DISEASES AND DISORDERS

Interferon β drives intestinal regeneration after radiation

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The cGAS-STING cytosolic DNA sensing pathway is critical for host defense. Here, we report that cGAS-STING–dependent type I interferon (IFN) response drives intestinal regeneration and animal recovery from radiation injury. STING deficiency has no effect on radiation-induced DNA damage or crypt apoptosis but abrogates epithelial IFN-β production, local inflammation, innate transcriptional response, and subsequent crypt regeneration. cGAS KO, IFNAR1 KO, or CCR2 KO also abrogates radiation-induced acute crypt inflammation and regeneration. Impaired intestinal regeneration and survival in STING-deficient mice are fully rescued by a single IFN-β treatment given 48 hours after irradiation but not by wild-type (WT) bone marrow. IFN-β treatment remarkably improves the survival of WT mice and Lgr5+ stem cell regeneration through elevated compensatory proliferation and more rapid DNA damage removal. Our findings support that inducible IFN-β production in the niche couples ISC injury and regeneration and its potential use to treat acute radiation injury.

INTRODUCTION

The intestinal epithelium carries out vital absorptive, secretory, and barrier functions, and has a rapid renewal cycle of 3 to 5 days necessitated by constant physical and mechanical loads. Intestinal stem cells (ISCs) drive homeostatic renewal and injury-induced regeneration (1–3). ISCs are intermingled with Paneth cells at the crypt bottom to continuously supply the four major epithelial lineages. ISCs are marked by the expression of several markers such as Lgr5 (4), and their proliferation and differentiation during homeostasis are regulated by Wnt, bone morphogenetic protein (BMP), epidermal growth factor (EGF), and Notch signals from the niche, often originating within a few cells’ distance (1, 5, 6). The ISC niche contains various cell types, such as Paneth cells and subepithelial myofibroblasts, and expands to include dynamic immune populations and the gut microbiota (1, 6). Specific roles of niche factors and cells in ISC regeneration after injury remain to be elucidated.

Rapid proliferation predisposes the intestinal epithelium to DNA damage–induced injury. Radiation-induced enteritis was first described in 1897 (7), and gastrointestinal acute radiation syndrome (GI-ARS) observed in patients with cancer and victims of accidental exposure has been reported since the 1940s (8, 9). However, no U.S. Food and Drug Administration (FDA)–approved treatment is currently available. High-dose [14 gray (Gy) or above] total body irradiation (TBI) or abdominal irradiation [(ABI), with major bone marrow (BM) sparing] models have been used extensively to study lethal GI injury occurring within 7 to 10 days. This is termed as the GI syndrome (10, 11), characterized by rapid loss of crypt cells, epithelial barrier dysfunction, and systemic inflammation that are not rescued by BM transplantation (9, 12, 13). Using these models, we and others have established the critical role of the p53 pathway in regulating intestinal damage and regeneration, which prevents delayed mitotic death through p53–upregulated modulator of apoptosis (PUMA)–dependent apoptosis and p21–dependent DNA repair of Lgr5+ stem cells (14–24). Stem cells show varying degrees of elevated proliferation after acute or with ongoing injury and cell loss, referred to as compensatory proliferation (1). However, underlying mechanisms coupling ISC injury with regeneration upon DNA damage remain largely undefined (25, 26), hampering the development of therapeutic agents.

Tissue injury and cell death leads to the production and release of damage-associated molecular patterns (27, 28), which are recognized by pattern recognition receptors (PRRs) found in immune and epithelial cells. Inflammation is critical to fight infections and repair tissue injury, while mediators of so-called “reparative” versus “damaging” inflammation are complex and believed to be context dependent (29–31). The endoplasmic reticulum–associated protein, stimulator of interferon genes (STING; mem173, Mita, Mpy5, and Eris), is a key signal transducer upon the detection of cytosolic DNA from pathogens or “self” by over a dozen nucleic acid sensors or PRRs (32, 33). Among them, cyclic GMP (guanosine monophosphate)–AMP (adenosine monophosphate) synthase (cGAS) produces the second messenger 2’,3’-cGAMP to activate STING directly. STING further activates innate immunity through interferon regulatory factor– and nuclear factor kappa B (NF-kB)–induced type 1 interferons (IFNs), particularly IFN-β and proinflammatory cytokines, which are essential to control viral infection. Prolonged STING/IFN activation leads to chronic inflammation and tissue destruction (32, 33).

