more frequently in PD-L1$^{-/-}$ recipients from days 14 to 28 ($p < 0.005$; Fig. 4G). In contrast, CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs from PD-L1$^{-/-}$ recipients were detected less frequently on day 14 than in WT recipients ($p < 0.05$), but levels were similar on days 21 and 28 (Fig. 4H). These results suggest that host PD-L1 deficiency exacerbated chronic GVHD in conjunction with IL-17$^+$IFN-γ$^+$ T cell expansion.

PD-L1 expression on host tissues contributes to chronic GVHD augmentation

To separate the role of PD-L1 on host APCs from host tissues, we generated chimeric recipients expressing PD-L1 on only hematopoietic cells or host tissues. Three types of chimeras were prepared: (WT→WT), (WT→PD-L1$^{-/-}$), and (PD-L1$^{-/-}$→WT). The three types of chimera mice were sublethally irradiated and then transplanted with 2 × 10^6 spleen T cells and 8 × 10^6 TCD-BM cells from B10.D2 mice. (PD-L1$^{-/-}$→WT) recipients showed

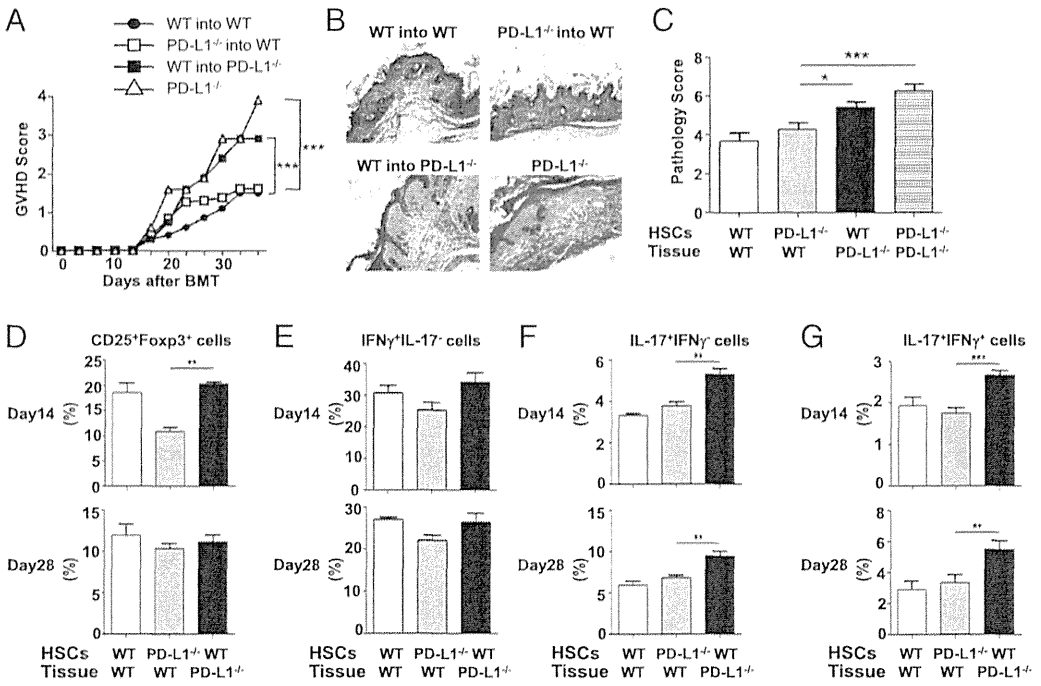

FIGURE 5. PD-L1 expression on host tissues contributes to chronic GVHD augmentation. (**A–G**) Sublethally irradiated (WT into WT), (PD-L1$^{-/-}$ into WT), (WT into PD-L1$^{-/-}$) chimera BALB/c recipients and PD-L1$^{-/-}$ BALB/c recipients were transplanted from WT B10.D2 donors. Clinical GVHD skin scores (A) are shown; data shown are from 1 representative of ≥3 independent experiments (n = 8 in each group). Skin tissues from the recipients were taken on day 36 after BMT. (B) Representative images are shown (original magnification ×100). (C) Pathology scores of skin on day 36 after BMT are shown. The percentages of donor-derived CD4$^+$ T cells expressing CD25$^+$ Foxp3$^+$ (D), IFN-γ$^+$IL-17$^-$ (E), IL-17$^+$IFN-γ$^-$ (F), and IL-17$^+$IFN-γ$^+$ cells (G) from pLNs of (WT into WT), (PD-L1$^{-/-}$ into WT), and (WT into PD-L1$^{-/-}$) recipients on days 14 and 28 are shown. The means (± SE) of each group are shown. Data shown are from 1 representative of ≥3 independent experiments (n = 6–8 in each group). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

similar clinical chronic GVHD to (WT→WT) recipients. In contrast, clinical chronic GVHD scores were exacerbated significantly in (WT→PD-L1$^{-/-}$) recipients compared with (WT→WT) recipients (Fig. 5A). Histopathologic examination also showed significantly exacerbated chronic GVHD pathology in (WT→PD-L1$^{-/-}$) recipients compared with (WT→WT) recipients (5.43 ± 0.30 versus 3.67 ± 0.42; $p < 0.05$; Fig. 5B, 5C).

We assessed CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs and Th17/Th1 expansion in pLNs of chimera recipients. CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs from (PD-L1$^{-/-}$→WT) recipients were detected less frequently on day 14 than in (WT→WT) and (WT→PD-L1$^{-/-}$) recipients ($p < 0.01$), but at similar levels on day 28 ($p = 0.36$; Fig. 5D). Intracellular staining also showed that IFN-γ$^+$IL-17$^-$ CD4$^+$ T cells from (WT→PD-L1$^{-/-}$) recipients were almost identical to (PD-L1$^{-/-}$→WT) and (WT→WT) recipients on days 14 and 28 after BMT (Fig. 5E). IL-17$^+$IFN-γ$^-$ and IL-17$^+$IFN-γ$^+$ CD4$^+$ T cells from (WT→PD-L1$^{-/-}$) recipients were increased and detected significantly more frequently than in (PD-L1$^{-/-}$→WT) recipients on days 14 and 28 after BMT (IL-17$^+$IFN-γ$^-$; day 14: $p < 0.01$; day 28: $p < 0.01$; Fig. 5E, IL-17$^+$IFN-γ$^+$; day 14: $p < 0.005$; day 28: $p < 0.01$; Fig. 5F). Collectively, these findings indicated that PD-L1 expression in host tissues was involved in suppressing the expansion of IL-17$^+$IFN-γ$^+$ T cells, attenuating chronic GVHD, and that PD-L1 expression on hematopoietic cells plays a role in the development of Tregs only during the early transplantation period but does not affect chronic GVHD severity.

Administration of Am80 overcomes the IL-17$^+$IFN-γ$^+$ T cell expansion caused by PD-L1 deficiency

Next, we examined whether the synthetic retinoid Am80 could alleviate chronic GVHD in PD-L1$^{-/-}$ recipients, because in a previous study we showed that Am80 suppressed Th17/Th1 cells (29). Recipients were administered Am80 orally (1.0 mg/kg) from day 0 after BMT. Am80 significantly ameliorated the clinical score not only in WT recipients, but also in PD-L1$^{-/-}$ recipients compared with the control group ($p < 0.005$; Fig. 6A). Histopathologic examination showed significantly reduced chronic GVHD skin damage in Am80-treated animals (WT vehicle: 5.50 ± 0.29 versus WT Am80: 2.83 ± 0.40, $p < 0.01$; PD-L1$^{-/-}$ vehicle: 8.13 ± 0.52 versus PD-L1$^{-/-}$ Am80: 3.29 ± 0.47, $p < 0.005$; Fig. 6B, 6C). CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs from the Am80-treated groups of WT and PD-L1$^{-/-}$ recipients were at similarly low frequencies only on day 14 but at similar levels on day 28 in each group (Fig. 6D). In contrast, the Am80-treated groups of both WT and PD-L1$^{-/-}$ recipients showed decreased IFN-γ$^+$IL-17$^-$, IL-17$^+$ IFN-γ$^-$ (day 28, WT vehicle versus WT Am80, $p < 0.05$; PD-L1$^{-/-}$ vehicle versus PD-L1$^{-/-}$ Am80, $p < 0.005$; Fig. 6D) and IL-17$^+$IFN-γ$^+$ cells (day 28, WT vehicle versus WT Am80, $p < 0.005$; PD-L1$^{-/-}$ vehicle versus PD-L1$^{-/-}$ Am80, $p < 0.005$; Fig. 6D) on days 14 and 28. These findings suggest that Am80 administration overcame the IL-17$^+$ IFN-γ$^+$ cell expansion caused by PD-L1 deficiency, resulting in reduced chronic GVHD damage in PD-L1$^{-/-}$ recipients.

