Immunity
Article

Interleukin-22 Protects Intestinal Stem Cells from Immune-Mediated Tissue Damage and Regulates Sensitivity to Graft versus Host Disease

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SUMMARY

Little is known about the maintenance of intestinal stem cells (ISCs) and progenitors during immune-mediated tissue damage or about the susceptibility of transplant recipients to tissue damage mediated by the donor immune system during graft versus host disease (GVHD). We demonstrate here that deficiency of recipient-derived IL-22 increased acute GVHD tissue damage and mortality, that ISCs were eliminated during GVHD, and that ISCs as well as their downstream progenitors expressed the IL-22 receptor. Intestinal IL-22 was produced after bone marrow transplant by IL-23-responsive innate lymphoid cells (ILCs) from the transplant recipients, and intestinal IL-22 increased in response to pre-transplant conditioning. However, ILC frequency and IL-22 amounts were decreased by GVHD. Recipient IL-22 deficiency led to increased crypt apoptosis, depletion of ISCs, and loss of epithelial integrity. Our findings reveal IL-22 as a critical regulator of tissue sensitivity to GVHD and a protective factor for ISCs during inflammatory intestinal damage.

INTRODUCTION

Although potentially curative for benign and malignant hematopoietic diseases, allogeneic hematopoietic transplantation continues to be limited by severe morbidity and mortality caused by graft versus host disease (GVHD) (Wingard et al., 2011). Acute GVHD occurs when alloreactive donor T cells activated against recipient antigens attack vital recipient organs, classically the skin, liver, and gastrointestinal (GI) tract (Shlomchik, 2007). GI GVHD in particular is a major contributor to transplant morbidity and mortality because skin GVHD can often be controlled without systemic immunosuppression and liver GVHD is much less frequent (Hill and Ferrara, 2000).

Although substantial progress has been made toward understanding the donor immune response directed against recipient tissues, there is little insight into the factors regulating sensitivity of recipient tissues to GVHD. Additionally, although the GVH immune response has long been felt to involve an attack against the intestinal stem and progenitor cell niche, there has been little direct evidence demonstrating the loss of intestinal stem cells (ISCs) during GVHD. Furthermore, there is little understanding of the role that tissue stem cell damage plays in other models of inflammatory epithelial injury. Given the limitations of existing immunosuppression-based therapies for GVHD, our currently poor understanding of the recipient response to GVHD, and our poor understanding of how recipient tissues recover from GVHD-induced damage, we sought to identify novel factors that could selectively regulate GI injury resulting from GVHD. Furthermore, we sought to do so by focusing on protection of recipients at the tissue level and at the level of ISCs. This rationale is supported by the recent demonstration that treatment with Wnt signaling agonists can provide a trophic signal for intestinal epithelium during GVHD (Takashima et al., 2011). Moreover, although steady state regulation of the intestinal stem and progenitor cell niche is thought to be a complex orchestration of Wnt, bone morphogenetic protein, Notch, and Hedgehog signaling (Medema and Vermeulen, 2011), there is little knowledge of ISC niche regulation during intestinal damage.

IL-22 is a recently characterized cytokine produced by several hematopoietic populations, including helper T (Th) cells and innate lymphoid cells (ILCs) (Liang et al., 2006; Spits and Di Santo, 2011), and expression of the IL-22 receptor (IL-22R) is

restricted to nonhematopoietic stromal cells (Pickert et al., 2009; Tachiiri et al., 2003; Witte et al., 2010; Wolk et al., 2004; Zenewicz et al., 2007). IL-22 has contrasting inflammatory and cytoprotective effects, leading to pathologic inflammation and excessive stimulation of keratinocytes in psoriasis while limiting toxicity in models of acute hepatitis and DSS-induced colitis (Pickert et al., 2009; Sonnenberg et al., 2011a; Wolk et al., 2006). IL-22 protects intestinal epithelium during inflammatory bowel disease (IBD) and experimental colitis by providing a signal for epithelial cell survival, proliferation, and wound healing (Pickert et al., 2009), but impacts of IL-22 on the stem and progenitor cell compartment are unknown. Given the parallels between IBD and GI GVHD (Asplund and Gramlich, 1998), we reasoned that IL-22 signaling could function to protect recipient intestinal tissue after bone marrow transplant (BMT) without limiting donor immunity and utilized clinically relevant mouse models of GVHD to evaluate the relationship between inflammatory tissue damage and epithelial regeneration.

RESULTS

Deficiency of Recipient-Derived IL-22 Increases GI GVHD

In order to test the hypothesis that IL-22 signaling protects recipients from GVHD, we first tested an aggressively lethal MHC-mismatched murine BMT model of C57BL/6 (B6, H-2^b) donor marrow and T cells transplanted into lethally irradiated BALB/c (H-2^d) recipients. Although transplantation of T cell-depleted allogeneic bone marrow (BM) rescues recipient mice from lethal total body irradiation (TBI), transplantation of allogeneic marrow and T cells leads to GVHD. Elimination of IL-22 with an IL-22-neutralizing antibody led to a significant increase in GVHD mortality (Figure 1A). However, transplantation with IL-22-deficient (*Il22*$^{-/-}$) donor marrow or T cells had no impact on GVHD survival (Figures 1B and 1C). In contrast, *Il22*$^{-/-}$ BMT recipients demonstrated significantly increased GVHD mortality (Figure 1D), suggesting a critical role for host cells in the production of protective IL-22 post-BMT. The importance of recipient-derived IL-22 was confirmed in a MHC-matched (H-2^b) BMT model with minor histocompatibility antigen (MiHA) disparity (LP→B6), which is similar to clinical matched unrelated donor transplantation. *Il22*$^{-/-}$ recipients again had significantly increased GVHD mortality and GVHD-associated organ pathology in the small and large intestine and liver (Figures 1E and 1F). The increased histopathologic scoring identified in the intestines of *Il22*$^{-/-}$ recipients was due to increased epithelial damage despite similar inflammatory activity during GVHD (Figure S1A available online). No differences in skin GVHD pathology were observed in *Il22*$^{-/-}$ recipients (Figure S1B).

Because certain host-derived hematopoietic cells can persist in recipient tissues post-BMT (Merad et al., 2004), we hypothesized that IL-22 could be produced by a subset of radio-resistant hematopoietic cells within recipient target organs. We thus generated hematopoietic chimeras for use as secondary transplant recipients by reconstituting CD45.1 B6 congenic mice with *Il22*$^{-/-}$ or wild-type (WT) CD45.2 B6 marrow (Figure 1G). Hematopoietic reconstitution was confirmed 3 months later by sampling peripheral blood (>90% in all cases). Chimeric recipients were then transplanted with allogeneic LP marrow and T cells in a reduced-intensity transplant model designed for mice undergoing secondary transplantation. IL-22 deficiency restricted to the recipient hematopoietic compartment resulted in increased GVHD morbidity and mortality (Figures 1G and 1H), indicating that the source of protective IL-22 was residual host-derived hematopoietic cells. In contrast, depletion of all donor-derived IL-22 by transplantation with both *Il22*$^{-/-}$ marrow and *Il22*$^{-/-}$ T cells did not increase GVHD mortality, further demonstrating that protective IL-22 was produced by a recipient-derived source (Figure 1I). This combined donor marrow and T cell IL-22 deficiency led to a statistically significant delay in GVHD mortality, suggesting a potentially additive proinflammatory effect of IL-22 produced by donor-derived cells.

Intestinal IL-22 Expression Is Increased after Pretransplant Conditioning but Reduced by GVHD

Given that recipient-derived IL-22 appeared to play a critical protective role in the development of GVHD, we next sought to determine the normal pattern of IL-22 expression post-BMT. Serum IL-22 concentrations were measured by ELISA in transplanted mice and untransplanted controls. Serum IL-22 concentrations were increased after both B6→BALB/c (MHC-mismatched) and LP→B6 (MiHA-mismatched) BMT. However, this increase in serum IL-22 concentrations post-BMT was not observed in transplanted mice with GVHD (Figure 2A). IL-22 ELISA on small and large intestine homogenates after LP→B6 BMT indicated that IL-22 protein was increased from baseline 3 weeks post-BMT in recipients without GVHD. However, similar to the serum expression pattern, intestinal IL-22 concentrations were lower in BMT recipients with GVHD than in recipients without GVHD (Figure 2B). Although reduced IL-22 protein in GVHD intestines could have been due to increased cytokine turnover in damaged tissues, this interpretation was refuted by the dual observations that IL-22 protein production was decreased during overnight culture of GVHD intestines and IL-22 mRNA was decreased in the lamina propria of GVHD mice (Figure S2). Consistent with increased host-derived IL-22 production post-BMT, sublethal TBI without transplantation also led to significantly elevated IL-22 protein in homogenized intestines (Figure 2C).

