Paradoxical role of the proto-oncogene Axl and Mer receptor tyrosine kinases in colon cancer

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The receptor tyrosine kinases Axl and Mer, belonging to the Tyro3, Axl and Mer (TAM) receptor family, are expressed in a number of tumor cells and have well-characterized oncogenic roles. The therapeutic targeting of these kinases is considered an anticancer strategy, and various inhibitors are currently under development. At the same time, Axl and Mer are expressed in dendritic cells and macrophages and have an essential function in limiting inflammation. Inflammation is an enabling characteristic of multiple cancer hallmarks. These contrasting oncogenic and anti-inflammatory functions of Axl and Mer posit a potential paradox in terms of anticancer therapy. Here we demonstrate that azoxymethane (AOM) and dextran sulfate sodium (DSS)-induced inflammation-associated cancer is exacerbated in mice lacking Axl and Mer. Ablation of Axl and Mer signaling is associated with increased production of proinflammatory cytokines and failure to clear apoptotic neutrophils in the intestinal lamina propria, thereby favoring a tumor-promoting environment. Interestingly, loss of these genes in the hematopoietic compartment is not associated with increased colitis. Axl and Mer are expressed in radioresistant intestinal macrophages, and the loss of these genes is associated with an increased inflammatory signature in this compartment. Our results raise the possibility of potential adverse effects of systemic anticancer therapies with Axl and Mer inhibitors, and underscore the importance of understanding their tissue and cell type-specific functions in cancer.

The proto-oncogenes *AXL* and *MER* were first cloned from chronic myelogenous and lymphoblastic leukemia cells (1–3). Increased expression of AXL has been reported in lung, breast, ovarian, gastric, pancreatic, and prostate cancers; leukemias and lymphomas; melanoma; and glioblastoma multiforme (4, 5). Increased MER expression is associated with leukemias, lymphomas, melanoma, rhabdomyosarcomas, and gastric and prostate cancers (4–6). AXL and MER expression also has been directly correlated with poor prognosis in cancer (6–9). Moreover, ectopic expression of AXL has been shown to confer resistance to EGF receptor therapy in lung cancer (10, 11). Multiple studies have demonstrated AXL and MER function in survival, invasion, and metastasis in a variety of tumors (12–15); thus, attention has focused on the pharmacologic targeting of AXL and MER in cancer. Axl and Mer share structural homology in the kinase domain with other tyrosine kinases, including conserved molecular interactions with ATP; however, several unique features of the active site allow for selective inhibition (16), and small-molecule inhibitors as well as biologics are in preclinical development (6, 16–19).

Axl and Mer are associated with another distinct feature of cancer—inflammation. Tumor-promoting inflammation has been described as an enabling characteristic that promotes the acquisition of cancer hallmarks and orchestrates tumor progression (20). Chronic inflammation increases the risk of colorectal cancer (21, 22). The relative risk of being diagnosed with colorectal cancer in the United States was ~0.05% in 2007 (www.cdc.gov). This risk is increased dramatically in patients with inflammatory bowel disease; for example, ulcerative colitis increases the risk by ~20-fold (23), and the reported risk in patients with pancolitis is 30% after 35 years of disease (24).

Recently, a direct role of innate immune cells and cytokines in colorectal cancer has been demonstrated using mouse models (25). One of the main physiological functions of Axl and Mer is in the inhibition of the innate immune response (26). Axl and Mer are expressed in dendritic cells and macrophages, and their activation limits toll-like receptor and cytokine receptor signaling (26–29); consequently, Axl and Mer should inhibit tumor-promoting inflammation and reduce the risk of colorectal cancer. In light of the oncogenic and anti-inflammatory functions of Axl and Mer, we sought to test the role of these receptor tyrosine kinases (RTKs) in inflammation-driven cancer.