Administration of anti–PD-1 agonistic Ab alleviates chronic GVHD

Donor CD4$^+$ and CD8$^+$ T cells in pLNs and spleen from both vehicle- and Am80-treated WT recipients showed similar expression levels of PD-1 (Supplemental Fig. 2A). Immunohistochemical analysis and mRNA quantitation of skin from Am80-treated recipients showed reduced PD-L1 expression compared with that from vehicle-treated recipients (Supplemental Fig. 2B, 2C). Thus, Am80 administration reduced chronic GVHD damage via suppressing IL-17$^+$IFN-γ$^+$ T cell expansion caused by impaired PD-L1 expression and did not directly affect the PD-1 pathway. Finally, to directly assess the role of therapeutic modu-

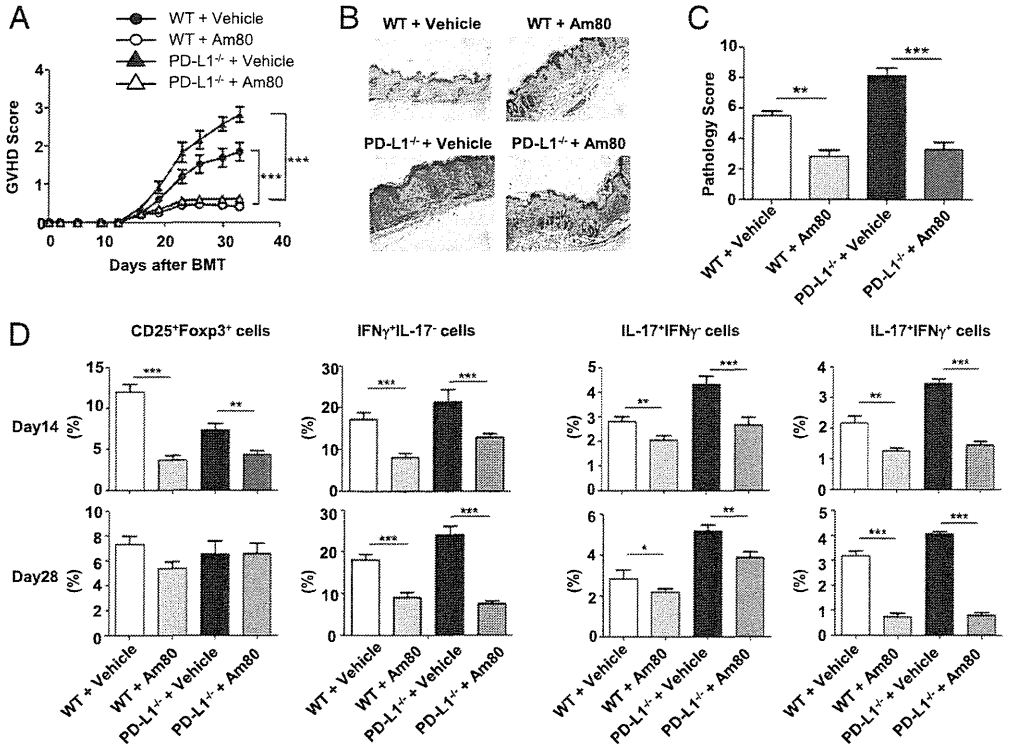

FIGURE 6. Administration of Am80 alleviates chronic GVHD enhanced by PD-L1 deficiency. (**A–D**) Sublethally irradiated WT and PD-L1$^{-/-}$ BALB/c recipients were transplanted from WT B10.D2 donors. These recipients received daily administration of Am80 (1.0 mg/kg body weight) or vehicle solution orally after BMT and were assessed for clinical signs of chronic GVHD every 3 d. Clinical GVHD skin scores (A) are shown; data shown are from 1 representative of ≥3 independent experiments (n = 8 in each group). Skin tissues from the recipients were taken on day 35 after BMT. (B) Representative images are shown (original magnification ×100). (C) Pathology scores of skin on day 35 after BMT are shown. (D) The percentages of donor-derived CD4$^+$ T cells expressing CD25$^+$ Foxp3$^+$, IFN-γ$^+$IL-17$^-$, IL-17$^+$IFN-γ$^-$, and IL-17$^+$IFN-γ$^+$ cells from pLNs of WT and PD-L1$^{-/-}$ BALB/c recipients with vehicle or Am80 treatment on days 14 and 28 are shown. The means (± SE) of each group are shown. Data shown are from 1 representative of ≥3 independent experiments (n = 6–8 in each group). $*p < 0.05$, $**p < 0.01$, $***p < 0.005$.

lation of PD-1 in chronic GVHD, we used an anti–PD-1 agonistic mAb in allogeneic recipients from day 14 after BMT. Stimulation of the PD-1 pathway ameliorated clinical chronic GVHD scores compared with the control group (anti–PD-1 agonistic Ab: 1.00 ± 0.24 versus rat IgG: 2.84 ± 0.42; $p < 0.05$; Fig. 7A), and pathologic scores of skin were improved (anti–PD-1 agonistic Ab: 3.50 ± 0.29 versus rat IgG: 6.20 ± 0.20; $p < 0.05$; Fig. 7B, 7C). These results suggest that the PD-1 pathway contributes to the development of chronic GVHD, and that stimulation of the PD-1 pathway alleviates clinical and pathologic chronic GVHD.

Discussion

The results of this study show that the PD-1 pathway is important in the alleviation of chronic GVHD. Blockade of the PD-1 pathway using anti–PD-1, anti–PD-L1, or anti–PD-L2 mAbs exacerbated chronic GVHD, and chimeric mice showed the importance of PD-L1 expression in host tissues in attenuating chronic GVHD. BMT into PD-L1–deficient recipients revealed IL-17$^+$IFN-γ$^+$ T cell expansion and Am80 administration of Am80 overcame the IL-17$^+$IFN-γ$^+$ T cell expansion caused by PD-L1 deficiency, resulting in reduced chronic GVHD damage in PD-L1$^{-/-}$ recipients. Stimulation of the PD-1 pathway with an agonistic anti–PD-1 mAb alleviated chronic GVHD, suggesting a new target for the prevention or treatment of chronic GVHD.