IL-23, which is produced by antigen presenting cells post-BMT (Das et al., 2009), has been reported as a major regulator of IL-22 production (Cella et al., 2009; Kreymborg et al., 2007; Sonnenberg et al., 2011b; Zheng et al., 2007). Consistent with this, we found that IL-22 protein was not elevated in homogenized intestines of p40-deficient mice (*Il12b*$^{-/-}$, lacking IL-23 and IL-12) 3 weeks after sublethal TBI (Figure 2D). Similarly to observations following MHC-mismatched transplantation (Das et al., 2009), IL-23 concentrations were elevated in tissue homogenates from lethally irradiated WT mice 2 weeks after MiHA-mismatched BMT (Figure 2E). Intestinal IL-23 was also elevated 1 week after lethal radiation without transplantation (Figure 2F), although IL-22 concentrations were not yet elevated at that time (data not shown). Kinetics of IL-23 expression thus preceded those of IL-22, suggesting that initial production of IL-23 post-BMT may have induced subsequent IL-22 expression.

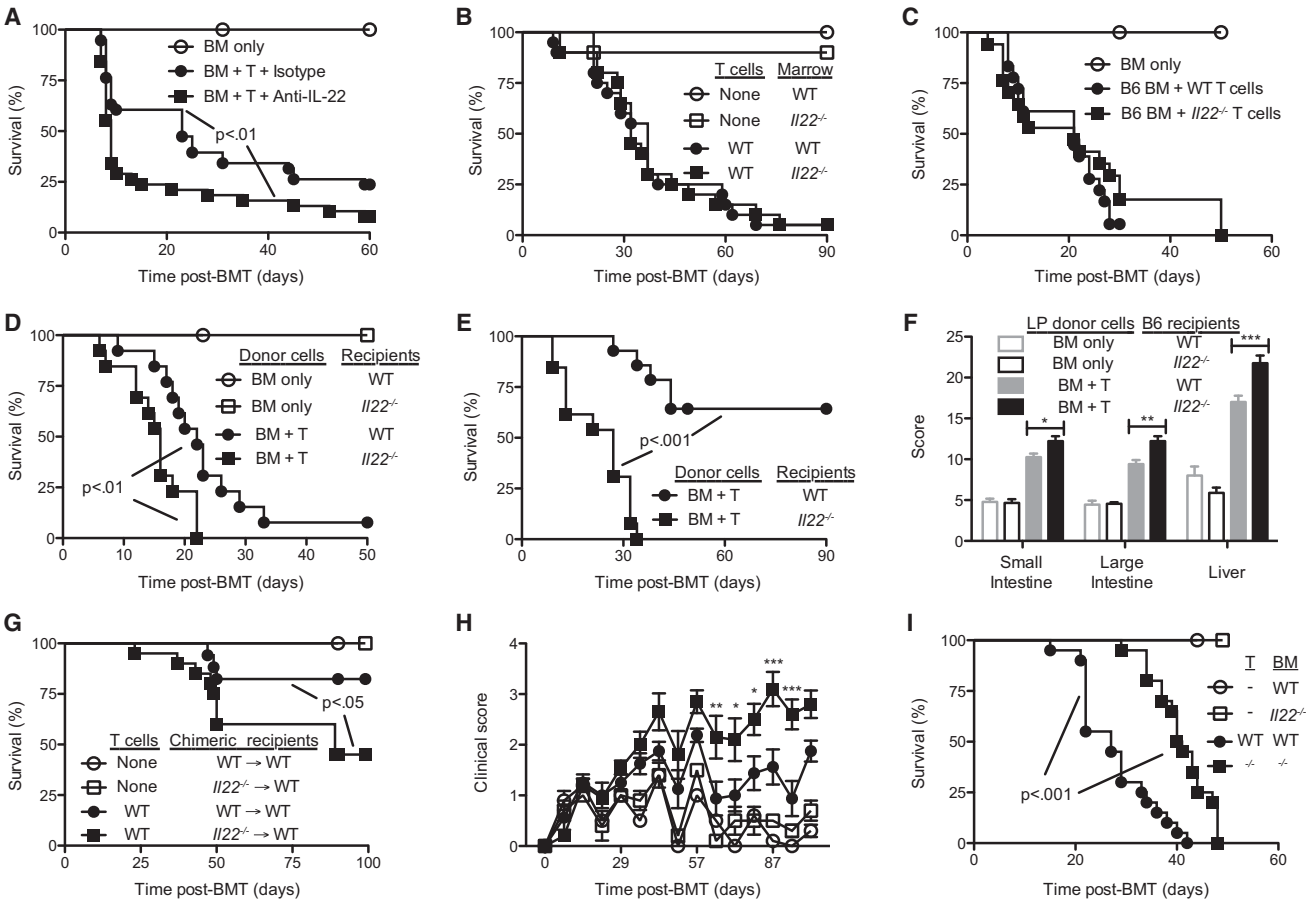

Figure 1. Host IL-22 Deficiency Increases GVHD

(A–D) BALB/c recipient mice were transplanted with B6 T cell-depleted marrow alone or with marrow and T cells (MHC mismatch). As shown in (A), IL-22 neutralizing antibody or isotype control was given to mice by intraperitoneal administration three times per week until day 30 post-BMT. The graph shows shortened median survival time in GVHD mice treated with neutralizing antibody. As shown in (B), mice were transplanted with *Il22*$^{-/-}$ or WT donor marrow with no change in median survival. As shown in (C), mice were transplanted with *Il22*$^{-/-}$ or WT donor T cells with no change in median survival. As shown in (D), BMT was performed into BALB/c *Il22*$^{-/-}$ or WT BMT recipients. The graph shows shortened median survival time in *Il22*$^{-/-}$ recipients with GVHD.

(E–H) B6 recipient mice transplanted with LP T cell-depleted marrow or marrow and T cells (minor antigen mismatch). As shown in (E), BMT was performed into B6 *Il22*$^{-/-}$ or WT BMT recipients. The graph shows shortened median survival time in *Il22*$^{-/-}$ recipients with GVHD. As shown in (F), the graph shows GVHD histopathology 3 weeks after BMT into *Il22*$^{-/-}$ or WT recipients. As shown in (G), BMT was performed with B6 WT chimeras (WT → WT) or hematopoietic *Il22*$^{-/-}$ chimeras (*Il22*$^{-/-}$ → WT) as recipients of LP WT donor cells. The graph shows survival time. In (H), the graph shows clinical GVHD scoring for chimeric BMT recipients shown in (G).

(I) BALB/c recipient mice were transplanted with either B6 WT marrow, *Il22*$^{-/-}$ marrow, WT marrow, and WT T cells or *Il22*$^{-/-}$ marrow and *Il22*$^{-/-}$ T cells. The graph shows survival time. Data are combined (A–G, I) or representative (H) of at least two independent experiments and at least nine mice per group; bar graphs show the mean and SE. *p < .05, **p < .01, ***p < .001.