In this study, we demonstrate a critical role of STING–dependent IFN-β production in the niche in driving intestinal regeneration after radiation. STING deficiency did not have any effect on radiation-induced crypt DNA damage or apoptosis, but it impaired animal survival, compensatory proliferation, acute inflammation, and DNA damage removal in the small intestine. A single dose of IFN-β given 48 hours after TBI fully rescued impaired intestinal inflammation and regeneration in STING-deficient mice and further boosted the survival of wild-type (WT) mice and Lgr5+ stem cell regeneration. These findings reveal an exciting role of spatial and temporal STING/IFN-β signaling in coupling DNA damage–provoked ISC injury with regeneration through a cross-talk between the stationary and mobile niche.
RESULTS

STING is required for intestinal regeneration after radiation injury

Crypt regeneration after 15 Gy TBI is characterized by highly enlarged crypts at 96 hours (more than 200% increase in size) driven by the rapid proliferation of single surviving clonogenic cells following the loss of existing crypts between 48 and 72 hours (Fig. 1A and fig. S1A). Crypt loss is caused by nonapoptotic cell death, which associated aberrant mitosis with highly elevated chromosomal segregation defects and micronuclei owing to unrepaired DNA damage upon replication (Fig. 1B). We performed RNA sequencing (RNA-seq) analysis of epithelial scraping to uncover mechanisms potentially linking cell death with this intensified regenerative response. TBI-regulated genes [up-regulated and down-regulated over twofold, adjusted P value (P_adj) < 0.05] increased significantly from 48 to 96 hours (under 2000 to over 4200) (Fig. 1C). Gene ontology (GO) analysis of the induced genes revealed that the top 10 most enriched pathways are related to innate immunity, including antiviral response, IFN, chemokine production (CxCL2), and translation (Fig. 1D). Gene Set Enrichment Analysis (GSEA) of differential genes identified IFN and tumor necrosis factor (TNF)/NF-κB as the top hits (Fig. 1E). As expected, induced genes were also significantly enriched in DNA damage–related pathways, including p53, apoptosis, and cell cycle (Fig. 1, D and E).

STING is a critical adaptor to activate innate immunity and IFN response (32, 33). We compared crypt regeneration in STING (golden-ticket, Gt/Gt thereafter) (I199N) mice (34) and WT mice. Gt/Gt mice showed markedly impaired crypt regeneration (by 77%) at 96 hours (Fig. 1, F and G, and fig. S1B), while displaying similar crypt loss (more than 93%) at 72 hours (Fig. 1H). Gt/Gt mice displayed shortened survival at multiple doses causing lethal GI injury, including 15 and 12 Gy TBI (gamma rays) and 15 Gy ABI (x-rays) (fig. S1C). ABI permits dose-dependent recovery from acute intestinal injury by major BM sparing (16, 19). WT mice fully recovered (100%) from 14 Gy ABI by week 8 following a significant (15–20%) weight loss on days 5 and 6, characteristic of acute GI damage. Gt/Gt mice showed 60% reduction in survival after a similar initial weight loss (Fig. 1, I and J). In addition, Gt/Gt ABI survivors showed a significant lag in weight gain over the 2 months, impaired progenitor (SOX9) and niche compartments (Paneth cells, MMP7), and elevated DNA damage in the crypts (γH2AX) (Fig. 1, J to L). A band of gray hair across the abdomen appeared in all WT and Gt/Gt ABI survivors by 3 months, which could be explained by an overall increase in abdominal fat and an increased activity of BM–derived Wnt16+ cells (35). Abdominal fat appears in all WT and Gt/Gt mice at 12 weeks (Fig. 1, F and G, and fig. S1B). These data demonstrate a selective role of STING in controlling intestinal recovery from radiation.

STING-dependent type 1 IFN response drives acute crypt regeneration and inflammation

High-dose TBI (15 Gy) induced peak DNA damage, apoptosis, and G2-M blockade at 4 hours in the crypts, followed by cell cycle release (p-H3) and delayed mitotic cell death (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL)) at 24 to 48 hours (Fig. 2, A and B, and fig. S2, A to C). Gt/Gt mice showed normal response in all markers but defective proliferation (p-H3) beginning at 48 hours (Fig. 2, A and B, and fig. S2, A to C). After 12 Gy TBI, Gt/Gt mice also showed defective crypt recovery and proliferation at 96 hours, with unaltered apoptosis at 4 and 24 hours or crypt loss by 72 hours (fig. S2, D and E). Cell death and tissue injury are known to cause acute inflammation (31). We examined the kinetics of BM–derived (CD45+) cells around the crypts after 15 Gy TBI. Peak CD45+ cell recruitment was detected at 48 hours around collapsed crypts in WT mice but was abrogated in Gt/Gt mice (Fig. 2C). Neutrophils (Ly-6B.2) and macrophages (CD68) are two major innate populations implicated in acute tissue damage and repair (30). Their peak recruitment to the crypts was also detected at 48 hours in WT mice and reduced by 70 and 50% in Gt/Gt mice, respectively (Fig. 2, D and E). Neutrophils displayed a rapid and sharp rise around collapsed crypts, compared to the slower and prolonged recruitment of macrophages (Fig. 2, D and E, and fig. S2F), consistent with their established roles in injury clearance and repair (30).