T cell activation via the TCR and costimulatory molecules has been well characterized, whereas coinhibitory pathways, which regulate T cell tolerance, are also known (32). The PD-1R and its ligands were identified and their inhibitory roles have become better understood (5–7, 9, 20, 21, 33). Previous studies have reported a role for PD-1/PD-L in acute GVHD, which is primarily

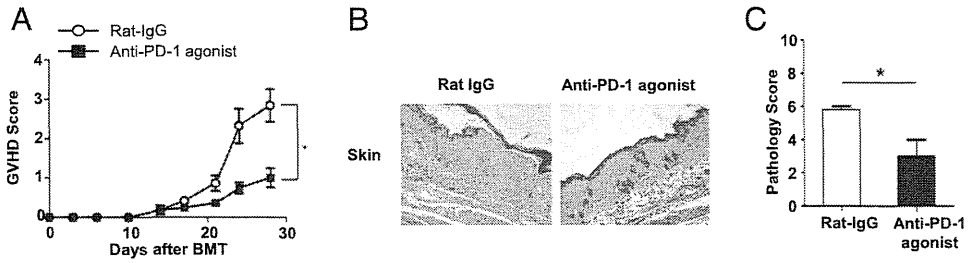

FIGURE 7. Administration of anti–PD-1 agonistic Ab alleviates chronic GVHD enhanced by PD-L1 deficiency. Sublethally irradiated BALB/c recipients were transplanted allogeneic B10.D2 donors. Recipients were injected with an anti–PD-1 agonist mAb or control rat IgG (200 μg/mouse) on days 14, 17, 20, 23, and 26 after BMT. (**A**) Clinical GVHD skin scores, (**B**) representative images (original magnification ×100), and (**C**) pathology scores of skin on day 30 after BMT are shown; data shown are from 1 representative of ≥2 independent experiments (n = 5 in each group). $*p < 0.05$.

Th1 biased and CD8 T cell mediated. PD-1/PD-L blockade accelerated donor $CD8^+$ T cell expansion and exacerbated acute GVHD (14–16). In our model, we found that IFN-γ^+ $CD8^+$ T cells were increased in PD-$L1^{-/-}$ recipients only during the early phase after BMT, but no difference was found between WT and PD-$L1^{-/-}$ recipients thereafter (Supplemental Fig. 3). In contrast, chronic GVHD is dependent primarily on $CD4^+$ T cells; the pathophysiology of chronic GVHD differs from that of acute GVHD. In this study, we investigated the PD-1 pathway in a well-defined chronic GVHD model. PD-$1^{-/-}$ mice on B10.D2 background were backcrossed for 10 generations and used as the donor. Lack of constitutive PD-1 signaling in donor T cells exacerbated GVHD and more than half died within 1 wk. Next, we used mAbs to inhibit the PD-1 pathway immediately before the development of chronic GVHD. Blockade of the PD-1 pathway using anti–PD-1, anti–PD-L1, or anti–PD-L2 mAbs exacerbated chronic GVHD and was confirmed by histopathologic examinations.

Donor tissue expression of PD-L1 provides protection against host T cell responses in cardiac and kidney allografts (34–36). More recently, Saha et al. (15) reported that PD-L1 expression in host tissues played an important role in the suppression of acute GVHD. In contrast, Yi et al. (18) reported that PD-L1 on host APCs, not tissues, was critical for Treg expansion in an autoimmune-like GVHD model. Host APCs, but not parenchymal cells, are replaced by donor cells, and we showed that even upregulated PD-L1 expression in host tissues in early phase was not enough to control or prevent chronic GVHD development and declined to basal levels in the late posttransplant period. In this study, to clarify the role of PD-L1 expression in host tissues during chronic GVHD, we used BM chimeric recipients. Transplantation of WT BM cells into PD-L1–deficient mice (WT→PD-$L1^{-/-}$ chimera) showed chronic GVHD exacerbation. In contrast, transplantation of PD-L1–deficient BM cells into WT mice (PD-$L1^{-/-}$→WT chimera) showed no exacerbation of chronic GVHD. This is consistent with previous observations that expression of PD-L1 on parenchymal cells inhibits self-reactive $CD4^+$ T cell–mediated autoimmune disease and $CD8^+$ T cell–mediated damage in chronic viral infection (24, 37). Taken together, our results indicated that PD-L1 expression in host tissues plays a critical role in alleviating chronic GVHD.

To clarify the mechanism of chronic GVHD exacerbation in PD-$L1^{-/-}$ recipients, we analyzed Treg reconstitution because PD-L1 regulates the development of induced Tregs (17). We found that Tregs were decreased significantly in PD-$L1^{-/-}$ recipients only during the early phase after BMT, and no difference was found between WT and PD-$L1^{-/-}$ recipients thereafter. We next identified the population of donor-derived Th1 and Th17 cells, because it has been shown that Th17 cells play a role in the pathogenesis of experimental autoimmune encephalomyelitis and chronic GVHD by our group and others (29, 38–40). IL-17^+ IFN-γ^- and IL-17^+IFN-γ^+ T cells were detected significantly more frequently in PD-$L1^{-/-}$ recipients than WT recipients. Furthermore, we showed the importance of PD-L1 expression on host tissues for expansion of IL-17^+IFN-γ^+ T cells. Treatment with Am80 overcame the IL-17^+IFN-γ^+ T cell expansion caused by PD-L1 deficiency and resulted in reduced chronic GVHD in PD-$L1^{-/-}$ recipients. D'Addio et al. (41) showed that PD-L1 blockade was associated with a switch in the Th1 balance toward Th17, leading to breakdown of fetomaternal tolerance. Recent clinical data reported augmentation of Th1 and Th17 responses in patients treated with anti–PD-1 therapy (42). Also, mesenchymal stem cells suppress Th17 proliferation via PD-L1 expression, and IL-27–primed $CD4^+$ T cells inhibit Th17 cell differentiation via PD-L1 (43). Therefore, PD-L1 deficiency plays an important role in Th17 expansion, and the PD-L1/Th17 axis may be a good therapeutic target for chronic GVHD.

In the acute GVHD model, PD-1/PD-L1 blockade accelerated acute GVHD via Th1 skewing; whereas during development of chronic GVHD, PD-L1 deficiency exacerbated histopathologically confirmed chronic GVHD via IL-17^+IFN-γ^+ T cell expansion, but not simply Th1 skewing. The pathophysiology of chronic GVHD includes defects in thymic function/negative selection (44), Tregs (45), clonal deletion (46–48), and clonal anergy (49, 50). In this study, we showed that the PD-1 pathway contributed to the development of chronic GVHD. Modulation of tissue expression of PD-L1 and/or stimulation of the PD-1 pathway of donor T cells may represent a new strategy for the prevention or treatment of chronic GVHD.

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Disclosures

The authors have no financial conflicts of interest.

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Anti–IL-12/23 p40 Antibody Attenuates Experimental Chronic Graft-versus-Host Disease via Suppression of IFN-γ/IL-17–Producing Cells

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Chronic graft-versus-host disease (GVHD) is a major cause of late death and morbidity after allogeneic hematopoietic cell transplantation. Recently, in addition to Th2 cells, Th1 and Th17 cells have been shown to contribute to chronic GVHD progression. IL-12 induces Th1 cells and IL-23 plays a role in stabilizing and/or amplifying Th17 cells, as well as in inducing IFN-γ/IL-17 double-producing cells. Because mAb targeting the p40 subunit common to both IL-12 and IL-23 can inhibit both IL-12R and IL-23R-mediated signaling, we investigated the effects of anti-p40 mAb on a well-defined chronic GVHD mice model. Treatment of anti-p40 mAb in allogeneic recipients significantly reduced the severity of clinical and pathological chronic GVHD. Intracellular staining revealed that IFN-γ single-positive (IL-17$^-$) and IFN-γ/IL-17 double-positive cells were suppressed in anti-p40 mAb–treated allogeneic recipients compared with control recipients. The cytokine levels of IFN-γ and IL-17 were also decreased in serum from anti-p40 mAb–treated allogeneic recipients. T-bet expression of donor IL-17$^+$ CD4$^+$ T cells was reduced significantly in anti-p40 mAb–treated recipients, and this reduction in T-bet expression was associated with IL-22 production by donor T cells. These results suggested that anti-p40 mAb attenuated chronic GVHD via suppression of IFN-γ/IL-17–producing cells, and that targeting the IL-12/IL-23 pathway may represent a promising therapeutic strategy for preventing and treating chronic GVHD.
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Recent progress with various sources of hematopoietic stem cells has enabled many more patients to receive treatment, and a number of patients have now survived for years posttransplant. However, such patients still experience development of graft-versus-host disease (GVHD), a major cause of late death and morbidity (1–3). Chronic GVHD occurs in approximately half of long-term survivors of allogeneic hematopoietic cell transplantation and presents with clinical manifestations similar to those typically observed in autoimmune disease, such as scleroderma and Sjögren syndrome. Although steroids remain the standard initial treatment of chronic GVHD, and half of patients respond to first-line treatment, steroid-refractory chronic GVHD, especially generalized scleroderma, carries a poor prognosis (4, 5).