Using a mouse model of AOM/DSS-induced colorectal cancer (30, 31), combined with genetic ablation of Axl and Mer, we found that Axl and Mer signaling prevents colitis and significantly reduces the number and size of colorectal adenomas. We detected a significant increase in the number of apoptotic neutrophils and in the production of proinflammatory cytokines in the lamina propria of $Axl^{-/-}Mer^{-/-}$ mice in the context of DSS-induced colitis. Axl and Mer are expressed primarily in the hematopoietic compartment (32). Surprisingly, the loss of Axl and Mer function in radiosensitive hematopoietic cells is not associated with increased colonic inflammation. Axl and Mer are expressed in a radioresistant population of lamina propria macrophages upon inflammation, and the absence of Axl and Mer results in an increased proinflammatory profile in lamina propria macrophages. $Axl^{-/-}Mer^{-/-}$ macrophages show increased production of inflammatory cytokines and reduced expression of genes associated with alternative activation—a distinct macrophage phenotype that decreases inflammation and promotes tissue repair (33). Our results demonstrate that although Axl and *Mer* can function as oncogenes in a number of cancers, these genes have a protective role against the development of colitis-associated cancer. Our findings underscore the potential adverse effects of systemic inhibition of Axl and Mer and highlight the importance of developing therapeutic strategies to spare these RTKs in macrophage populations relevant to the regulation of local inflammation and tissue homeostasis.

Results

Genetic Ablation of Axl and Mer Promotes Colitis-Associated Colon Cancer. We compared the development of colitis-associated colorectal cancer in WT and $Axl^{-/-}Mer^{-/-}$ mice. Colorectal cancer was induced by the administration of a single i.p. dose of the procarcinogen AOM, followed by three cycles of DSS in the drinking water separated by treatment-free intervals (Fig. 1*A*). Endoscopic analysis revealed that the $Axl^{-/-}Mer^{-/-}$ mice

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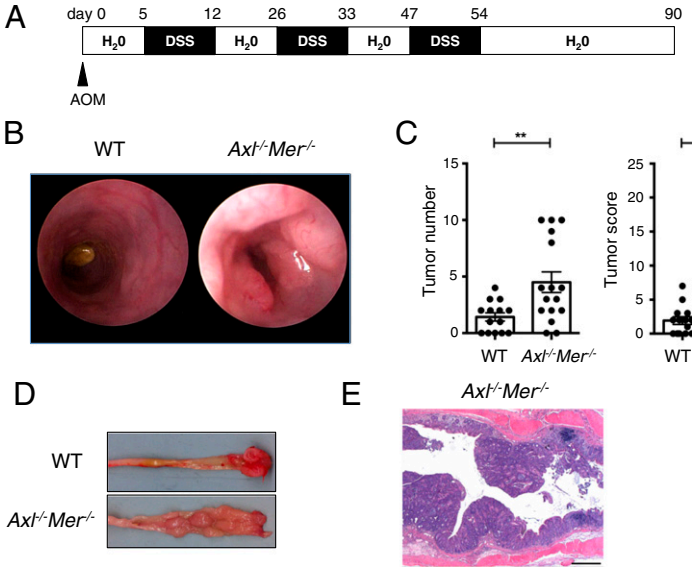

Fig. 1. Genetic ablation of Axl and Mer leads to increased susceptibility to AOM/DSS-induced colon cancer. (*A*) Schematic representation of AOM/DSS-induced colon cancer treatment. In brief, WT and $Axl^{-/-}Mer^{-/-}$ mice were injected with the DNA-methylating agent AOM and were subsequently treated with the indicated cycles of DSS. (*B*) Representative colonoscopy images after 90 d of treatment in WT and $Axl^{-/-}Mer^{-/-}$ mice. (*C*) Tumor number and tumor score in WT and $Axl^{-/-}Mer^{-/-}$ mice after 90 d of treatment as determined by colonoscopy. (*D*) Representative luminal views of WT and $Axl^{-/-}Mer^{-/-}$ colons treated as indicated in *A*. Tumors in the rectum and distal colon of $Axl^{-/-}Mer^{-/-}$ can be seen. (*E*) Representative image of colon sections stained with H&E, showing adenomas in $Axl^{-/-}Mer^{-/-}$ mice. (Scale bar: 500 μm.) Data are presented as representative individual samples or as the mean of 14–16 independent samples per group. $^{**}P < 0.01$.

developed significantly more polyps compared with WT mice (Fig. 1*B*). Moreover, the polyps were more numerous and significantly larger in the $Axl^{-/-}Mer^{-/-}$ mice, contributing to a higher tumor score in these mice (Fig. 1*C*). Dissection of the gut at the end of treatment confirmed the presence of macroscopic polyps in the distal colon and rectum of $Axl^{-/-}Mer^{-/-}$ mice (Fig. 1*D*). Histopathological analyses revealed the presence of adenomas in these mice (Fig. 1*E*).