Our RNA-seq data indicated strong activation of IFN and NF-κB signaling in the intestinal epithelium 48 hours after TBI (Fig. 1E). To determine the role of STING in their activation and contribution to crypt regeneration, we analyzed their classical transcriptional targets by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in a detailed time course before the emergence of regenerated crypts (0 to 72 hours) in WT and Gt/Gt mice. We found a complete loss of induction in type 1 IFN targets IFNB, IFNA, and chemokines CCL2 and CXCL2 in the intestinal epithelium of Gt/Gt mice, with elevated expression of NF-κB targets IL6, TNFA, and TGFB (Fig. 2F and fig. S2G). The basal expression of these genes was not notably affected in Gt/Gt mice (0 hour). Chemokines Ccl2 and Cxcl2 are potent chemoattractants of neutrophils and macrophages (30). Genetic ablation of receptors of type 1 IFNs [IFNARI knockout (KO)] or CCL2 (CCR2 KO), but not TNFAO or TNFR1 KO, impaired crypt regeneration, proliferation, and recruitment of neutrophils and macrophages at 48 and 96 hours (Fig. 2, G to J, and fig. S2, H to J). cGAS can detect micronuclei to activate STING-dependent type 1 IFN response (32, 33). cGAS KO mice were found to be defective in acute crypt regeneration, proliferation, and inflammation to an extent similar to Gt/Gt mice (Fig. 2, G to J, and fig. S2, H to J). These data demonstrate an essential and previously unidentified role of the cGAS-STING cytosolic DNA sensing pathway and type 1 IFN response in acute crypt regeneration after radiation-induced cell death.

IFN-β restores crypt inflammation and regeneration in STING-deficient mice

We then directly tested the role of type 1 IFNs in STING-deficient crypt regeneration. A single intraperitoneal injection of IFN-β (25 μg/kg) at 48 hours fully restored crypt regeneration in Gt/Gt mice, compared to limited rescue by IFN-β given at 24 hours (Fig. 3, A and B, and fig. S3A). IFN-β treatment at 48 hours fully restored crypt proliferation (p-H3), peri-cryptal infiltration of neutrophils (Ly6B.2) and macrophages (CD68), and epithelial expression of chemokines CCL2 and CXCL2 by 96 hours (Fig. 3, C to E). In contrast, IFNα given to Gt/Gt mice at 48 hours at the same or fourfold excess dose (100 μg/kg) showed a partial rescue on proliferation but limited or no rescue on crypt regeneration or innate cell recruitment (Fig. 3, A and B, and fig. S3, A and B).

IFNs can regulate the expression of thousands of genes in tissue- and cell type–specific manners (35), so we used RNA-seq analysis to further characterize TBI and STING–dependent signaling that is lost in Gt/Gt mice and recovered by IFN-β treatment. A significant fraction of TBI–induced genes in WT mice were lost in Gt/Gt mice at 48 hours (more than 70%, 697) and 96 hours (by ~50%, 722) (Figs. 1C
Fig. 1. STING deficiency impairs acute crypt regeneration and intestinal recovery after radiation. (A to H) The small intestine of WT and Gt/Gt mice after 15 Gy TBI was analyzed at indicated times (0 to 96 hours). (A) Representative H&E images of crypts at 0, 48, and 96 hours in WT mice. Scale bar, 100 μm. Arrows indicate characteristic single crypts. (B) Scores of abnormal mitoses (bridge, lagging chromosome, multipolar spindle, and micronuclei) in the crypts, with representative H&E images with micronuclei (arrows) at 24 hours in WT mice. Scale bar, 25 μm. (C) TBI-induced (red) or suppressed (green) genes (P_{adj} < 0.05) in epithelial scraping at 48 and 96 hours identified by RNA-seq, n = 2 mice per group. (D) Gene ontology (GO) analysis of TBI-induced genes with the top 10 nonredundant enriched pathways shown. (E) GSEA of differential genes with the top three enriched pathways shown. (F) Representative BrdU immunohistochemistry (IHC) at 96 hours to visualize regeneration. Scale bar, 500 μm. (G) Quantitation of regenerated crypts per cross section from (F). (H) Quantitation of crypt counts per cross section at indicated times. (I to L) WT and Gt/Gt mice after 14 Gy ABI were followed for 8 weeks. (I) Survival. Log-tank test. (J) Body weight change of all mice alive on day 40 compared to day 0. (K) Representative SOX9, γH2AX, and MMP7 immunofluorescence (IF) in the crypts on day 56. Scale bars, 50 μm. (L) Quantitation of SOX9⁺, γH2AX⁺, and MMP7⁺ crypt cells. (B), (G), (H), and (L) values are means ± SEM, n = 3 mice per group with six to eight full cross sections per mouse. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Student’s t test, two-tailed), WT versus Gt/Gt or as indicated.
and 3F and fig. S3C), which were highly enriched in IFN-associated antiviral responses and innate immunity (fig. S3D). IFN-β treatment strongly increased the number of differentially expressed genes in Gt/Gt mice at 96 hours compared to 48 hours and recovered a minor fraction of TBI-induced genes lost in Gt/Gt mice at either 48 hours (200 of 607) or 96 hours (302 of 722) (Fig. 3F and fig. S3C). IFN-β–rescued genes were highly enriched in antiviral response and translation at 48 hours and progressed to infection and inflammation at 96 hours, dominated by innate mucosal immunity (50%), macrophage chemotaxis (10%), and IFNγ (40%) (Fig. 3F and fig. S3E). In addition, IFN-β treatment at 48 hours strongly improved survival of Gt/Gt mice after 14 Gy ABI and enhanced weight gain during the
recovery phase (after days 5 and 6) (Fig. 3, G and H). These data support the idea that STING-dependent and temporal IFN-β production is required for acute crypt inflammation and regeneration.