The pathogenesis of chronic GVHD remains elusive, but recent studies have provided some insights. Donor T cells play a central role in the immunologic attack on host tissues in both acute and chronic GVHD. It has traditionally been assumed that the predominant cytokines produced during acute GVHD are Th1 cytokines, whereas those produced during chronic GVHD are Th2 cytokines. In addition to Th2 cells, recent studies have suggested that multiple cytokines secreted by Th1 and Th17 cells are involved in the pathogenesis of chronic GVHD (6–10). Our previous study showed that Th1 cell and Th17 cell expansion occurred during chronic GVHD and contributed to chronic GVHD progression using a mouse model (10). These results were consistent with clinical studies showing that Th1 and Th17 cells increased in patients with active chronic GVHD (11–15). We also identified a population of donor-derived IFN-γ/IL-17 double-positive cells after only allogeneic transplantation, not syngeneic transplantation, suggesting that this population is generated by allogeneic stimulation (10). These IFN-γ/IL-17 double-producing cells are found in both mice and humans, and much attention is currently focusing on elucidating the role of those cells in various inflammatory diseases (16–25).

The Th17 cell spectrum has been shown to range from "classical" to "alternative" Th17 cells (25). McGeachy et al. (22) showed that Th17 cells generated via TGF-β and IL-6 are nonpathogenic, whereas "alternative" Th17 cells produce IFN-γ and are more pathogenic (16–26). IL-12 induces Th1 cells, and IL-23 promotes the generation of "alternative" Th17 cells (22–28). mAb targeting the p40 subunit common to IL-12 (a heterodimer of p35 and p40) and IL-23 (a heterodimer of p19 and p40) can inhibit both IL-12R and IL-23R-mediated signaling. Anti-p40 mAb is

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S.O. and H.F. conducted the experiments, analyzed the data, and wrote the manuscript; Y.M. designed the experiments, supervised the research, and wrote the manuscript; H.N. performed the research; T.T. performed histopathologic analyses of the organs; A.Y. provided mAbs for the study; and K.-i.M., N.F., E.K., and M.T. supervised the research.

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Abbreviations used in this article: BMT, bone marrow transplantation; GVHD, graft-versus-host disease; PLN, peripheral lymph node; TCD-BM, T cell–depleted bone marrow.

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clinically available as ustekinumab and showed marked efficacy for the treatment of chronic inflammatory disorders such as psoriasis, psoriatic arthritis, and Crohn's disease (29–32). We hypothesized that Th1 and "alternative" Th17 play a role in the pathophysiology of chronic GVHD. We show in this study that blockade of IL-12/IL-23 by anti-p40 mAb reduces chronic GVHD damage using a well-defined mouse model of chronic GVHD.

Materials and Methods

Bone marrow transplantation

Female B10.D2 (H-2^d) donor mice were purchased from Japan SLC (Shizuoka, Japan). Female BALB/c (H-2^d) recipient mice were purchased from Charles River Japan (Yokohama, Japan). All mice were maintained under specific pathogen-free conditions and used at 8–12 wk of age. All animal experiments were performed according to the regulations of the Institutional Animal Care and Research Advisory Committee, Okayama University Advanced Science Research Center. Mice received transplants according to standard protocols described previously (10). In brief, recipient BALB/c mice received a single dose of 5.8-Gy X-ray total body irradiation and were injected with 2×10^6 spleen T cells and 8×10^6 T cell–depleted bone marrow (TCD-BM) cells from B10.D2 donors. T cell depletion and purification were performed using anti-CD90.2 microbeads and an AutoMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Donor cells were injected i.v. into the recipients on day 0.

Evaluation of chronic GVHD

After bone marrow transplantation (BMT), animals were weighed twice a week and scored for skin manifestations of GVHD. The following scoring system was used (10): healthy appearance, 0; skin lesions with alopecia <1 cm^2 in area, 1; skin lesions with alopecia 1–2 cm^2 in area, 2; and skin lesions with alopecia >2 cm^2 in area, 3. In addition, animals were assigned 0.3 point for skin disease area (lesions or scaling) on each of the ears, tails, and paws. The minimum score was zero, and the maximum was 3.9.

Tissue histopathology

Shaved skin from the interscapular region (~2 cm^2), liver, and salivary gland specimens of recipients were fixed in 10% formalin, embedded in paraffin, sectioned, mounted on slides, and stained with H&E. Skin slides were scored based on dermal fibrosis, fat loss, inflammation, epidermal interface changes, and follicular dropout (0–2 for each category; the maximum score was 10) (10). Liver slides were scored based on bile duct injury and inflammation (0–4 for each category), and the maximum score was 8 (33). Salivary slides were scored on mononuclear cell infiltration and follicular destruction, and the maximum score was 4 (34). All slides were scored by a pathologist (T.T.) blinded to the experimental group.

Intracellular cytokine staining and cytokine analysis

Peripheral lymph nodes (PLNs) were removed from the mice and processed into single-cell suspensions. Skin-infiltrating cells were obtained from skins of the recipient interscapular region (2 cm^2) with a gentleMACS Dissociator (Miltenyi Biotec) and Whole Skin Dissociation Kit (Miltenyi Biotec) according to the manufacturer's protocol. Cells were stimulated in vitro with 50 ng/ml PMA (Sigma-Aldrich, St. Louis, MO) and 100 ng/ml ionomycin (Sigma-Aldrich) at 37°C for 3 h or with anti-CD3 (1 μg/ml) at 37°C overnight (24). Cells were then incubated with GolgiStop (BD Biosciences, Franklin Lakes, NJ) for an additional 2 h. mAbs conjugated to FITC, PE, peridinin–chlorophyll protein complexes, allophycocyanin, Alexa Fluor 647, or Brilliant Violet 421 were used to assess the cell populations and were purchased from BD Pharmingen (Franklin Lakes, NJ) or eBioscience (San Diego, CA). Cells were analyzed on a MACS Quant flow cytometer (Miltenyi Biotec) with FlowJo software (TreeStar, Ashland, OR) that was housed in the Central Research Laboratory, Okayama University Medical School. The total PLN cells were adjusted to 1×10^6/ml in culture. The supernatants were removed, and the cytokine levels were measured by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol.

Anti-p40 mAb

mAbs (clone C17.8) against p40 (a common subunit of IL-12 and IL-23), provided by Dr. Akihiko Yoshimura, Keio University School of Medicine, were purified by protein G-column chromatography from ascites of nude mice transplanted with hybridoma. Mice were injected i.p. with anti-p40 mAb (500 μg/mice) or rat IgG (Sigma-Aldrich) every 3 d from day 0 after BMT.

Real-time PCR

Total RNA from snap-frozen skin tissues was extracted using the TRIzol reagent (Life Technologies, Tokyo, Japan) according to the manufacturer's protocol. cDNA was synthesized using oligo(dT) primers and SuperScript II reverse transcriptase (Invitrogen). Target cDNA levels were quantified using real-time quantitative PCR with an ABI Prism 5300 system (Applied Biosystems, Tokyo, Japan). TaqMan Universal PCR Master mix, primers, and the fluorescent TaqMan probe specific for murine Tbx21 (Mm00450960_m1), murine ifng (Mm00801778_m1), murine Rorc (Mm00441139_m1), and a housekeeping gene, mGAPDH (Mm99999915-g19), were purchased from Applied Biosystems. The mRNA levels of individual genes were normalized relative to GAPDH, using the cycle threshold (Ct) equation: $\Delta Ct = Ct_{target} - Ct_{GAPDH}$.