Distinct mechanisms accounting for an increased incidence of tumors have been reported in this model. Although cancer is commonly caused by colitis, inflammation-independent mechanisms have been reported (34). Thus, we directly tested whether the loss of Axl and Mer results in increased colitis. WT and $Axl^{-/-}Mer^{-/-}$ mice were acutely treated with DSS in the drinking water. The DSS dose was adjusted to induce minimal inflammation in WT mice as judged by colonoscopy (Fig. 2*B*). $Axl^{-/-}Mer^{-/-}$ mice showed a significant reduction in body weight after DSS treatment compared with WT mice (Fig. 2*A*). Colonoscopy in the $Axl^{-/-}Mer^{-/-}$ mice revealed enhanced colitis as demonstrated by increased granularity, loss of apparent vasculature, decreased translucency, and looser stool consistency (Fig. 2*B*). Consistent with increased inflammation after DSS administration, colon length was significantly reduced in the $Axl^{-/-}Mer^{-/-}$ mice (Fig. 2*C*). Histopathological analysis following established methods (35) confirmed the increased severity of colitis (i.e., mucosal ulceration, crypt loss, crypt hyperplasia, inflammatory cell infiltration, and edema) in the $Axl^{-/-}Mer^{-/-}$ mice compared with WT mice after DSS administration (Fig. 2*D*). Colons from untreated $Axl^{-/-}Mer^{-/-}$ mice were unremarkable and within normal limits by both colonoscopy and histopathological analysis (Fig. 2 *B* and *D*). Taken together, these results demonstrate that the increased severity of colitis and tissue injury in the $Axl^{-/-}Mer^{-/-}$ mice is associated with an increased risk of colorectal cancer.

Axl- and Mer-Dependent Phagocytosis of Apoptotic Neutrophils and Inhibition of Cytokine Production Prevent Colitis. We next evaluated additional features of intestinal inflammation in WT and $Axl^{-/-}Mer^{-/-}$ mice. The percentages of $Ly6G^+$ neutrophils and $F4/80^+CD11b^+$