**Epithelial IFN-β production initiates intestinal regeneration after radiation injury**

IFN-β can be produced by nearly all cell types (35), while ISC activation is believed to be regulated by niche signals often within the distance of a few cells (1, 6). We used IFN-β–yellow fluorescent protein (YFP) reporter mice to monitor IFN-β production in the intestine in response to radiation. Baseline IFN-β–YFP level was low in WT mice and strongly induced in the villi and some collapsed crypts at 48 hours after 15 TBI. Using serial sections, we found that IFN-β–YFP is highly induced in macrophages (CD68+) but not neutrophils, which remained mostly in the villi with a minor fraction moving toward collapsed crypts (Fig. 4A). Intense IFN-β–YFP in the crypts was completely...
negative for CD68 and costained with radioresistant Paneth cells (MMP7+ and TUNEL−) and some crypt cells (MMP7− and TUNEL+) (Fig. 4, B to D). IFN-β production in the crypts gradually increased from 24 to 72 hours, followed by a quick drop at 96 hours in WT mice (Fig. 4C), paralleling the kinetics of crypt loss and mitotic death (Fig. 1, A and B). Gt/Gt mice showed virtual absence of IFN-β–YFP staining or induction by TBI in the intestine, in contrast to normal induction of crypt cell death (Figs. 2A and 4C). IFN-β–YFP reporter and apoptosis were also induced in colonic crypts after 15 Gy TBI at 48 hours, albeit at much lower levels compared to the small intestine (fig. S4, B and C). We detected no regenerated crypt at 96 hours in the colon of either WT or Gt/Gt mice with little change in proliferation or crypt numbers, which is in sharp contrast to marked changes in the small intestine (fig. S4D). These findings are consistent with the well-known radiation resistance and lacking acute regeneration in mouse colon after TBI (10, 36), likely attributable to limited mitotic death or IFN-β production.

To test the role of non-BM STING and IFN-β production in crypt regeneration, we made BM chimeras in lethally irradiated WT and Gt/Gt mice (10 Gy TBI) (16). BM chimeras after 8 weeks of recovery were
then subjected to 15 Gy TBI and analyzed for acute crypt regeneration and inflammation. *Gr/Gr* hosts phenocopied *Gr/Gr* mice in defective crypt regeneration, inflammation (CD45⁺), and proliferation (p-H3), which were not corrected by WT BM (Fig. 4, E to H, and fig. S4, E and F). In contrast, *Gr/Gr* BM did not impair crypt regeneration in WT hosts and only slightly reduced intestinal proliferation and inflammation at 48 and 96 hours. Baseline crypt proliferation and peri-cryptal CD45 counts were comparable across chimeric groups, while a minor reduction in crypt counts was observed in *Gr/Gr* hosts (less than 3%) but irrespective of the BM genotype (Fig. 4, G and H, and fig. S4, E to G). *Gr/Gr* BM did not compromise the survival of WT hosts after 14.5 Gy ABI (fig. S4H). Together with findings in IFNAR1 KO, CCR2 KO, and cGAS KO mice, these data support that STING-dependent epithelial IFN-β production is the major mechanism underlying reparative inflammation in the gut after radiation injury.