IL-22 Is Produced Post-BMT by Radio-Resistant Host ILCs that Are Eliminated during GVHD

Because IL-22 was expressed post-BMT and was derived from recipient hematopoietic cells, we next sought to identify the cellular source of intestinal IL-22 after BMT. ILCs contribute to lymphoid ontogeny and intestinal immunity and are potent sources of IL-22 (Sonnenberg et al., 2011a; Spits and Di Santo, 2011). Lamina propria ILCs were identified post-BMT by flow cytometry on the basis of their expression of RORγt in the absence of CD3 (Figure S3). We observed that CD45^{+}CD3^{-} RORγt^{+} ILCs could persist post-BMT in the lamina propria of transplanted mice without GVHD, but were reduced in frequency and total numbers in BMT recipients with GVHD (Figure 3A). ILCs produced IL-22 in response to IL-23 stimulation in vitro (Figure 3B) and were of host origin (Figure 3C). However, host IL-22-producing ILCs were eliminated during GVHD (Figure 3D). This was confirmed in MiHA-disparate recipients with GVHD (Figure 3E), and similar results were observed in large intestine as well (data not shown). Although IL-22 can be produced by other lymphoid populations such as Th17 cells and natural killer cells (Witte et al., 2010), no significant IL-22 production was identified in lamina propria subsets expressing the T cell marker CD3 or lacking RORγt (Figure S3). Phenotypic assessment of IL-22^{+} ILCs post-BMT indicated NKp46^{-} cells with expression of IL-7Rα and CCR6 (Figure 3F), consistent with the phenotype of a lymphoid tissue inducer-like cell (Sawa et al., 2010). Thus,

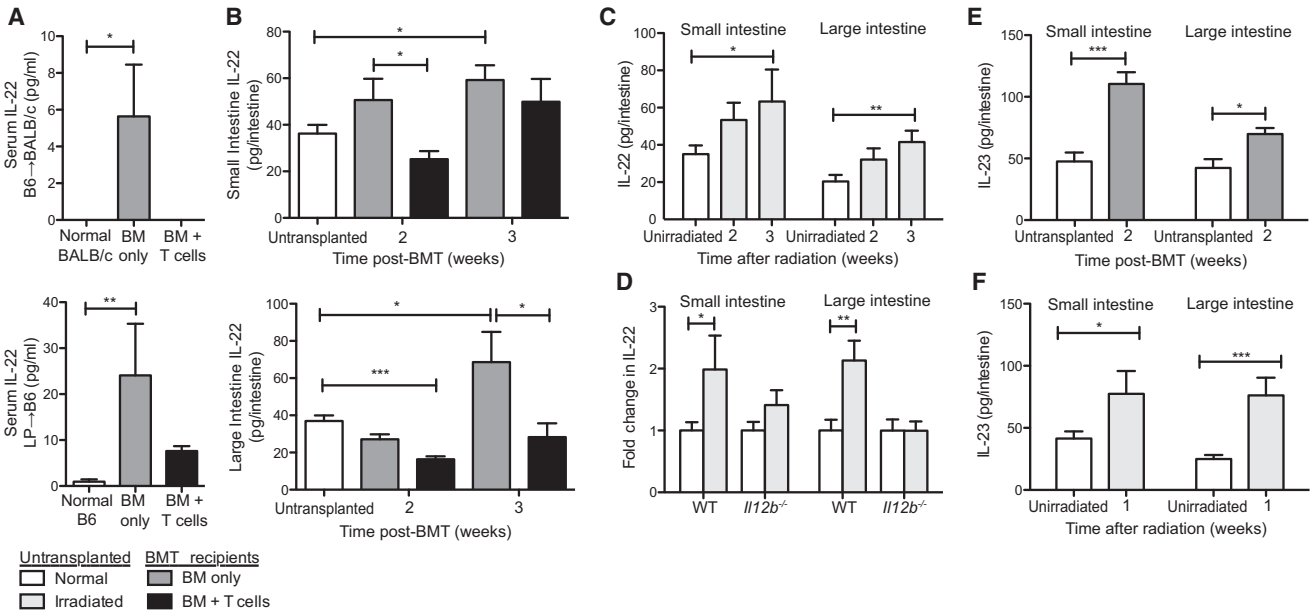

Figure 2. IL-22 and IL-23 Are Produced after Pretransplant Conditioning

(A) IL-22 ELISA was performed on serum from BALB/c and B6 mice 2 weeks after B6→BALB/c BMT or 3 weeks after LP→B6 BMT. Graphs show serum IL-22 concentrations. Data are representative of two independent experiments with at least four mice per group in each experiment.

(B–F) Cytokine ELISA was performed on homogenized intestinal tissue from B6 mice. In (B), the graph shows intestinal IL-22 concentrations after LP→B6 BMT. In (C), the graph shows intestinal IL-22 concentrations after sublethal 550 cGy TBI of WT B6 mice. In (D), the graph shows fold increase in intestinal IL-22 concentrations after sublethal 550 cGy TBI of WT versus p40-deficient (*Il12b*$^{-/-}$) B6 mice lacking IL-23 and IL-12. In (E), the graph shows intestinal IL-23 concentrations after LP→B6 BMT without T cells. In (F), the graph shows intestinal IL-23 concentrations after lethal 1100 cGy TBI, split dose. In all panels, data are combined from at least two independent experiments and at least seven mice per group; bar graphs show mean and SE. *p < .05, **p < .01, ***p < .001.

ILCs are the recipient-derived source of IL-22 post-BMT, and they are reduced during aggressive experimental (MHC-mismatched) and clinically modeled (MHC-matched) GVHD.

In order to determine how recipient-derived ILCs could persist post-BMT despite lethal pretransplant conditioning, we evaluated the sensitivity of lamina propria ILCs to radiation treatment. Assessment of ILCs in B6 lamina propria 24–72 hr after lethal (1100 cGy) TBI indicated no significant change in the absolute numbers of ILCs postradiation (Figure 4A). ILC frequency in lamina propria increased 24 hr after radiation treatment (Figure 4B), further indicating a relative resistance of ILCs to radiation as compared to other CD45$^+$ lamina propria cells. We also assessed the sensitivity of intestinal T cells to radiation treatment. In contrast to the ILCs, lamina propria CD4$^+$ T cells decreased in frequency 72 hr after TBI (Figure 4C). This led to an increase in the ILC-to-CD4 ratio after TBI, providing further evidence of the radio resistance of ILCs (Figure 4D).

Given the short-term radio resistance of ILCs and the persistence of recipient-derived ILCs early after T cell-depleted BMT, we performed a long-term assessment of the ILC chimerism up to 3 months post-BMT. In the periphery, the CD11b$^+$ myeloid cells were fully of donor origin by 2 months post-BMT, indicating donor hematopoietic stem and progenitor cell engraftment (Figure 4E). Consistent with this donor hematopoietic engraftment, lamina propria T cells were primarily donor derived and new donor-derived ILCs were also identified in recipient lamina propria 2 months post-BMT (Figure 4E). Interestingly, despite the donor hematopoietic engraftment in both myeloid cells and in T cells in the periphery and in T cells within the GI lamina propria, the ILCs in recipient lamina propria remained primarily recipient-derived even 3 months post-BMT (Figures 4F and 4G).

Host IL-22 Deficiency Does Not Significantly Alter the Donor Immune Response

Because recipient-derived IL-22$^+$ ILCs could persist post-BMT and IL-22 expression increased post-BMT in mice without GVHD, we sought to determine how deficiency of recipient-derived IL-22 could lead to increased GVHD. Given that GVHD is mediated by donor T cells, we assessed for differences in the donor T cell compartment after transplant into WT or *Il22*$^{-/-}$ recipients (Figure 5). Although host IL-22 deficiency led to increased GVHD mortality and intestinal pathology, no differences were observed in lymphocyte infiltration of recipient intestines (Figure 5A). Furthermore, no major differences were observed in frequencies of donor lymphoid subsets within recipient spleen or mesenteric lymph nodes (Figure 5B), and no differences were observed in donor T cell production of IFN-γ or TNF-α (Figure 5C). Therefore, although increased GVHD was observed in *Il22*$^{-/-}$ recipients, this did not appear to be due to increased donor alloreactivity.