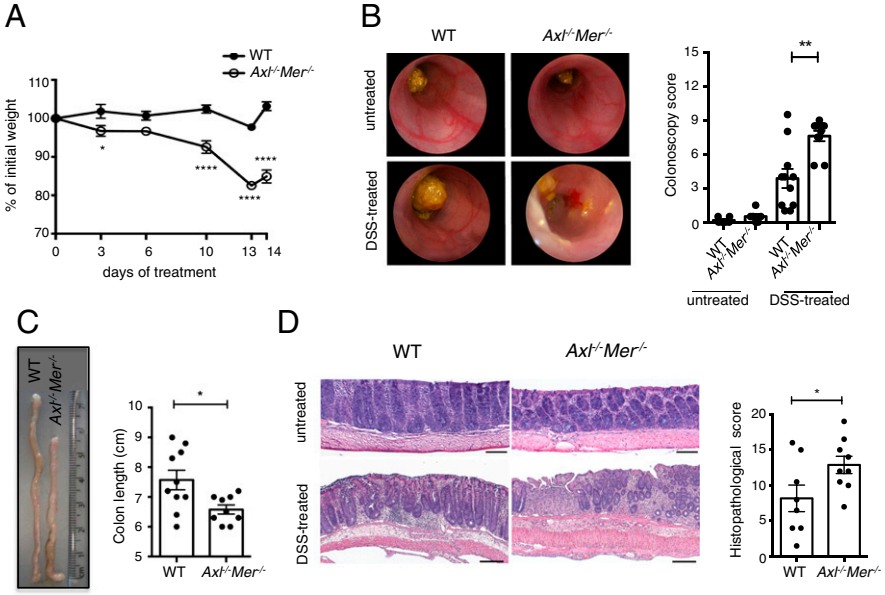

Fig. 2. Genetic ablation of Axl and Mer leads to increased susceptibility to DSS-induced colitis. (*A* and *B*) Body weight (*A*) and representative colonoscopy images and colonoscopy scores (*B*) in WT and $Axl^{-/-}Mer^{-/-}$ mice in the steady-state condition (untreated) and after DSS treatment (DSS-treated). (*C*) Representative images and colon length in WT and $Axl^{-/-}Mer^{-/-}$ mice after DSS treatment. (*D*) Representative images of H&E-stained colon sections showing normal colon morphology in untreated mice and increased colitis in $Axl^{-/-}Mer^{-/-}$ mice compared with WT after DSS treatment. Histological scoring of colitis is reported. (Scale bars: 500 μm.) Data are representative images or mean ± SEM of at least six independent samples per group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$.

macrophages were not altered in WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice at steady state (Fig. 3*A* and Fig. S1). The induction of colitis was associated with a significant increase in Ly6G^{+} cells or neutrophils in both WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice (Fig. 3*A*). Interestingly, immunohistochemical and FACS analyses revealed a significant increase in the number of TUNEL^{+}/Ly6G^{+} apoptotic neutrophils in the lamina propria of *Axl*$^{-/-}$*Mer*$^{-/-}$ mice compared with WT mice (Fig. 3 *B* and *C*). Clearance of apoptotic neutrophils is essential for the resolution of inflammation and tissue repair (36).

Tyro3, Axl and Mer (TAM) receptors function in the removal of apoptotic membranes of photoreceptors and apoptotic germ cells in the testis (32); thus, we directly compared the ability of bone marrow (BM)-derived macrophages from WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice to phagocytose apoptotic neutrophils in in vitro assays. Apoptotic neutrophils were labeled with CellTracker dye and cocultured with BM-derived macrophages at a ratio of 5:1. *Axl*$^{-/-}$*Mer*$^{-/-}$ macrophages demonstrated a significant deficit in the ability to phagocytose apoptotic neutrophils compared with WT macrophages (Fig. 3*D*). The loss of Axl and Mer also was associated with increased inflammatory cytokine production by lamina propria leukocytes. CD45^{+} cells were sorted from the lamina propria of WT or *Axl*$^{-/-}$*Mer*$^{-/-}$ mice after 7 d of DSS treatment. Quantitative real-time PCR (qPCR) analyses demonstrated an increase in the expression of IFN-γ and TNF-α in *Axl*$^{-/-}$*Mer*$^{-/-}$ leukocytes (Fig. 3*E*). Taken together, our results indicate that Axl and Mer function in limiting inflammation in the intestinal lamina propria through the phagocytosis of apoptotic neutrophils and regulation of proinflammatory cytokine production.

Hematopoietic Function of Axl and Mer Does Not Confer Increased Susceptibility to Colitis. Because Axl and Mer are expressed primarily in BM-derived dendritic cells and macrophages (32), we tested whether the loss of Axl and Mer in the hematopoietic compartment accounted for the increased susceptibility to induced colitis. For this, CD45.1 C57B6 WT mice were lethally irradiated and their hematopoietic compartments reconstituted with CD45.2 WT or *Axl*$^{-/-}$*Mer*$^{-/-}$ hematopoietic progenitors (Fig. 4*A*). Efficient reconstitution of the hematopoietic compartment was confirmed by the analysis of CD45.1^{+} and CD45.2^{+} leukocytes in the peripheral blood at 2 mo after BM transfer (Fig. 4*B*). Treatment with DSS did not cause enhanced colitis in