**STING/IFN-β promotes intestinal recovery through compensatory proliferation and local inflammation**

Recent studies suggest that intestinal recovery in the first 1 and 2 weeks is critical for animal recovery from TBI (9 to 10 Gy)–induced lethal hematopoietic injury within 30 days (37–40). WT mice showed gradual crypt loss (2 to 30%) from days 4 to 12 after 9.1 Gy sublethal TBI, which was accompanied by increased proliferation and peri-cryptal macrophages (CD68⁺), and decreased DNA damage (H2AX) and cell death (TUNEL) over time (Fig. 5, A to F, and fig. S5). Increased or compensatory proliferation was evident on day 4 and was about 50% more than that on day 0 (p-H3⁺) (Fig. 5D and fig. S5B). In contrast, *Gr/Gr* mice showed significantly reduced survival after 9.1 Gy TBI (Fig. 5A), with more severe loss of crypts on days 8 and 12, and nearly complete loss of compensatory proliferation and macrophage recruitment (CD68⁺) as early as day 4 (Fig. 5, B to E).

**Fig. 5. STING/IFN-β drives reparative inflammation after TBI.** Survival and the small intestine of mice were analyzed after 9.1 to 9.5 Gy TBI at indicated times (0 to 12 days). Vehicle or IFN-β (25 µg/kg) was given 48 hours after TBI. (A) Survival of WT and *Gr/Gr* mice. Log-rank test. (B) Representative H&E and p-H3 IF in the crypts. Scale bar, 100 µm. (C to F) Quantitation of crypt counts, p-H3⁺ crypt cells, and peri-cryptal CD68⁺ cells per cross section and yH2AX⁺ cells per crypt. (G) Representative IFN-β–YFP and CD68 IF in serial sections of WT mice. Scale bar, 100 µm. Insets showed enlarged CD68⁺/IFN⁺ crypt cells. Scale bar, 50 µm. (H) Quantitation of IFN-β (YFP) expression intensity in the crypts of WT mice. N = 2 per group. 5 cross sections per mouse. (I) Survival of WT mice after 9.1, 9.25, and 9.5 Gy TBI with or without IFN-β. Log-rank test, n = 15 per group. (C) to (F) and (H) values are means + SEM for n = 3 mice per group, six to eight full cross sections per mouse or as specified. *P < 0.05, ++P < 0.01, +++P < 0.001 (one-way ANOVA with Tukey post hoc test).
and fig. S5, A to D). *Gt/Gt* mice also showed a delayed increase in DNA damage and cell death on days 8 and 12, not on days 1 and 4, indicative of defective DNA damage removal but not induction (Fig. 5F and fig. S5, E to H).

Using the IFN-β-YFP reporter and serial sections, we confirmed gradual increase of IFNβ⁺ CD68⁺ crypt cells (0.5- to 6-fold) from days 4 to 12 after 9.1 Gy TBI only in WT and not in *Gt/Gt* mice (Fig. 5, G and H), with a minor fraction of CD68⁺ IFNβ⁺ cells moving from the villi toward or into the crypts (Fig. 5G). In comparison, we observed the massively increased proliferation 1400% (or 14-fold) from days 3 and 4 after 15 Gy TBI, following a sharp rise of IFN-β-YFP production (more than 10-fold) and crypt loss (more than 90%) from days 2 and 3, which could not sustain because of lethality on days 6 to 8 (Figs. 1, G and H, and 4C). The single IFN-β treatment given 48 hours after TBI fully corrected impaired survival, crypt recovery, and other deficiencies in *Gt/Gt* mice to levels comparable to WT mice (Fig. 5, A to F, and fig. S5). IFN-β treatment prevented crypt loss in WT mice, enhanced proliferation (~30% on day 4) and CD68⁺ cell recruitment (~40% on day 4), and reduced DNA damage (γH2AX) (~50% on day 12) (Fig. 5, C to F, and fig. S5, B to F). Of note, significant γH2AX reduction with IFN-β treatment was observed in WT and *Gt/Gt* mice on day 12, not earlier, after the increase in proliferation and CD68⁺ cell infiltration, further supporting accelerated DNA damage removal and repair rather than induction. IFN-β treatment also significantly improved the survival of WT mice after lethal and sublethal TBI, from 10 to 70% (9.1 Gy), 0 to 30%, and 20% (9.25 and 9.5 Gy) by day 35 (Fig. 5I). These data strongly support the idea that STING-dependent local IFN-β production is calibrated to continuously respond to dose-dependent DNA damage and cell loss upon cell division, which specifically mounts compensatory proliferation and inflammation to facilitate intestinal recovery. IFN-β is therefore both necessary and sufficient to enhance intestinal recovery.