Intestinal Stem Cells Are Targets of GVHD

Consistent with direct protection of recipient epithelium by IL-22 after transplant, IL-22R expression was significantly increased

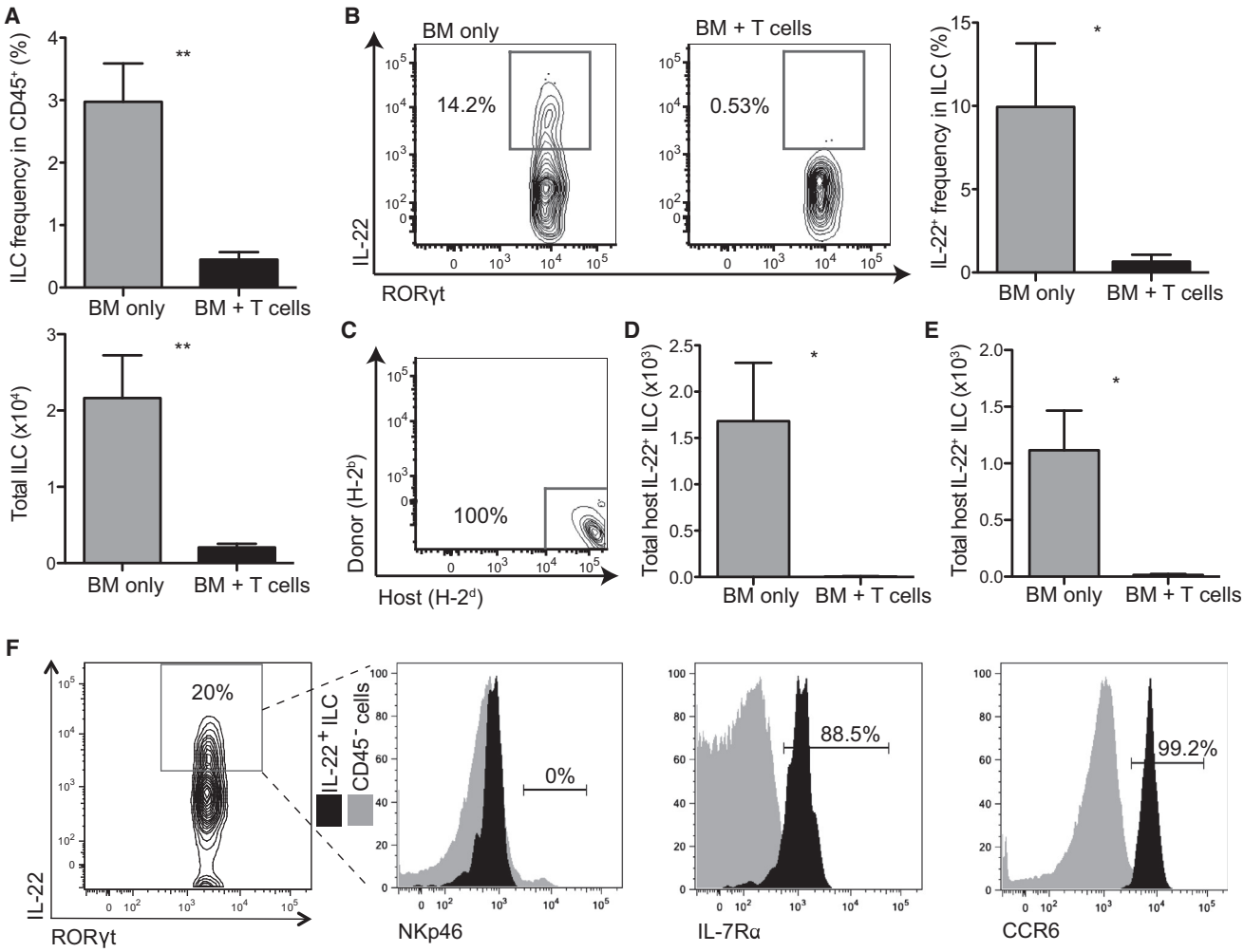

Figure 3. IL-22 Is Produced Post-BMT by IL-23-Responsive Host ILCs that Are Eliminated during GVHD

Detection of CD45$^+$CD3$^-$RORγt$^+$ ILCs 2 weeks after BMT by flow cytometry of small intestine lamina propria cells after 5 hr of IL-23 stimulation in vitro. (A–D) B6→BALB/c BMT was performed with T cell-depleted marrow alone or with marrow and T cells. In (A), the graph shows frequency of CD3$^-$RORγt$^+$ ILCs among CD45$^+$ lamina propria cells and total numbers of CD45$^+$CD3$^-$RORγt$^+$ lamina propria ILCs from recipients with (BM + T cells) or without (BM only) GVHD. In (B), plots show concatenated frequencies of IL-22-producing RORγt$^+$ ILCs. The bar graph shows frequencies of IL-22$^+$RORγt$^+$ ILCs among all RORγt$^+$ ILCs. In (C), the plot shows detection of H-2^{d+} host-derived cells among IL-22-producing ILCs identified after BM-only BMT shown in (B). In (D), the graph shows total host IL-22-producing ILCs after B6→BALB/c BMT. Data are representative of three independent experiments and at least 13 mice per group. (E and F) LP→B6 BMT was performed with T cell-depleted marrow alone or with marrow and T cells. In (E), the graph shows total host IL-22-producing ILCs after LP→B6BMT. In (F), plots show NKp46$^-$IL-7Rα$^+$CCR6$^+$ phenotype of IL-22-producing CD45$^+$CD3$^-$RORγt$^+$ ILCs after BM-only BMT. In all panels, data are representative of two independent experiments and at least six mice per group; bar graphs show mean and SE. *p < .05, **p < .01.

post-BMT in small and large intestines of WT recipients regardless of GVHD (Figures 6A and 6B). In addition to mature villus epithelium, immunohistochemistry identified IL-22R expression within intestinal crypts where the epithelial stem and progenitor cell niche is located (Figure 6C) (Medema and Vermeulen, 2011; Simons and Clevers, 2011). Lgr5$^+$ ISCs, termed crypt base columnar (CBC) cells for their histologic location and morphology, are capable of generating entire crypt-villus structures in vitro and in vivo (Barker et al., 2007; Sato et al., 2009). Paneth cells provide a supportive microenvironment for ISCs in vivo at least in part by activating Wnt signaling within ISCs (Sato et al., 2011). Immunohistochemistry thus appeared to identify IL-22R expression on both epithelial progenitors at the apex and ISCs at the crypt base between Paneth cells (Figure 6C). Immunofluorescence further demonstrated IL-22R expression on ISCs located between lysozyme-expressing Paneth cells, in addition to IL-22R expression on ISC progeny higher in the crypt (Figure 6D). This was confirmed by flow cytometry for IL-22R on Lgr5-GFP reporter crypt epithelial cells (Barker et al., 2007), in which GFP$^+$ cells were also found to express IL-22R (Figure S4). IL-22R expression is thus increased post-BMT, and IL-22R is expressed within the intestinal stem and progenitor cell compartment.

GVHD attacks recipient intestinal epithelium, and it is thought to include an attack against epithelial precursors. However, definitive data demonstrating the elimination of ISCs during