Statistical analyses

Group comparisons of skin chronic GVHD scores and pathology scores were performed using the Mann–Whitney *U* test or Kruskal–Wallis test. Cell populations, cytokine levels, mean weights, and gene expression data were analyzed by unpaired Student *t* tests. Survival was evaluated using the log-rank test. All data were analyzed using GraphPad Prism software (version 5.0). The p values <0.05 indicate statistical significance.

Results

Anti–IL-12/23 p40 mAb attenuates murine chronic GVHD

To examine whether anti–IL-12/23 p40 mAb can alleviate chronic GVHD, we used a common chronic GVHD model, the MHC-compatible, murine minor histocompatibility Ag–incompatible allogeneic BMT model (B10.D2 into BALB/c). Sublethally irradiated (5.8 Gy) BALB/c mice were transplanted with 2×10^6 spleen T cells and 8×10^6 TCD-BM cells from B10.D2 mice. As previously reported (10), full donor chimerism (<5% recipient cells) of $CD3^+$ T cells and $B220^+$ B cells were recognized in the spleens, PLNs, and peripheral blood from both the control IgG and the anti-p40 groups on day 14 (Fig. 1A). Anti-p40 mAb was injected peritoneally on every third day from day 0 of BMT. Allogeneic recipients treated with control IgG showed significantly increased clinical chronic GVHD scores compared with syngeneic recipients (Fig. 1B). Allogeneic recipients also showed obvious histopathological damage to the skin and other organs, such as the salivary gland and liver (Fig. 2A). However, we found that anti-p40 mAb significantly ameliorated the clinical score compared with the controls ($p = 0.002$; Fig. 1B). Histopathological examination of the skin on day 28 showed significantly reduced chronic GVHD damage in anti-p40 mAb–treated animals (2.8 ± 0.4 versus 6.0 ± 0.3; $p < 0.01$; Fig. 2B). A dry mouth is one of the distinctive features of chronic GVHD, and lymphocytic inflammation, fibrosis, and atrophy of acinar tissue were observed in the salivary glands of control-treated allogeneic recipients. Histopathological examination of the salivary glands showed reduced chronic GVHD pathology in the anti-p40 mAb–treated recipients (2.0 ± 0.4 versus 3.2 ± 0.2; $p < 0.05$; Fig. 2B). Pathological scores of the liver also tended to be less in anti-p40 mAb–treated recipients compared with control-treated recipients, although it was not statistically significant (Fig. 2B). These findings suggest that anti-p40 mAb attenuated clinical and pathological chronic GVHD. We also examined whether anti-p40 mAb could be used for the treatment of chronic GVHD. Anti-p40 mAb was injected i.p. to mice from day 21 of BMT, when mice had developed clinical signs of chronic GVHD, and anti-p40 mAb significantly improved the clinical scores ($p = 0.03$; Fig. 2C).

Anti-p40 mAb treatment reduced IFN-γ and IL-17 production in donor T cells of PLNs

Cells isolated from PLNs were harvested on day 28 after BMT and analyzed for cytokine expression of those stimulated with PMA and ionomycin (Fig. 3A). Intracellular staining showed that IFN-γ^+ (IL-$17A^-$) $CD4^+$ T cells from PLNs of allogeneic recipients were increased and were detected more frequently than PLNs from

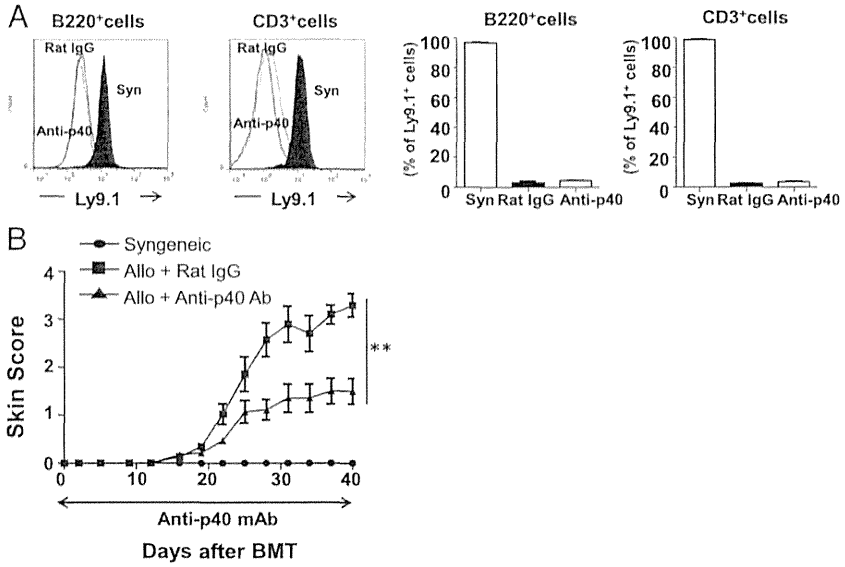

FIGURE 1. Anti-p40 mAb attenuates murine chronic GVHD. Sublethally irradiated (5.8 Gy) BALB/c mice were transplanted with 2×10^6 spleen T cells plus 8×10^6 TCD-BM from WT B10.D2 mice. The syngeneic group received transplantation of the same dose of splenocytes and TCD-BM from BALB/c mice. The allogeneic recipients were treated i.p. with anti-p40 mAb (500 μg/mice) or rat IgG (Sigma-Aldrich) every 3 d from day 0 after BMT. (**A**) Representative histograms of Ly9.1 expression on $B220^+$ cells and $CD3^+$ cells from peripheral blood are shown (*left panels*). Percentages of $Ly9.1^+$ cells among $B220^+$ and $CD3^+$ cells from peripheral blood from day 14 after BMT are shown (*right panels*). Data shown are from one representative of three independent experiments ($n = 4$–6 in each group). (**B**) Clinical GVHD skin scores are shown. Anti-p40 mAb or rat IgG were administered every 3 d from day 0; data shown are from one representative of three independent experiments ($n = 6$ in each group). The means ± SE of each group are shown. Data are representative of at least two independent experiments. $^{**}p < 0.01$.

syngeneic recipients, as expected (58 ± 6 versus 3.3 ± 0.3%; $p = 0.0005$; Fig. 3A). IFN-γ^+ (IL-17A$^-$) $CD4^+$ T cells of anti-p40 mAb–treated recipients tended to be decreased compared with control-treated allogeneic recipients (38 ± 9 versus 58 ± 8%; $p = 0.1$; Fig. 3A). The IFN-γ/IL-17A double-positive cells were reduced and detected significantly less frequently in anti-p40 mAb–treated recipients (1.5 ± 0.2 versus 4.0 ± 0.4%; $p = 0.0003$; Fig. 3A). In contrast, IL-17A$^+$ (IFN-γ^-) $CD4^+$ T cells of anti-p40