**STING/IFN-β improves Lgr5⁺ stem cell regeneration after TBI**

Genome integrity plays a key role in stem cell recovery and longevity (13, 41). We used Lgr5 lineage marking and tracing mice (4) to analyze the effects of IFN-β on radiation-induced ISC recovery and regeneration. TBI (9.1 Gy) rapidly depleted Lgr5⁺ cells by day 4 [more than 90%, mostly lower crypt base columnar cells (CBCs)], which recovered to 20 to 30% of pre-TBI levels on days 8 and 12. IFN-β treatment significantly increased the number of Lgr5⁺ cells (by ~100%) on days 8 and 12 to an average of four (versus two) Lgr5⁺ cells per crypt (Fig. 6, A to C). Lgr5-driven regeneration was significantly increased throughout the small intestine and colon, as evidenced by X-galactosidase (X-Gal)-stained intestinal whole mounts and entirely blue crypts in the sections (50 to 100%) (Fig. 6, D to F, and fig. S6, A to D). IFN-β treatment also increased Lgr5 lineage, crypt regeneration, and proliferation on day 4 after 15 Gy TBI (Fig. 6G and fig. S6, E to H) and significantly improved the survival of mice from 6.6 to 53% after 16 Gy ABL (Fig. 6H). The efficiency of Lgr5-driven regeneration after TBI was drastically lower in the colon compared to the small intestine and still enhanced by IFN-β, likely reflecting limited IFN-β production (fig. S4, B to D) and labeling efficiency in the colon (1, 10). IFN-β given to unirradiated mice increased crypt proliferation but not crypt numbers after 48 hours (fig. S6I). These data support the idea that injury-inducible IFN-β plays a key role in intestinal regeneration and animal recovery after radiation, which can be effectively boosted by exogenous IFN-β (Fig. 6I).

**DISCUSSION**

The rapid renewal of intestinal epithelium, structural compartmentalization of stem cells and niche, and uniform, dose-dependent DNA damage induced by radiation provide a powerful model to mechanistically dissect injury-induced regeneration. Wnt, BMP, EGF, and Notch are niche signals required for ISC maintenance and regeneration during homeostasis and following injury (1, 5, 6). Previous studies established that the rapid activation of the p53 pathway removed DNA damage through apoptosis or DNA repair, which suppresses delayed mitotic cell death caused by sustained DNA damage upon replication (14, 19, 20). This study demonstrates that the delayed mitotic death upon DNA replication selectively activates cGAS-STING–dependent IFN-β production in the niche to promote ISC and intestinal recovery through compensatory proliferation and acute inflammation. Rapid IFN-β production therefore explains the robust regeneration observed in the small intestine, not colon, in mice after high-dose TBI, which is the basis of the crypt microcolony assay widely used since the 1970s (42, 43). The lack of rescue by IFNα on acute crypt inflammation and regeneration in *Gt/Gt* mice might reflect the differences of IFNα and IFN-β in receptor binding affinity, priming of type 1 IFN response, and controlling cell type–specific targets (35). Therefore, we propose that STING/IFN-β–driven acute crypt proliferation and inflammation is reparative following DNA damage and works with p53–dependent mechanisms to preserve the genome integrity of the ISC pool over multiple cell divisions (Fig. 6I). IFN-β is required for chemokine expression in the intestinal epithelial cells to recruit innate cells (i.e., CCR2⁺ cells) and likely the proliferation of Lgr5⁺ stem cells. These two events are likely linked to facilitate the removal of DNA damage and defective cells produced by activated ISCs and progenitors. This model is consistent with an emerging role of mobile immune niche in tissue repair (31, 44) and further supports Paneth cells as an origin of intestinal inflammation (45) signaling between ISCs and immune cells during regeneration.

Compensatory proliferation is commonly observed after injury (1). Our data show that STING-dependent and inducible IFN-β production underlies compensatory proliferation in the gut upon radiation injury, while dispensable under hemostasis. This response is dose dependent and accounts for a minor fraction of the overall proliferation (~30%) on day 4 after 9.1 Gy TBI, which persists with the gradual rise in epithelial IFN-β (IFN-β-YFP) and Lgr5⁺ cell recovery through day 12. In contrast, IFN-β is the primary driver of the acute crypt regeneration on day 4 after 15 Gy TBI, following peak production between 48 and 72 hours upon rapid crypt and cell loss to massively increase proliferation (1400%). Therefore, IFN-β given at 48 hours, not 24 hours, rescued intestinal regenerative defects caused by STING deficiency. Our findings are consistent with an injury-inducible role of STING/IFN-β (46, 47) or JAK/STAT (Janus kinase/signal transducer and activator of transcription) (48) in intestinal recovery in mice and flies. Previous work has established that pharmacologic induction of quiescence before, but not after, irradiation potently protected hematopoietic stem cells (49) and ISC (19) against radiation injury through p53–dependent survival and DNA repair (13, 41, 50). Together, these findings establish a powerful role of proliferation kinetics of tissue stem cells in DNA damage repair and recovery through cell-intrinsic programs and niche cross-talk and the potential of cell-cycle modulators in ISC preservation (13, 41, 50).