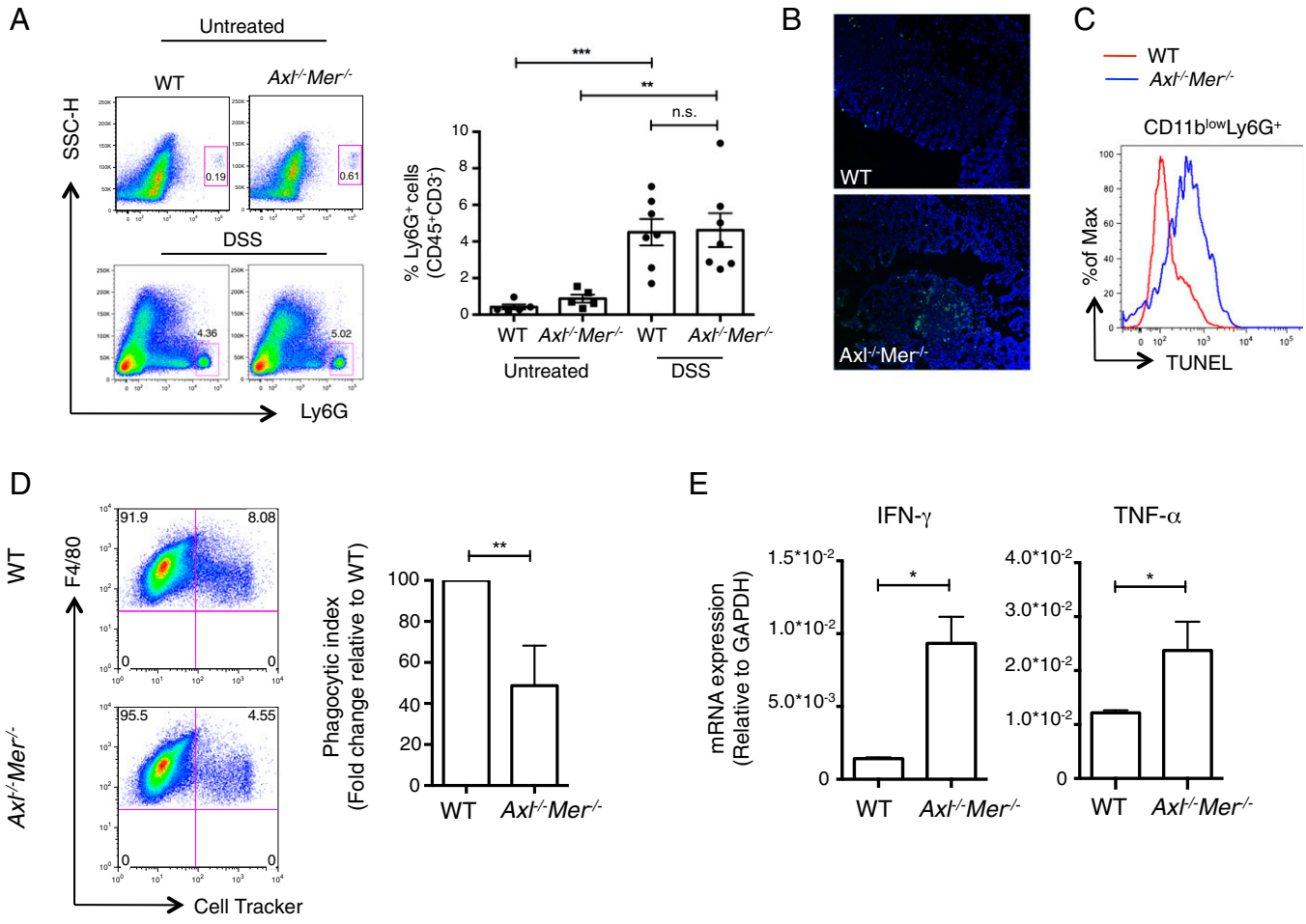

Fig. 3. Increased number of apoptotic neutrophils and production of proinflammatory cytokines in the lamina propria of *Axl*$^{-/-}$*Mer*$^{-/-}$ mice. (*A*) Percentage of neutrophils (CD45^{+}CD3^{-}Ly6G^{+}) in the lamina propria of WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ before (untreated) and after 7 d of DSS treatment (DSS) as detected by FACS analysis. Representative FACS plots and independent data are shown. (*B*) Representative immunofluorescence staining for TUNEL in colon sections from WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice after 7 d of DSS treatment. Nuclei are counterstained with DAPI. (Scale bar: 75 μm.) (*C*) TUNEL staining in CD11b^{low}Ly6G^{+} cells in the lamina propria leukocytes of WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice as detected by FACS analysis. (*D*) Rate of phagocytosis of apoptotic neutrophils, labeled with CellTracker and incubated with WT or *Axl*$^{-/-}$*Mer*$^{-/-}$ BM macrophages for 60 min. FACS plots show WT or *Axl*$^{-/-}$*Mer*$^{-/-}$ F4/80^{+} BM macrophages that have engulfed CellTracker-labeled apoptotic neutrophils. The phagocytic index normalized to WT macrophages is shown on the right. (*E*) Expression of IFN-γ and TNF-α in the lamina propria leukocytes from WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice after 7 d of DSS treatment as detected by qPCR. Data are presented as representative images or as mean ± SEM of at least five samples per group. n.s., nonsignificant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