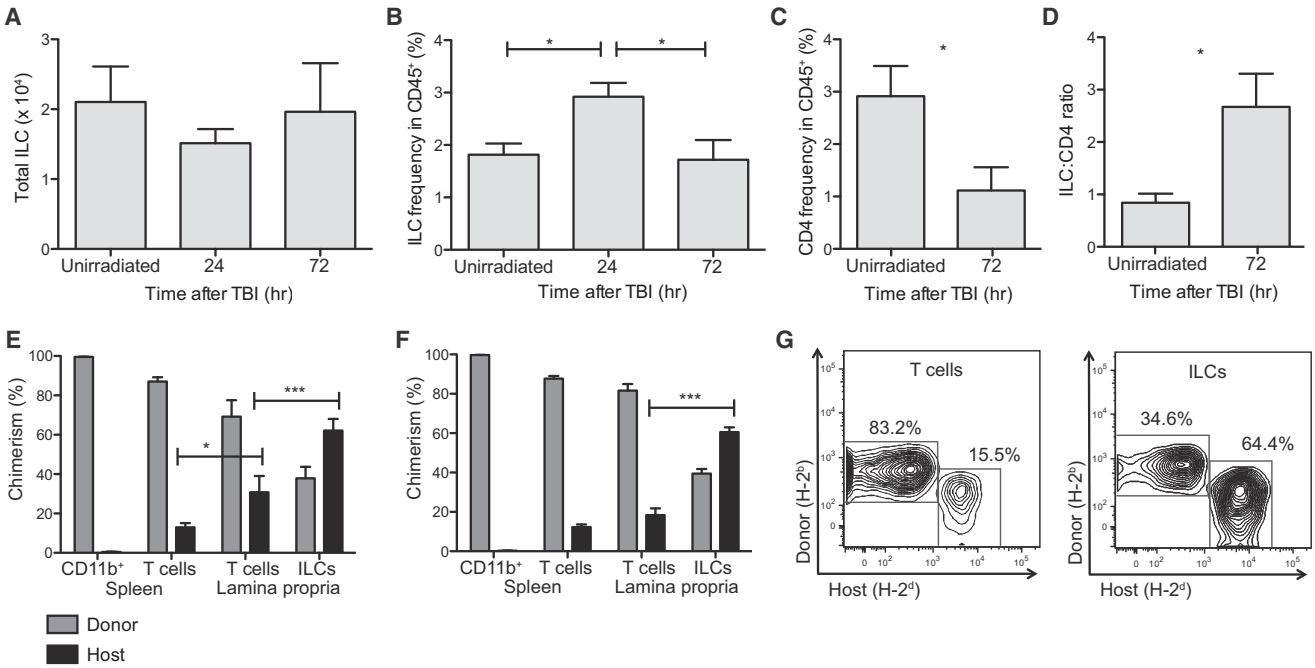

Figure 4. ILCs Are Radio Resistant and Persist Long-Term Post-BMT

(A–D) Flow cytometry was performed on lamina propria cells from B6 small intestine 24–72 hr after lethal TBI (550cGy × 2). In (A), the graph shows total numbers of intestinal CD45$^+$CD3$^-$RORγt$^+$ ILCs after TBI. In (B), the graph shows frequency of CD3$^-$RORγt$^+$ ILCs among lamina propria CD45$^+$ cells after TBI. In (C), the graph shows frequency of CD4$^+$ T cells among lamina propria CD45$^+$ cells after TBI. In (D), the graph shows ratio of ILCs to CD4$^+$ T cells after TBI.

(E–G) Donor and host chimerism of peripheral myeloid cells, peripheral T cells, small intestine lamina propria T cells, and small intestine lamina propria ILCs was measured by flow cytometry after T cell-depleted B6 → BALB/c allogeneic BMT. In (E), the graph shows chimerism 2 months post-BMT. In (F), the graph shows chimerism 3 months post-BMT. Data are combined (A–F) or representative (G) of at least two independent experiments with at least five mice per group; bar graphs show mean and SE. *p < .05, ***p < .001.

GVHD have been limited prior to the identification of ISC-specific markers. Takashima et al. (2011) reported that GVHD impaired the recovery of ISCs from radiation injury and that treatment with the ISC growth factor R-spondin1 could promote epithelial recovery and reduce systemic GVHD. In order to examine ISC damage during GVHD, we utilized B6 Lgr5-LacZ reporter mice as recipients during MiHA-mismatched transplantation. Lgr5-expressing cells were identified by LacZ staining, and transplantation demonstrated a significant reduction of LacZ-expressing CBC cells in BMT recipients with GVHD (Figure 6E). This indicates that ISCs are eliminated during GVHD despite their expression of IL-22R, which is consistent with our findings that IL-22 and IL-22-producing ILCs were reduced during GVHD.

IL-22 Deficiency Leads to Increased Epithelial Damage during GVHD and Loss of ISCs

Given the lack of a major discernible impact on alloreactive donor immunity during IL-22 deficiency, the expression of IL-22R on intestinal stem and progenitor cells, and the loss of ISCs during GVHD, we sought to determine how IL-22 could act upon recipient epithelium to protect against GVHD. In addition to having a greater overall GVHD score post-BMT, blinded histopathologic assessment indicated an increase in epithelial apoptosis within intestinal crypts of *Il22*$^{-/-}$ recipient mice during GVHD (Figure 7A). Given that the intestinal stem and progenitor niche is located within epithelial crypts, and that these cells express IL-22R, we next compared the stem cell compartment in WT and *Il22*$^{-/-}$ recipient mice during GVHD (Figure 7B). Similar to what was observed with Lgr5-LacZ reporter mice, blinded histologic assessment of the ISC niche indicated that WT recipients without the β-galactosidase reporter also demonstrated a loss of CBC cells during GVHD (Figure 7B, WT recipients transplanted +/− donor T cells), confirming that ISCs were GVHD targets. Moreover, loss of ISCs in *Il22*$^{-/-}$ recipients with GVHD was significantly worse than the loss of ISCs in WT mice (Figure 7B). These findings indicate that IL-22 is not only capable of protecting mature intestinal epithelium, but that IL-22 can also protect ISCs.

Given the regulation of epithelial cell production of Reg family molecules by IL-22 (Sanos et al., 2011), we examined the expression of Reg3γ and Reg3β mRNA during GVHD in WT and *Il22*$^{-/-}$ recipients. Both molecules are known to be produced by intestinal epithelium in response to IL-22, and, in addition to their direct antimicrobial properties, Reg3β has also been reported to promote epithelial cell proliferation and protection from apoptosis in response to tissue injury (Kinnebrew et al., 2012; Okamoto, 1999; Pickert et al., 2009; van Ampting et al., 2012). Consistent with the overall increased mortality and epithelial damage of *Il22*$^{-/-}$ mice during GVHD, expression of Reg3γ and Reg3β mRNA were both reduced during GVHD in *Il22*$^{-/-}$ mice as compared to WT GVHD mice (Figures 7C and 7D). Finally, in order to test the epithelial consequences of increased

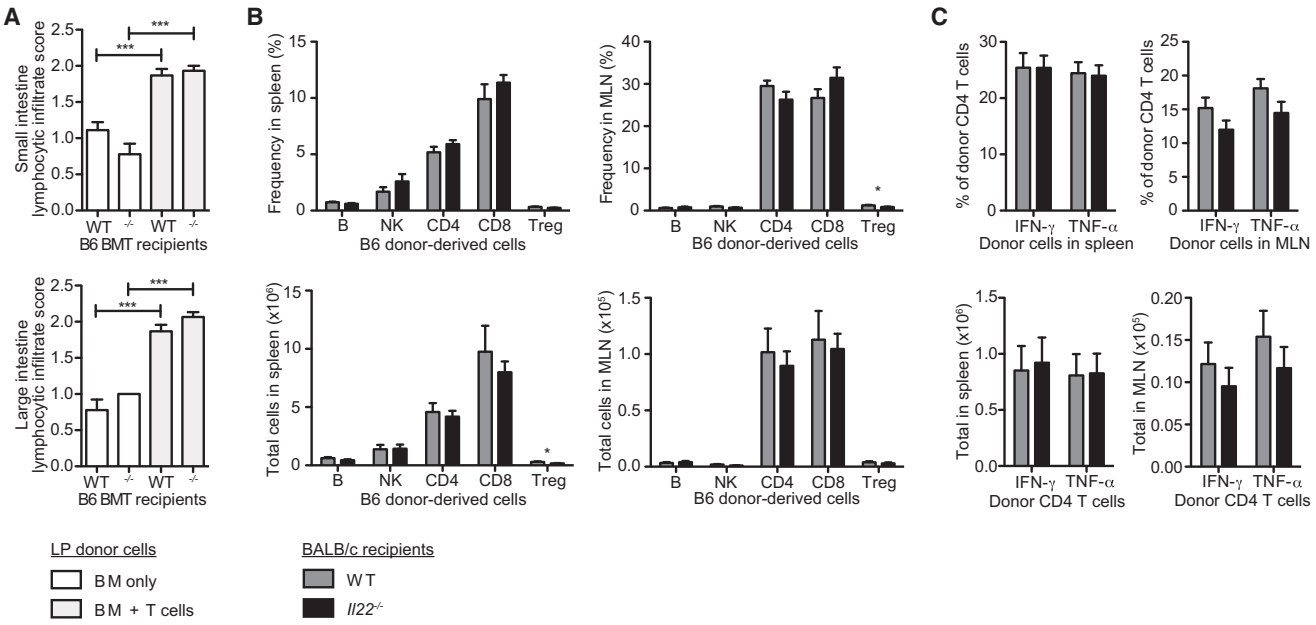

Figure 5. Donor Immunity Is Not Significantly Altered by Host IL-22 Deficiency

(A) Histopathologic assessment of lymphocyte infiltration was performed on intestinal tissues from WT or *Il22*$^{-/-}$ recipients 3 weeks after LP→B6 BMT. Graphs show histopathologic scoring of lymphocytic infiltrates.