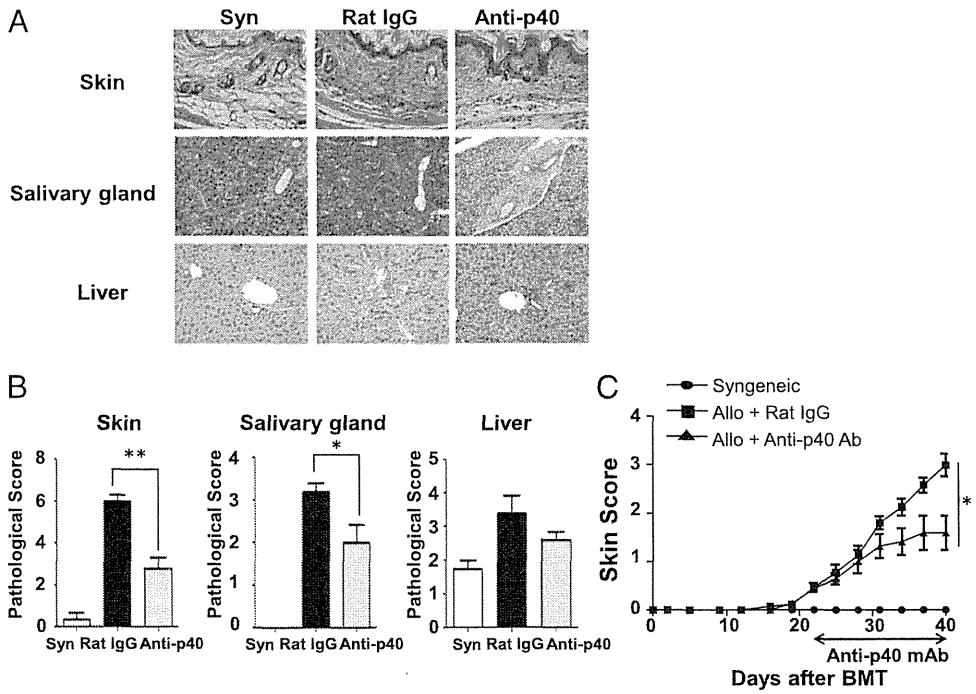

FIGURE 2. Anti-p40 mAb reduces pathological GVHD damages. Sublethally irradiated BALB/c recipients were transplanted from B10.D2 or syngeneic BALB/c donors. The allogeneic recipients were treated i.p. with anti-p40 mAb or rat IgG every 3 d from day 0 after BMT. (**A**) Histopathology of the skin, salivary gland, and liver of syngeneic and allogeneic recipients 28 d after BMT (H&E staining, original magnification ×100). (**B**) Skin, salivary gland, and liver pathology score from syngeneic (white bar), control-treated allogeneic (black bar), or anti-p40 mAb–treated allogeneic (gray bar) recipients are shown. Data shown are from one representative of three independent experiments ($n = 6$ in each group). (**C**) Clinical GVHD skin scores are shown. Anti-p40 mAb or rat IgG were administered every 3 d from day 21 after BMT. Three to six mice per group were used. The means (±SE) of each group are shown. Data are representative of at least two independent experiments. $^*p < 0.05$, $^{**}p < 0.01$.

mAb–treated recipients did not decrease compared with control-treated allogeneic recipients (7.7 ± 0.9 versus 4.9 ± 1%; $p = 0.1$; Fig. 3A). We also determined intracellular stainings of IFN-γ^+ (IL-17A$^-$), IL-17A$^+$ (IFN-γ^-), and IFN-γ/IL-17A double-positive cells isolated from PLNs stimulated with anti-CD3. Results with anti-CD3 stimulation were similar to those with PMA and ionomycin, and confirmed that anti-p40 mAb treatment reduced both IFN-γ^+ (IL-17A$^-$) cells and IFN-γ/IL-17A double-positive cells (IFN-γ^+ [IL-17A$^-$] cells: 0.33 ± 0.039 versus 3.33 ± 0.97%, $p <$ 0.05; IFN-γ/IL-17A double-positive cells: 0.86 ± 0.10 versus 1.72 ± 0.33%, $p < 0.05$; Fig. 3B). Next, we evaluated Th1 and Th17 cell cytokine development after BMT. Cells isolated from PLNs of allogeneic recipients secreted significantly greater amounts of IL-17, IFN-γ, and IL-23 than syngeneic recipients after stimulation with PMA and ionomycin. However, cells isolated from PLNs of anti-p40 mAb–treated allogeneic recipients secreted significantly less IFN-γ and IL-17 than controls (IFN-γ: 1000 ± 200 versus 1500 ± 30 pg/ml, $p = 0.04$; IL-17: 280 ± 50 versus 620 ± 50 pg/ml, $p = 0.002$; Fig. 4A). Although levels of cytokine, especially IFN-γ, from PLNs with anti-CD3 stimulation were lower than those with PMA and ionomycin, results of anti-CD3 stimulation revealed similar results showing that cells isolated from anti-p40 mAb–treated groups secreted less IFN-γ and IL-17 (IFN-γ: 199.4 ± 74 versus 654.1 ± 215 pg/ml, $p = 0.04$; IL-17: 327 ± 135 versus 497 ± 279 pg/ml, $p = 0.58$; Fig. 4A). IL-23 productions from anti-p40 mAb–treated groups also tend to be suppressed, but with no significance. These cytokine levels were also decreased in serum from anti-p40 mAb–treated allogeneic recipients 28 d after BMT (IFN-γ: 10.0 ± 0.6 versus 35 ± 7 pg/ml, $p = 0.03$; IL-17: 2.8 ± 2 versus 7.5 ± 2 pg/ml, $p = 0.2$; Fig. 4B). Collectively, these findings indicated that anti-p40 mAb reduced IFN-γ and IFN-γ/IL-17A double-positive cells, leading to alleviation of chronic GVHD.

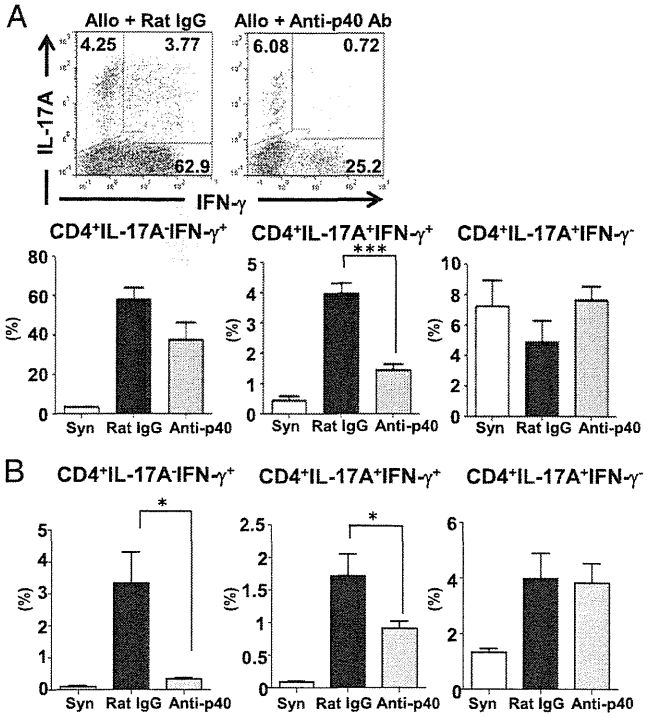

FIGURE 3. Anti-p40 mAb treatment downregulates IFN-γ/IL-17 double-positive cells. Sublethally irradiated BALB/c recipients were transplanted as in Fig. 1. (**A**) Representative staining for intracellular IFN-γ and IL-17A on CD4$^+$ T cells on day 28 for control-treated allogeneic or anti-p40 mAb–treated allogeneic mice stimulated with PMA/ionomycin are shown. PLN cells from syngeneic (white bar), control-treated allogeneic (black bar), or anti-p40 mAb–treated allogeneic (gray bar) recipients were stained for intracellular IFN-γ and IL-17A on day 28 after BMT. The percentages of IL-17A$^-$/IFN-γ^+ cells, IL-17A$^+$/IFN-γ^+ cells, and IL-17A$^+$/IFN-γ^- cells stimulated with PMA/ionomycin (A) and with anti-CD3 (**B**) are shown. Four to seven mice per group were used. The means (± SE) of each group are shown. Data are representative of at least two independent experiments. $*p < 0.05$, $***p < 0.001$.