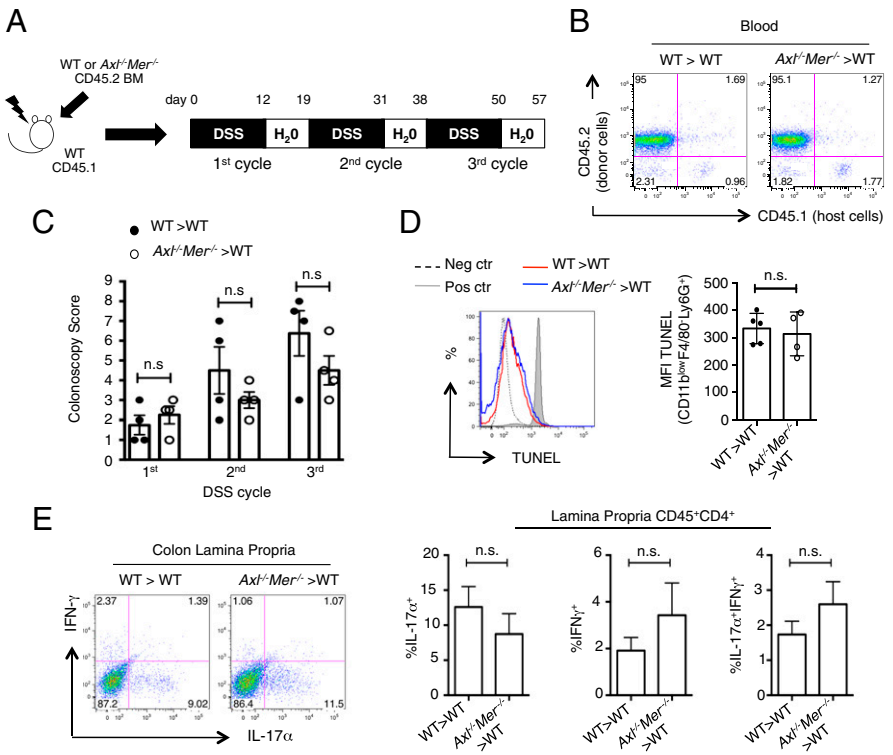

Fig. 4. Loss of Axl and Mer in the hematopoietic compartment does not confer susceptibility to colitis. (*A*) Schematic representation of BM chimeras and chronic DSS treatment. (*B*) FACS analysis of chimerism of blood leukocytes in BM chimeras as indicated in *A*. (*C*) Colonoscopy score determined at the end of each DSS cycle in the indicated BM chimeras. (*D*) TUNEL staining in $CD11b^{low}F4/80^{-}Ly6G^{+}$ cells in the lamina propria leukocytes of WT > WT and $Axl^{-/-}Mer^{-/-}$ >*WT* BM chimera mice, as detected by FACS, after 7 d of DSS treatment. Representative histograms (*Left*) and bar graphs (*Right*) indicating mean fluorescence intensity (MFI) of TUNEL expression are shown. (*E*) Representative FACS plots (*Left*) and bar graphs (*Right*) showing the frequency of IL17-α^{+}, IFN-γ^{+}, and IL17-α^{+}IFN-γ^{+} $CD4^{+}$ T cells in the lamina propria of BM chimeras at the end of the chronic DSS treatment. Data are presented as representative images or as mean ± SEM of at least four independent samples per group. n.s., non-significant.