(B and C) Splenocytes and mesenteric lymph node (MLN) cells from WT or *Il22*$^{-/-}$ recipients were analyzed with flow cytometry 2 weeks after B6→BALB/c BMT. In (B), graphs show frequencies and total numbers of donor lymphoid subsets among all splenocytes or among all MLN cells. In (C), graphs show frequencies among donor CD4$^+$ T cells and total numbers of all donor CD4$^+$ T cells expressing IFN-γ or TNF-α after stimulation in vitro with PMA and ionomycin. In all panels, data are combined from two independent experiments with at least nine mice per group; bar graphs show mean and SE. *p < .05, ***p < .001.

apoptosis, loss of CBC cells, and reduced Reg3γ and Reg3β in *Il22*$^{-/-}$ recipient mice, we tested epithelial barrier function in BMT recipients by performing oral administration of FITC-dextran, a nonabsorbed carbohydrate that enters the bloodstream if the epithelial barrier has been compromised (Brandl et al., 2009; Kabashima et al., 2002; Yan et al., 2009). Challenge with oral FITC-dextran indicated increased GVHD-related epithelial damage and loss of epithelial integrity in *Il22*$^{-/-}$ BMT recipients (Figure 7E). Thus, with an equivalent donor immune response, *Il22*$^{-/-}$ recipient mice suffered increased mortality, intestinal pathology, damage to the ISC compartment, and compromised epithelial barrier function.

DISCUSSION

Little is known about the susceptibility of transplant recipients to tissue damage mediated by the donor immune system and the regenerative mechanisms employed by recipient tissues to recover from this immune-mediated damage. Although alteration of GVHD by recipient-derived factors has previously been described, the primary effect of these molecules has been on the activation of the donor immune system (Lin et al., 2003; Penack et al., 2010). A clinical parameter of transplant patients that has been linked to their risk for developing GVHD is increased recipient age (Flowers et al., 2011; Nash et al., 1992). However, specific mechanistic etiologies for the susceptibility of older adults to GVHD are unclear, and acute GVHD susceptibility can be difficult to separate from toxicity because of pretransplant conditioning (Jagasia et al., 2012). Factors such as DNA repair mechanisms may regulate damage from conditioning (Dalle, 2008), and indoleamine 2,3-dioxygenase may regulate repair mechanisms as well as immune effects (Jasperson et al., 2008), but specific regulation of GVHD susceptibility remains poorly understood. In our study, host IL-22 deficiency led to increased GVHD morbidity and mortality. With the lethal pretransplant conditioning doses used here, IL-22-deficient recipients demonstrated increased intestinal pathology specifically in the inflammatory setting of GVHD, but not after conditioning and transplantation in the absence of donor T cells. Host IL-22 deficiency did not significantly alter the alloreactive donor immune response; however, it did increase GVHD in two distinct model systems, worsen epithelial apoptosis, and compromise the integrity of the intestinal barrier function. These findings indicate that IL-22 is an important recipient-derived factor with a primary role in regulating recipient sensitivity to GVHD.

In our study, deficiency of recipient-derived IL-22 led to increased GVHD pathology in the intestines and liver, although no differences were observed in GVHD of the skin. The importance of IL-22 in protecting recipients from GVHD was clearly demonstrated by the use of an IL-22 neutralizing antibody after allogeneic BMT. Although deficiency of IL-22 derived from either donor marrow or from donor T cells was not found to impact mortality after transplantation with *Il22*$^{-/-}$ donor cells, we identified that combined transplantation with marrow and T cells that were both deficient in IL-22 led to a modest but statistically

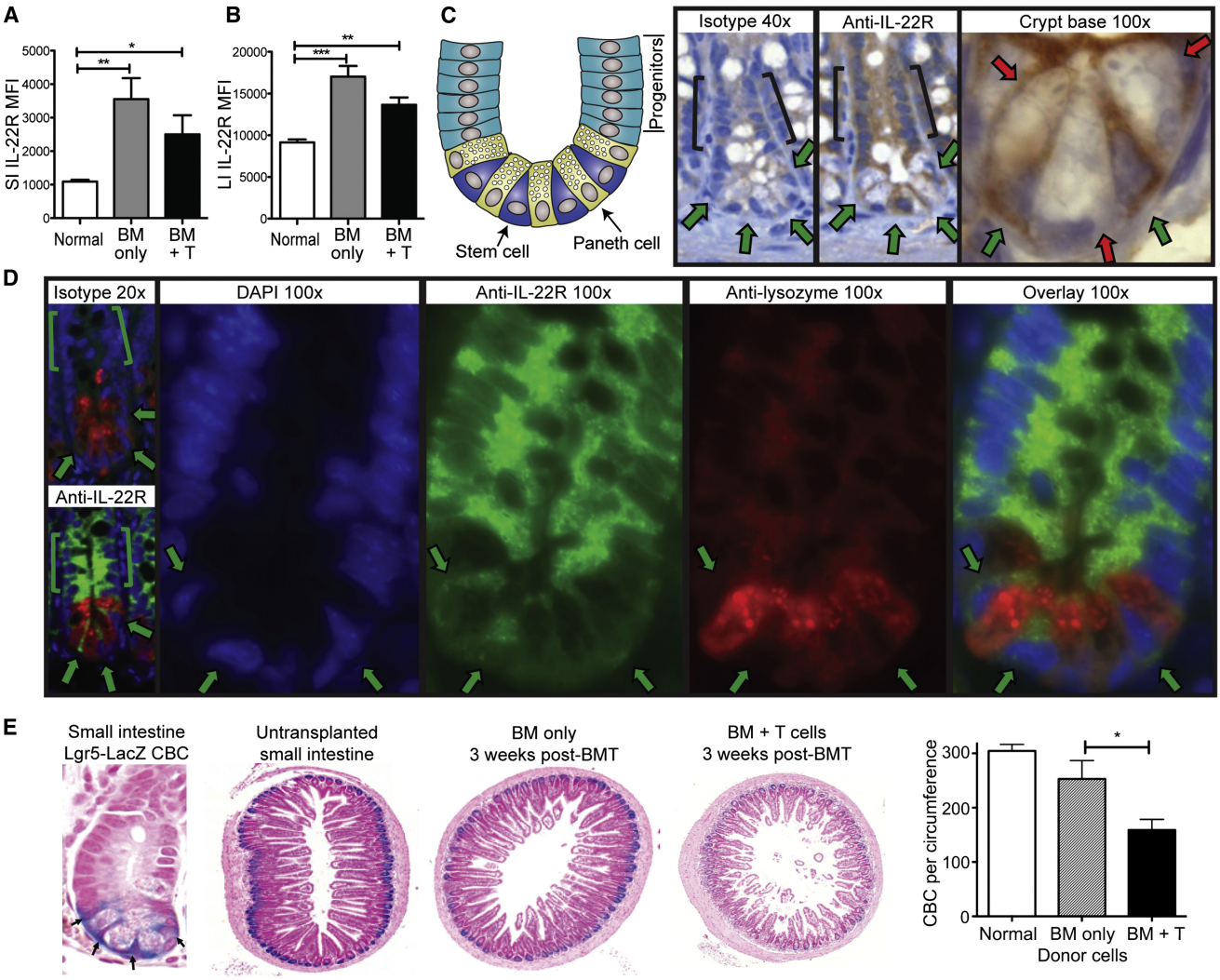

Figure 6. IL-22R Is Expressed in Intestinal Crypts, and Intestinal Stem Cells Are Targets of GVHD

(A and B) LP→B6 BMT was performed with T cell-depleted marrow alone or with marrow and T cells. IL-22R expression was measured by flow cytometry performed on epithelial cells from normal or transplanted WT mice 3 weeks post-BMT. In (A), the graph shows small intestine (SI) epithelial cell IL-22R MFI. In (B), the graph shows large intestine (LI) epithelial cell IL-22R MFI.

(C) Immunohistochemistry was performed on tissue sections from untransplanted B6 small intestine. A schematic is shown depicting intestinal stem cells (ISCs), progenitor cells, and Paneth cells within a small intestine crypt. ISCs expressing Lgr5 are histologically referred to as crypt base columnar (CBC) cells on the basis of their location and morphology. Immunohistochemistry images show serial sections stained with either an IL-22R antibody or isotype control. Brown staining indicates IL-22R expression in progenitor layer (brackets) and on CBC cells (green arrows) between Paneth cells (red arrows) at crypt base.