The intestinal epithelium and barrier is under the highest proliferation demand in an adult animal and interacts extensively with the
immune system (~70%) and gut microbiota to regulate host physiology (51). Barrier defects therefore have a strong systemic effect and can impair injury recovery if prolonged (51, 52). Radiation-induced acute intestinal injury can be lethal and lead to late effects in survivors characterized by barrier defects, elevated systemic inflammation, and multiple organ impairment (9, 12, 26), and currently has no FDA-approved treatment (8, 9). Persistent DNA damage causes chronic STING/IFN-β activation and inflammation that are associated with increased risk of cancer and stem cell exhaustion (32, 33, 53). TBI induces patchy IFN-β production and local inflammation compared to uniform cell death and DNA damage in the crypts, suggesting limiting or tightly regulated production of repair factors. This raises an interesting possibility that a transient IFN-β boost could mimic a more severe injury, accelerate barrier recovery, and, in turn, help prevent chronic inflammation and STING activation (Fig. 6I). IFN-β has long been used to manage patients with multiple sclerosis (54). It will be interesting to test whether IFN-β can be used to treat radiation-induced acute intestinal injury and prevent late effects (Fig. 6I) in the setting of repeated radiotherapy and single accidental exposure. It should also be noted that on the basis of the critical interaction of the GI with BM-derived cells, BM STING and systemic IFN-β are likely important for animal recovery when BM injury is substantial.
Inflammation and tissue repair are coordinated through highly complex and dynamic immune subpopulations, cytokines, and chemokines (29, 30). Our study certainly supports this notion and calls for a better mechanistic understanding of reparative inflammation in the gut. For example, how do radio-resistant Paneth cells sense DNA damage from dying ISCs? Do Paneth cells make additional cGAS/STING-dependent factors that act differentially on ISCs and immune cells? Does Ccl2/Cxcl2-dependent local inflammation deliver immune-derived ISC growth factors in addition to dead cell clearance (Fig. 6I)? It will also be interesting to test whether the manipulation of cell death or proliferation favors ISC regeneration by influencing immune population kinetics and DNA repair pathway choice (19, 41).

Our current study focuses on acute ISC regeneration after DNA damage, while improving ISC regeneration during chronic inflammation likely requires different strategies with proinflammatory signals and cells already elevated (29, 30). Future studies using cell type and conditional KOs will help better define these complex interactions during ISC injury and regeneration and identify potential targets for therapeutic intervention.

In summary, our study demonstrates a critical role of STING-dependent production of IFN-β in the niche to promote ISC recovery and regeneration after radiation injury through compensatory proliferation and acute inflammation. IFN-β might be a useful agent to treat radiation-induced acute intestinal injury and help improve long-term barrier function in the setting of radiotherapy or accidental exposure. Our findings also support a broader role of the cytokine niche and Paneth cells in regulating ISC regeneration.

MATERIALS AND METHODS

Mice and treatment

The procedures for all animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. C57BL/6J (WT), C57BL/6J- Tmem173β (βGt/Gt) (34), and various strains IFNAR1 KO (55), CCR2 KO (56), cGAS KO (57), TNFA KO (58), TNFR1 KO (59), IFNB-YFP (60), Lgr5-GFP (Lgr5-EGFP-IRES-creERT2) (4), and B6.129S4-Gt(ROSA)26Sortm1Sor/J (ROSA B/B) have been used. All strains are in or have been backcrossed with the C57BL/6 background for more than 10 generations (F10). Mice were housed in microisolator cages in a room illuminated from 07:00 to 19:00 hours (12:12-hour light-dark cycle), with access to water and chow ad libitum. TBI was administered at a rate of 320 cGy/min in a 137Cs irradiator (Mark I, JL Shepherd and Associates). ABI was performed on the TrueBeam system (Varian Medical Systems) at a rate of 300 MU/min (~275 cGy/min, x-ray). IFN-β (PropepTech, Rocky Hill, NJ) was administered to mice by intraperitoneal injection at 25 μg/kg. INFα (Sigma-Aldrich, St. Louis, MO) was administered to mice by intraperitoneal injection at 25 or 100 μg/kg. Some mice were injected with bromo-deoxy-uridine (BrdU) (100 mg/kg; Sigma-Aldrich) 2 hours before sacrifice. Details on strains, genotyping, and BM transplantation are found in the Supplemental Materials.

BM transplantation

Seven- to 9-week-old female WT and STING mice were irradiated with 10 Gy TBI the day before transplantation as previously described (16). The following morning, ~1 million cells (whole BM) from male WT or STING donor mice were injected via the tail vein into each recipient mouse. Transplanted mice were allowed 8 weeks of recovery for full BM engraftment and then irradiated again as indicated.