recipients of $Axl^{-/-}Mer^{-/-}$ hematopoietic progenitors compared with recipients of WT hematopoietic progenitors, as confirmed by colonoscopy (Fig. 4*C*). We continued the DSS treatment for two additional cycles. Surprisingly, even chronic DSS treatment failed to demonstrate a significant difference in colonic inflammation between recipients of $Axl^{-/-}Mer^{-/-}$ hematopoietic progenitors and recipients of WT hematopoietic progenitors (Fig. 4*C*). In line with these results, we did not detect a significant increase in the number of $TUNEL^{+}/Ly6G^{+}$ apoptotic neutrophils in the lamina propria of recipients of $Axl^{-/-}Mer^{-/-}$ BM compared with recipients of WT BM after 7 d of DSS treatment (Fig. 4*D*). Furthermore, recipients of WT and $Axl^{-/-}Mer^{-/-}$ hematopoietic progenitors demonstrated similar induction of IL-17^{+}, IFN-γ^{+}, and IL-17^{+}IFN-γ^{+} colon lamina propria $CD4^{+}$ T cells (Fig. 4*E*). These results demonstrate that loss of Axl and Mer in the radiosensitive hematopoietic compartment is not responsible for the increased susceptibility to induced colitis observed in $Axl^{-/-}Mer^{-/-}$ mice.

Loss of Axl and Mer Is Associated with Increased Inflammatory Profile of a Radioresistant Macrophage Population. The foregoing surprising findings suggest that an Axl- and Mer-expressing radioresistant population inhibits intestinal inflammation and inflammation-associated colon cancer. Analyses of colonic lamina propria isolates from BM recipients of WT or $Axl^{-/-}Mer^{-/-}$ hematopoietic progenitors after DSS treatment revealed the presence of a small but significant fraction of radioresistant $CD45.1^{+}$ cells (Fig. 5*A*). Approximately 70% of these cells were positive for the macrophage markers F4/80 and CD11b (Fig. 5*B*). Interestingly, Mer-expressing cells were identified within this radioresistant macrophage population (Fig. 5*C*), suggesting Mer as a marker distinctly associated with mature intestinal tissue resident macrophages.

We next investigated the expression of Axl and Mer in lamina propria macrophages during inflammation. Compared with day 0 (untreated), at day 3 we detected two distinct subpopulations, $F4/80^{high}CD11b^{+}$ and $F4/80^{low}CD11b^{+}$. We found a similar pattern after 7 d of DSS administration, whereas at day 14 (at 2 d after completion of the DSS regimen), the macrophage population pattern resembled that at day 0. These distinct subpopulations of macrophages were cell-sorted, and qPCR analyses of Axl and Mer during the course of DSS treatment revealed robust induction on expression of *Axl* and *Mer* mRNA at days 3 and 7 of DSS treatment. *Axl* and *Mer* expression was significantly higher in the $F4/80^{high}CD11b^{+}$ subpopulation compared with the $F4/80^{low}CD11b^{+}$ subpopulation (Fig. 5*D*). Expression of Mer was confirmed at the protein level by FACS analysis (Fig. S2). In addition, Mer was selectively expressed by $F4/80^{high}CD11b^{+}$ macrophages in the lamina propria, as shown by FACS analysis performed on different cell populations of the colon after 7 d of DSS treatment (Fig. S3*A*) as well as by immunofluorescence analysis (Fig. S4). Upon inflammation, *Axl* expression was enriched in lamina propria macrophages compared with $CD45^{+}CD11b^{-}CD11c^{-}$ and $CD45^{-}$ cells sorted from the lamina propria, as detected by qPCR (Fig S3*B*).

We previously described the essential functions of TAM receptors in limiting cytokine production and in regulating the magnitude of the overall immune response (26). Thus, we compared the levels of activation of lamina propria macrophages in DSS-treated WT and $Axl^{-/-}Mer^{-/-}$ mice. Our results show that loss of Axl and Mer signaling led to a significant increase in the production of multiple proinflammatory mediators (i.e., Nos2, IL-6, IL-17α, TNF-α, and IL-12p35), along with a reduction of negative regulators of inflammation associated with the alternative activation of macrophages (i.e., RELM-α, IL-10, and TGF-β) by lamina propria macrophages (Fig. 5*E*). These results suggest that the increased proinflammatory response and a lack of alternative activation in radioresistant lamina propria macrophages account for the increased colitis in $Axl^{-/-}Mer^{-/-}$ mice.