(D) Immunofluorescence was performed on serial sections from untransplanted B6 small intestine stained with either an IL-22R antibody or isotype control. Images demonstrate IL-22R (green), lysozyme (red), and nuclear (blue) staining, indicating IL-22R expression by progenitors (brackets) above and CBC cells (green arrows) between lysozyme-expressing Paneth cells (red granules).

(E) LP→B6 allogeneic BMT was performed with Lgr5-LacZ reporter mice used as recipients. Images show a close-up of Lgr5-expressing small intestine CBC cells from untransplanted B6 Lgr5-LacZ reporter mice and circumference views of small intestines from B6 Lgr5-LacZ reporter mice without transplantation, post-BMT without GVHD, and post-BMT with GVHD. The graph shows small intestine $Lgr5^+$ CBC frequencies for B6 reporter mice without transplantation and post-BMT. In all panels, data are representative of at least two independent experiments and at least seven mice per group; bar graphs show mean and SE. *$p < .05$, **$p < .01$, ***$p < .001$.

significant increase in median survival. Although the dominant effect appears to be epithelial protection by IL-22 given the results of IL-22 neutralizing antibody treatment, these findings suggest that the same cytokine produced by donor versus recipient-derived sources may have opposing effects on GVHD-related tissue damage. Further investigation will be required to determine which effect is dominant in clinical transplantation and whether exogenous IL-22 administration will yield a protective or proinflammatory outcome.

Significant progress has recently been made in the understanding of IL-22 and immune-mediated regulation of epithelial maintenance and regeneration (Sonnenberg et al., 2011a). An

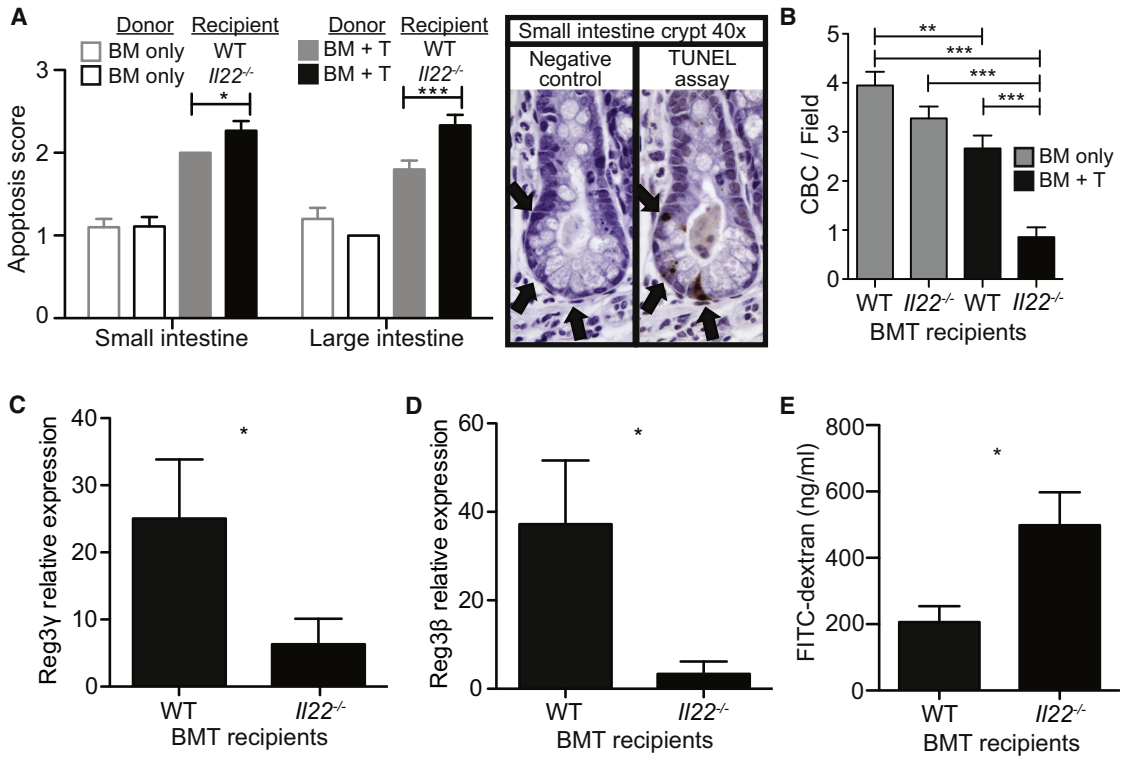

Figure 7. IL-22 Deficiency Leads to Increased Epithelial Damage and Loss of Intestinal Stem Cells

LP→B6 BMT was performed with WT or *Il22*$^{-/-}$ recipients and analyzed 3 weeks post-BMT.

(A) Histopathologic assessment of apoptosis in crypt epithelium was performed on tissue sections from transplanted mice. The graph shows histopathologic apoptosis score for intestinal tissues from BMT recipients transplanted with or without T cells. The image shows representative TUNEL staining on serial sections from small intestine post-BMT, confirming apoptosis (black arrows) in crypt epithelium of *Il22*$^{-/-}$ recipients during GVHD.

(B) Histologic assessment of small intestine CBC cells was performed on tissue sections from WT and *Il22*$^{-/-}$ recipients with (BM + T) or without (BM only) GVHD. The graph shows small intestine CBC frequencies in nonreporter mice post-BMT.

(C and D) Quantitative PCR for Reg3γ and Reg3β mRNA expression was performed on colon samples from WT versus *Il22*$^{-/-}$ recipients with GVHD. Graphs show Reg3γ and Reg3β relative expression in colons after BMT with marrow and T cells.

(E) BMT recipients of marrow and T cells were challenged with oral gavage of FITC-dextran 3 weeks post-BMT. The graph shows plasma FITC-dextran concentrations in WT versus *Il22*$^{-/-}$ recipients with GVHD. In all panels, data are combined or representative (TUNEL assay) of at least two independent experiments and at least eight mice per group; bar graphs show mean and standard error. *p < .05, **p < .01, ***p < .001.

unaddressed topic of central importance is the effect of the immune system on the precursor compartment of epithelial stem and progenitor cells. Several cell types have been proposed as the earliest stem cell population of intestinal epithelium (Barker et al., 2007; Breault et al., 2008; Montgomery et al., 2011; Sangiorgi and Capecchi, 2008; Sato et al., 2011; Simons and Clevers, 2011; Zhu et al., 2009), with the Lgr5^{+} CBC cell having demonstrated the capacity to regenerate entire crypt-villus structures in vitro from just a single cell (Sato et al., 2011; Sato et al., 2009; Simons and Clevers, 2011). In our study, GI GVHD was found to specifically damage the ISC compartment, and loss of ISCs was even more severe in the absence of host-derived IL-22. These findings demonstrate that IL-22 can maintain intestinal epithelium during settings of inflammatory damage at the level of the ISC. This IL-22-mediated protection of ISCs during GVHD and our identification of IL-22R expression on intestinal stem and progenitor cells further elucidate the finding that ILCs can regulate intestinal epithelium (Sawa et al., 2011) and extend the influence of ILCs and IL-22 to the ISC compartment. We propose that Paneth cells in the stem cell niche may provide a constitutive basal signal for ISC maintenance, whereas ILCs can provide an IL-22-dependent signal to maintain the stem and progenitor cell pool and epithelial barrier in the context of tissue damage and regeneration.