Anti-p40 mAb treatment suppressed IFN-γ/IL-17A double-positive cells

Because IFN-γ/IL-17A double-positive cells are enriched in the target organs of several autoimmune disease models, it has been suggested that these double producers are particularly pathogenic in tissue inflammation and autoimmunity. These double-positive cells show higher expression of T-bet than IL-17A single-positive T cells (24, 35). Therefore, we examined ROR-γt and T-bet expression in donor IL-17A$^+$ CD4$^+$ T cells isolated from PLNs harvested on day 28 after BMT. ROR-γt and T-bet$^+$ cells in Th17 cells were significantly higher in allogeneic recipients than in syngeneic recipients (Fig. 5A, 5B). Anti-p40 mAb–treated recipients showed significantly lower T-bet expression than controls (PMA/ionomycin: 0.77 ± 0.2 versus 1.6 ± 0.3%, $p = 0.03$; CD3: 0.038 ± 0.012 versus 0.88 ± 0.35%, $p = 0.043$; Fig. 5A); however, both anti-p40 mAb–treated and control-treated allogeneic recipients displayed similar levels in ROR-γt expression after stimulation with either PMA/ionomycin or CD3 (Fig. 5B). This reduction in T-bet expression was associated with IL-22 production by CD4$^+$ T cells from anti-p40 mAb–treated recipients (42 ± 18 versus 110 ± 17 pg/ml, $p = 0.03$; Fig. 5C). IL-22 was reported to be secreted by pathogenic Th17 cells, $\gamma\delta$ T cells, NK cells, and innate lymphoid cell 3 (36). IL-22$^+$ cells were mainly CD4$^+$ cells (>70%) in this allogeneic model, and IL-22$^+$ CD4$^+$ T cells of anti-p40 mAb–treated recipients were decreased compared with control-treated allogeneic recipients (3.07 ± 0.54 versus 7.65 ± 0.70%, $p <$ 0.001; Fig. 5D). The levels of IL-22 were also decreased in serum from anti-p40 mAb–treated allogeneic recipients 28 d after BMT (19 ± 5 versus 206 ± 78 pg/ml, $p = 0.04$; Fig. 5E). These results suggested that anti-p40 mAb treatment suppressed Th1 cells and "alternative" Th17 cells, but not "classical" Th 17 cells, during chronic GVHD.

Skins targeted by GVHD showed direct clues of GVHD alleviation

Cytokine production profiles of CD4$^+$ T cells in the PLNs and skin might differ. Analysis of lymphocyte extraction from skin homogenates revealed that anti-p40 mAb treatment reduced IFN-γ^+ (IL-17A$^-$) cells, but not significantly, and reduced IFN-γ/IL-17A double-positive cells significantly (IFN-γ^+ [IL-17A$^-$] cells: 1.08 ± 0.41 versus 1.94 ± 0.28%, $p = 0.16$; IFN-γ^+/IL-17A$^+$ double-positive cells: 0.34 ± 0.072 versus 0.67 ± 0.10%, $p < 0.05$; Fig. 6A). To confirm cytokine gene expression, we also checked gene expression of ROR-γt, Tbx21, and ifng by RT-PCR from skins (Fig. 6B). Tbx21 and ifng expression significantly decreased in the anti-p40 mAb group compared with the allogeneic group. In contrast, ROR-γt expression did not differ in both groups. ELISA analyses of skin homogenates showed that only IFN-γ increased significantly in the control IgG group. IL-17 and IL-23 were similar levels in both control IgG and anti-p40 groups (Fig. 6C). These results suggested that anti-p40 mAb treatment suppressed mainly skin-infiltrating Th1 cells and "alternative" Th17 cells.

Discussion

The results of this study show that anti–IL-12/IL-23 p40 mAb attenuated clinical and pathological chronic GVHD using a well-

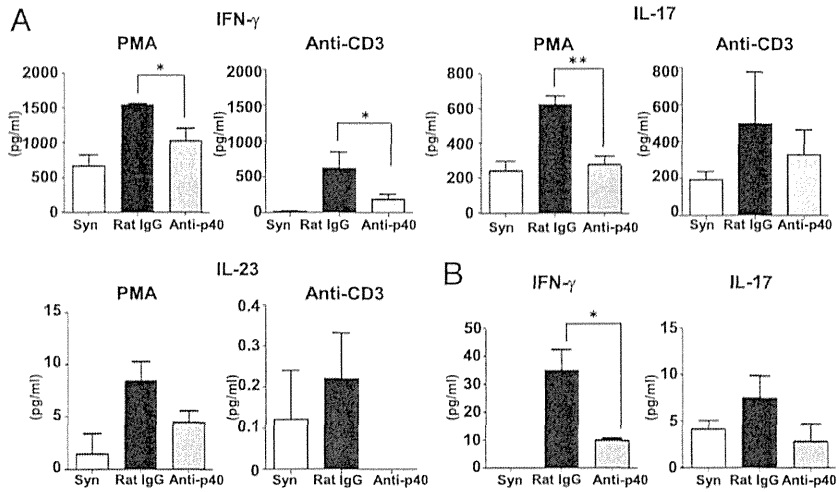

FIGURE 4. Anti-p40 mAb treatment downregulates Th1 and Th17 cell cytokine development. Sublethally irradiated BALB/c recipients were transplanted as in Fig. 1. (**A**) PLN cells from syngeneic (white bar), control-treated allogeneic (black bar), or anti-p40 mAb–treated allogeneic (gray bar) recipients on day 28 were stimulated with PMA/ionomycin or with anti-CD3 in vitro. The supernatants were collected and the cytokine (IFN-γ, IL-17, and IL-23) levels determined by ELISA. Graphs indicate the levels of cytokines secreted per 1×10^6 total stimulated PLN cells. (**B**) Levels of these cytokine were also determined in serum from syngeneic, control-treated allogeneic, or anti-p40 mAb–treated allogeneic recipients on day 28 after BMT. Four to seven mice per group were used. The means (±SE) of each group are shown. Data are representative of at least two independent experiments. $^*p < 0.05$, $^{**}p < 0.01$.

defined mouse model of chronic GVHD. Anti-p40 mAb suppressed IFN-γ single-positive (IL-17$^-$) and IFN-γ/IL-17 double-positive cells, whereas IL-17 single-positive (IFN-γ$^-$) cells were not altered compared with control-treated allogeneic recipients. Donor IL-17$^+$ CD4$^+$ T cells of anti-p40 mAb-treated recipients tended to show similar ROR-γt expression compared with control-treated allogeneic recipients. By contrast, T-bet expression of IL-17$^+$ CD4$^+$ T cells was abrogated significantly in anti-p40 mAb–treated recipients. These results suggested that anti-p40 mAb treatment attenuated chronic GVHD via suppression of IFN-γ and IL-17 production.

Th17 cells were divided into two groups: IL-17 single-positive cells that are nonpathogenic "classical" Th17 cells and IFN-γ/IL-17 double-positive cells that are more pathogenic "alternative" Th17 cells. Previously, we showed that IFN-γ/IL-17 double-positive donor cells were detected only in allogeneic recipients with chronic GVHD, but not in syngeneic recipients (10). Recent studies have revealed that IFN-γ/IL-17 double-producing cells are associated with infection or isolated from sites of inflammation (16–25). IFN-γ/IL-17 double-producing cells are isolated from the gut of patients with Crohn's disease and the skin of patients with psoriasis (19, 20). Dander et al. reported that Th17 cells in the