Tissue processing and histological analysis

Immediately after sacrifice, the proximal jejunum was removed, carefully rinsed with ice-cold saline, and cut into six to eight 1-cm sections that were bundled together with 3M Micropore tape. Tissue bundles were fixed overnight in 10% neutral-buffered formalin (Sigma-Aldrich) before paraffin embedding. Tissue sections (5 μm) were deparaffinized and rehydrated through graded ethanols. Histological analysis was performed by hematoxylin and eosin (H&E) staining. Protocols on tissue preparation, crypt microcolony assay, various staining (14, 16, 40), and Lgr5 lineage tracing (19) have been described with more details found in the Supplemental Materials.

Quantitative real-time PCR

RNA was prepared as previously described (20). Briefly, fresh epithelial scraping from 10 cm of jejunum was washed in cold phosphate-buffered saline (PBS), resuspended in 700 μl of RNA lysis buffer, and homogenized in a Dounce homogenizer, and RNA was isolated using the Quick-RNA MiniPrep Kit (Zymo Research, Irvine, CA) according to the manufacturer’s instructions. cDNA was generated from 2 μg of total RNA pooled from two to three mice using Superscript III reverse transcriptase (Invitrogen) and random primers. Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase levels. Details on mouse primers are found in table S3.

Transcriptomics and bioinformatics

RNA-seq analysis

RNA was prepared as previously described with minor modifications (20). Briefly, fresh epithelial scraping from 10 cm of jejunum was washed in cold PBS and resuspended in 700 μl of TRizol reagent (catalog no. 15596018, Thermo Fisher Scientific), and total RNA was isolated according to the manufacturer’s instructions. RNA was quantitated and quality controlled using Bioanalyzer. Bulk RNA-seq (20M reads per sample, 3 μg per sample provided) was performed by Novogene (Sacramento, CA) using Illumina HiSeq platform, and a combination of software was used to map the reads to reference genome and obtain gene expression levels in Fragments Per Kilobase Million (FPKM).

Differential expression analysis

For DESeq2 with biological replicates (2), differential expression analysis between two conditions/groups was performed using the DESeq2 R package (2_1.6.3). The resulting gene expression levels in Fragments Per Kilobase Million (FPKM). For DESeq2 with biological replicates (2), differential expression analysis between two conditions/groups was performed using the DESeq2 R package (2_1.6.3). The resulting gene expression levels in Fragments Per Kilobase Million (FPKM).

Gene Set Enrichment Analysis

GSEA was performed with Molecular Signature Database (MsigDB. v7.1) (62, 63). The input was differential genes from RNA-seq analysis (P < 0.05). “Signal-to-Noise” ratio statistic was used to rank the genes per their correlation with either irradiated (48 or 96 hours) or control (unirradiated) (blue). The green curve is the enrichment score (ES) curve, which is the running sum of the weighted ES obtained from the GSEA software 4.0.3 (https://gsea-msigdb.org/gsea/msigdb/index.jsp). The normalized ES and the corresponding P value are reported on the plot. The top three (significant) pathways were shown.
GO enrichment analysis of differentially expressed genes

GO enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected $P$ value less than 0.05 were considered significantly enriched by differentially expressed genes. The input is the genes of interest and the output is the enriched pathways with the significant $P$ values ($\leq 0.05$). The display was limited to the top 10 nonoverlapping pathways with the most significant $P$ values and those containing 4 to 400 genes (for specificity). ClueGO plugin in Cytoscape (http://apps.cytoscape.org/apps/cluego) was additionally used to visualize GO in immune-related pathways, and the significance of the terms and groups is automatically calculated with kappa statistics (64).

Data access

RNA-seq data with raw read counts of all called genes and differentially expressed genes (DEG_all, DEG_up, and DEG_down) in paired samples and associated pathway analysis have been deposited at DRYAD. This dataset has been assigned a unique identifier or DOI (doi:10.5061/dryad.pvmvdmn2) and will become public after the publication of the manuscript.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism software (VIII, GraphPad Software Inc., La Jolla, CA). Multiple comparisons were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test, whereas those between two groups were made by two-tailed, unpaired $t$ test. Survival data were analyzed by log-rank test. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($P < 0.05$). The means ± SEM are displayed in the figures. Sample size was determined using a combination of published work and power calculations. For ANOVA, we have computed the power for a test of interaction in a two-way factorial design applied by constructing mixed linear growth models to calculate the needed sample size. We estimated that usually 5 to 10 per group, with 3 mice per group and six to eight cross sections per mouse, will provide 80% power to detect a standardized interaction of 1.5 SDs as previously described (19, 20).

SUPPLEMENTARY MATERIALS

Supplemental material for this article is available at https://science.org/doi/10.1126/sciadv.abi5253

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

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