We found that recipient-derived IL-22 is critical for reducing tissue damage during GVHD. In order for IL-22 and ILCs to exert their protective functions within recipient epithelium post-BMT, ILCs must be able to persist post-BMT despite lethal pretransplant conditioning and hematopoietic reconstitution. Merad et al. (2004) have described how recipient Langerhans cells can persist in the skin of mice after T cell-depleted allogeneic BMT despite donor hematopoietic reconstitution, and there is increasing evidence for the importance of tissue-specific cells in mediating local immune responses (Jiang et al., 2012). We demonstrate here that intestinal ILCs are resistant to systemically lethal doses of TBI and that these ILCs can persist within the lamina propria 3 months post-BMT, well after donor hematopoietic engraftment has occurred and even after the lamina propria T cell compartment has reconstituted with donor-derived cells. Interestingly, the T cell reconstitution in the periphery

appeared to occur more rapidly than within the GI tract, given that donor T cell chimerism was greater in the spleen than in the small intestine lamina propria 2 months post-BMT. Although donor-derived ILCs could be identified by 2 months post-BMT, they represented the minority of the lamina propria ILC pool. Moreover, even once the lamina propria T cell chimerism had equilibrated to the T cell chimerism in the periphery by 3 months post-BMT, the lamina propria ILCs remained largely of host origin. Recipient-derived cells therefore comprise the majority of the intestinal ILC compartment for at least 3 months after BMT, further highlighting the significance of the loss of these cells during GVHD.

In conclusion, IL-22 was produced post-BMT by IL-23-responsive ILCs that were eliminated during GVHD, and deficiency of IL-22 led to substantial loss of ISCs, increased intestinal pathology, and loss of epithelial barrier function. These findings reveal a reciprocal mucosal immunity network between the epithelial compartment and the immune system, where tissue damage leads to induction of IL-23, which can stimulate the production of IL-22 by ILCs. This IL-22 production can then protect the intestinal stem and progenitor cell compartment and mature epithelium from inflammatory tissue damage. However, during GVHD, there is loss of the IL-22-producing ILCs necessary for limiting that inflammatory tissue damage. The loss of tissue-protective ILCs represents a pathophysiologic mechanism contributing to tissue damage in GVHD. These findings could lead to targeted approaches for reduction of clinical GVHD without limiting critical posttransplant functions of the donor immune system such as graft versus leukemia or lymphoma responses, antimicrobial immunity, facilitation of engraftment, and promotion of immune reconstitution. Further study of the IL-22-IL-22R axis may even facilitate the pretransplant identification of patients who are at increased risk for the development of GVHD. Finally, these findings may have broad implications for pathogenesis and protection of ISCs during other intestinal inflammatory diseases.

EXPERIMENTAL PROCEDURES

Mice and Bone Marrow Transplantation

C57BL/6 (CD45.2 B6, H-2^b), B6.SJL-Ptprca Pepcb/BoyJ (CD45.1 B6 congenic, H-2^b), *Il12b*$^{-/-}$ B6, BALB/c (H-2^d), and LP (H-2^b) mice were obtained from the Jackson Laboratory. B6 and BALB/c *Il22*$^{-/-}$ mice were provided by Genentech, as was anti-IL-22 neutralizing antibody 8E11. Lgr5-LacZ and Lgr5-GFP B6 mice were provided by H. Clevers (Barker et al., 2007). The BMT procedure was performed as previously described (Petrovic et al., 2004), with 850 cGy split-dosed lethal irradiation of BALB/c hosts receiving bone marrow (5 × 10^6), T cell-depleted with anti-Thy-1.2 and low-TOX-M rabbit complement (Cedarlane Laboratories), or performed with 1100 cGy split-dosed lethal irradiation of B6 hosts receiving T cell-depleted bone marrow (5 × 10^6) as well. Donor T cells (typically 1 × 10^6 B6 or 4 × 10^6 LP, unless otherwise specified) were prepared for transplantation by harvesting donor splenocytes and enriching for T cells by either nylon wool passage (routinely >70% T cell purity) or by Miltenyi MACS purification of CD5 (routinely > 90% purity). Recipient mice were monitored for survival and clinical GVHD symptoms and were sacrificed for blinded histopathologic and flow cytometric analysis as previously described (Petrovic et al., 2004). For chimeric experiments, CD45.1 B6 congenic mice were lethally irradiated and reconstituted with either wild-type or *Il22*$^{-/-}$ CD45.2 marrow. Three months later, donor reconstitution was confirmed by FACS of peripheral blood for CD45.1 versus CD45.2, and chimeric mice were irradiated again (900 cGy, split dose) and transplanted with LP marrow (10 × 10^6) and T cells (3 × 10^6). BMT protocols were approved by the MSKCC Institutional Animal Care and Use Committee.

Histopathologic Analysis of GVHD Target Organs

GVHD mice were sacrificed, and the small intestine, large intestine, and liver were removed, formalin-preserved, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Scoring was performed as previously described (Petrovic et al., 2004).

Tissue Analyses and Flow Cytometry

Lymphoid organs from GVHD mice were processed into single-cell suspensions, and lamina propria lymphocytes were isolated after dissociation of the epithelium and digestion in DNaseI (Roche) and Collagenase D (Roche). Surface staining was performed with the corresponding cocktail of antibodies, and an eBioscience Fixation/Permeabilization kit was used per the manufacturer's protocol for intracellular staining. FACS for IL-22R (rat-anti-mouse IL-22Rα antibody 496514, R&D Systems) included both surface and intracellular staining. Intracellular cytokine staining was performed with anti-IL-22 (1H8PWSR, eBioscience), anti-IFN-γ (XMG1.2, BD PharMingen), or anti-TNF-α (MP6-XT22, BD PharMingen) after 5 hr of restimulation with BD Golgi Plug (1 ul/ml) and IL-23 (40 ng/ml) for IL-22 expression or phorbol-12-myristate-13-acetate (50 ng/ml) and ionomycin (500 ng/ml) for IFN-γ and TNF expression. IL-22 and IL-23 ELISA were performed per the manufacturer's protocol (BioLegend) on either homogenates from small and large intestines or on supernatants from intestines that were cultured at 37°C overnight in RPMI with FBS.

Immunohistochemistry, Immunofluorescence, TUNEL, LacZ, and ISC Histology

Intestines were harvested from normal mice, formalin-fixed, and stained with rat-anti-mouse IL-22Rα antibody 496514 (R&D Systems) versus isotype control and/or polyclonal rabbit anti-human lysozyme 3.2.1.17 (Dakocytomation). Immunofluorescence secondary staining was performed with AF488 for IL-22R and AF568 for lysozyme. TUNEL assay was performed on formalin-fixed/paraffin-embedded tissue as described by Gavrieli et al. (1992). For Lgr5-LacZ transplants, 2.5 cm segments of ileum were obtained, and staining for presence of β-galactosidase (LacZ) was performed as per Barker et al. (2007). In brief, small intestines were fixed in 1% formaldehyde, 0.2% glutaraldehyde, and 0.02% NP40 in PBS and then incubated with β-galactosidase substrate. Tissues were then fixed again in 4% PFA in PBS and paraffin-embedded, and sections were counterstained with Nuclear Fast Red (Vector). For assessing ISCs in nonreporter mice, crypt base columnar intestinal stem cells were identified as reported by their morphology and their location at the crypt base between Paneth cells (Barker et al., 2007).

Quantitative PCR

Reverse transcription-PCR was done with a QuantiTect reverse transcription kit (QIAGEN). For real-time PCR, specific primer and probe sets were obtained from Applied Biosystems as follows: Beta-actin: Mm01205647_g1, Reg3b: Mm00440616_g1, and Reg3g: Mm00441127_m1. PCR was done on ABI 7500 (Applied Biosystems) with TaqMan Universal PCR Master Mix (Applied Biosystems). Relative amounts of Reg3g and Reg3b mRNA were calculated by the comparative ΔC(t) method.

FITC-Dextran Assay

Mice were kept without food and water for 4 hr and then FITC-dextran (#FD4-1G, Sigma) was administered by oral gavage at a concentration of 40 mg/ml in phosphate-buffered saline (PBS) in 400 ul (16mg) per mouse (~800mg/kg). Four hours later, plasma was collected from peripheral blood, then mixed 1:1 with PBS and analyzed on a plate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Statistics

Bars and error bars represent the means + SEM, respectively, for the various groups. Survival data were analyzed with the Mantel-Cox log-rank test. For nonsurvival pointwise analyses, unpaired t test was used for comparisons between two experimental groups, or nonparametric Mann-Whitney U test was used for non-Gaussian distributions, and ANOVA was used for

comparisons with more than two groups. Survival transplants were performed with 10–38 mice per group, and all other experiments were performed at least twice with at least six mice per group.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2012.05.028.

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