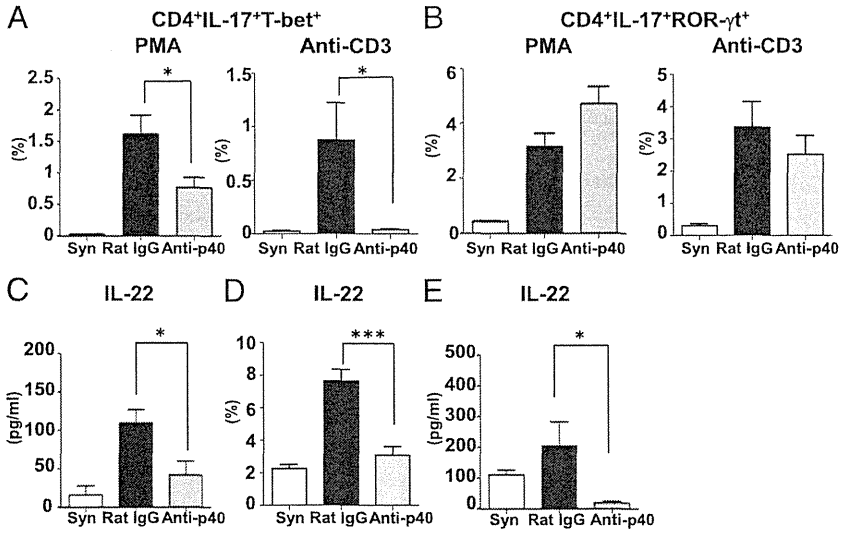

FIGURE 5. Anti-p40 mAb treatment suppresses Th1 cells. Sublethally irradiated BALB/c recipients were transplanted as in Fig. 1. PLN cells from syngeneic (white bar), control-treated allogeneic (black bar), or anti-p40 mAb–treated allogeneic (gray bar) recipients were stained for intracellular IL-17A, T-bet, and ROR-γt of CD4 T cells stimulated with PMA/ionomycin or with anti-CD3 in vivo on day 28 after BMT. The percentages of IL-17A$^+$/T-bet$^+$ cells (**A**) and IL-17A$^+$/ROR-γt$^+$ cells (**B**) are shown. (**C**) PLN cells from syngeneic and allogeneic recipients on day 28 were stimulated with PMA and ionomycin in vitro. Five hours later, the supernatants were collected to determine cytokine levels of IL-22 by ELISA. Graphs indicate the levels of cytokines secreted per 1×10^6 total stimulated PLN cells. (**D**) The percentages of IL-22$^+$ cells on CD4$^+$ cells are shown. (**E**) Levels of IL-22 were also determined in serum from syngeneic, control-treated allogeneic, or anti-p40 mAb–treated allogeneic recipients on day 28 after BMT. Four to seven mice per group were used. The means (±SE) of each group are shown. Data are representative of at least two independent experiments. $^*p < 0.05$, $^{***}p < 0.001$.

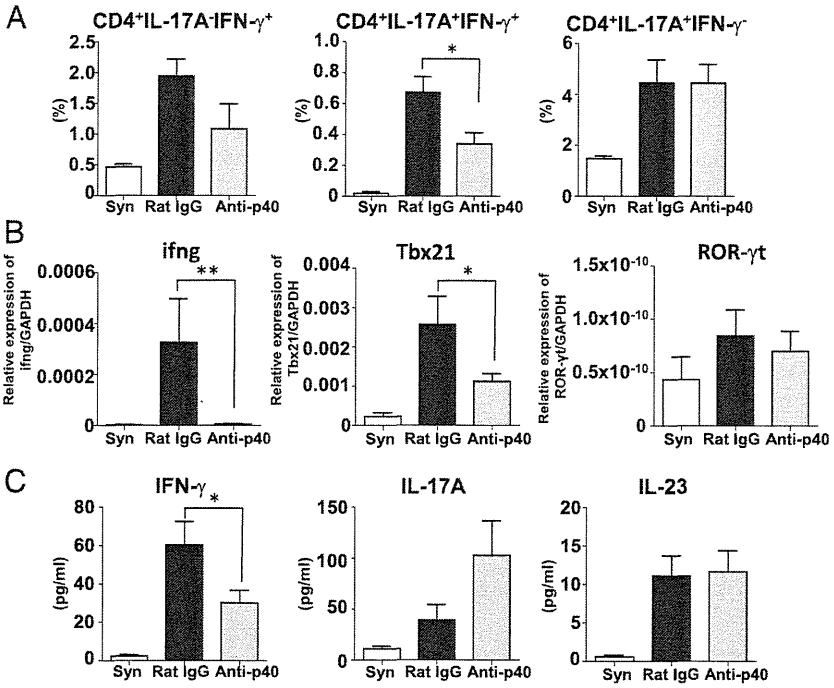

FIGURE 6. Anti-p40 mAb treatment alleviates Th1 cells of the skin. Sublethally irradiated BALB/c recipients were transplanted as in Fig. 1. Cutaneous infiltration cells from syngeneic (white bar), control-treated allogeneic (black bar), or anti-p40 mAb–treated allogeneic (gray bar) recipients were stained for intracellular IFN-γ and IL-17A on day 28 after BMT with PMA/ionomycin. (**A**) The percentages of IL-17A$^-$/IFN-γ$^+$ cells, IL-17A$^+$/IFN-γ$^+$ cells, and IL-17A$^+$/IFN-γ$^-$ cells are shown. (**B**) Ifng, Tbx21, and ROR-γt mRNA from skins of syngeneic, control-treated allogeneic, or anti-p40 mAb–treated allogeneic recipients on day 28 after BMT are shown. (**C**) The supernatants of skin homogenates on day 28 were collected and the cytokine (IFN-γ, IL-17, and IL-23) levels were determined by ELISA. Four to seven mice per group were used. The means (±SE) of each group are shown. Data are representative of at least two independent experiments. $*p < 0.05$, $**p < 0.01$.

peripheral blood of patients with active chronic GVHD expressed IL-23R, and IFN-γ/IL-17 double-positive cells were detected in the liver and skin GVHD lesions (11). IFN-γ/IL-17 double-producing cells arise from Th17 cells and have lower RORγt expression than IL-17 single-positive cells (24). T cells that lack the IL-23R fail to develop into IFN-γ/IL-17 double-producing cells and do not trigger colitis in the T cell transfer model (23). Thus, these cells develop through IL-23–driven upregulation of T-bet and may play an important role in disease pathogenesis (24). In this study, anti-p40 mAb reduced IFN-γ/IL-17 double-positive cells, and donor IL-17$^+$ CD4 T cells showed decreased T-bet expression. Production of IL-22, "alternative" Th17-type cytokine, was also reduced in T cells from anti-p40 mAb–treated recipients. These results suggested that IFN-γ/IL-17 double-producing cells play a role in the pathophysiology of chronic GVHD, and anti-p40 mAb treatment might have shifted the "alternative" Th17 cells to "classical" Th17 cells.

Psoriasis is a chronic, relapsing, immunoinflammatory dermatosis, and Crohn's disease is a chronic inflammatory bowel disease. IL-12 and IL-23, as well as IL-12– and IL-23–mediated Th1 and Th17 cells, are involved in the pathophysiology of psoriasis and Crohn's disease. The human mAb ustekinumab binds the p40 subunit common to IL-12 and IL-23, and shows marked efficacy for the treatment of chronic inflammatory disorders such as psoriasis (29, 30), psoriatic arthritis (31), and Crohn's disease (32). IL-23, rather than IL-12, seems to be essential for the pathogenesis of experimental autoimmune encephalomyelitis, arthritis, and inflammatory bowel disease because IL-23 knockout mice are protected from disease (26, 37, 38). By contrast, Becker et al. (39) reported that because IL-23 cross-regulates IL-12 production, IL-23 knockout mice produce increased levels of IL-12 and were highly susceptible to the development of experimental T cell–mediated 2,4,6-trinitrobenzenesulfonic acid colitis. Blockade of p40 rescued IL-23 knockout mice from lethal colitis. In this study, although blockade of IL-12/IL-23 by anti-p40 mAb effectively suppressed chronic GVHD, further investigation is needed to clarify whether blockade of only the IL-23 pathway can reduce chronic GVHD.

In conclusion, anti-p40 mAb attenuated clinical and pathological chronic GVHD. Anti-p40 mAb suppressed IFN-γ single-positive and IFN-γ/IL-17 double-positive cells, but not IL-17 single-positive cells. T-bet expression in donor IL-17$^+$ CD4 T cells was decreased after anti-p40 mAb treatment, suggesting that anti-p40 mAb suppressed mainly the "alternative" Th17 cells. Modulation of the IL-12/IL-23 pathway may represent a new strategy for the treatment of chronic GVHD, and anti-p40, which is clinically available as ustekinumab, might be a promising therapeutic agent for chronic GVHD.

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Disclosures

The authors have no financial conflicts of interest.

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