Discussion

Here we identify a paradoxical function of the proto-oncogenes Axl and Mer RTKs in preventing excessive colonic inflammation and inhibiting inflammation-associated colorectal cancer. These RTKs are well-defined proto-oncogenes in various types of cancer, with established molecular functions in promoting cancer hallmarks. Based on the association of Axl and Mer with cancer and their suitability as RTKs for therapeutic targeting with small-

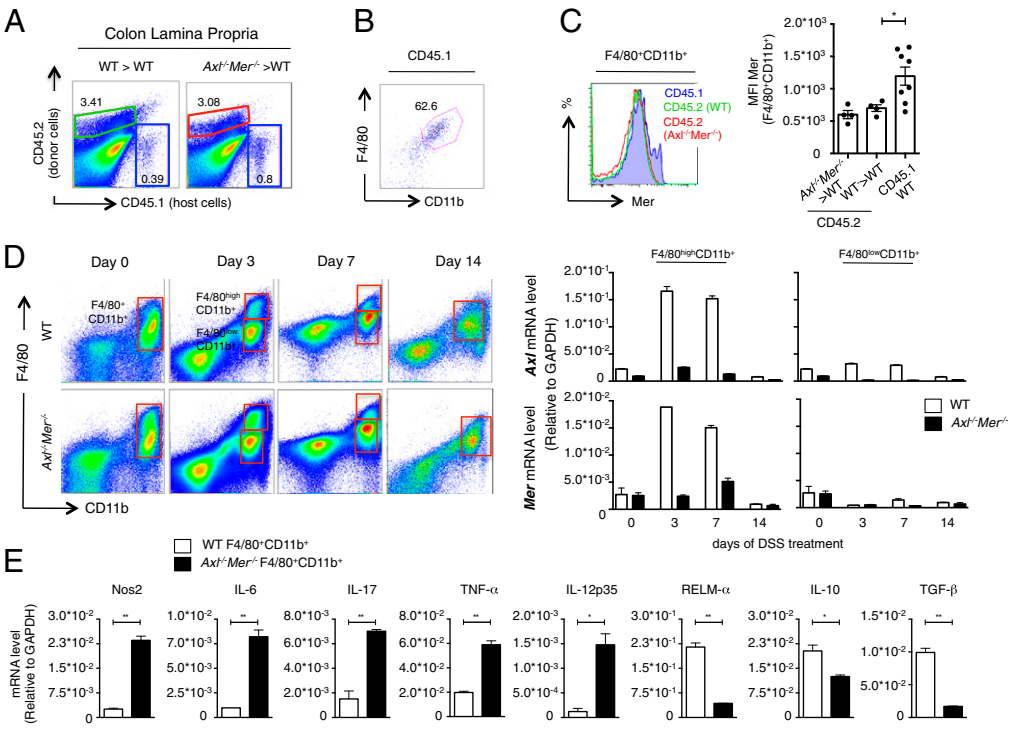

Fig. 5. Axl and Mer are expressed in radioresistant intestinal macrophages, limiting the inflammatory response to DSS-induced colitis. (*A*) FACS analysis of chimerism of lamina propria leukocytes in the indicated BM chimeras, showing the persistence of a radioresistant CD45.1$^+$ population. (*B*) Percentage of F4/80$^+$CD11b$^+$ cells in the radioresistant CD45.1$^+$ host population, as shown by FACS analysis. (*C*) Representative histogram showing Mer expression in host CD45.1$^+$ (blue), donor CD45.2$^+$ WT (green), and donor CD45.2$^+$ *Axl*$^{-/-}$*Mer*$^{-/-}$ (red) F4/80$^+$CD11b$^+$ lamina propria leukocytes and the MFI of Mer in the indicated F4/80$^+$CD11b$^+$ populations isolated at 2 d after the completion of DSS treatment (day 14). MFI of Mer expression in each population is represented on the right. (*D*) Expression of *Axl* and *Mer* in indicated cell-sorted macrophages from the lamina propria of WT (open bar) and *Axl*$^{-/-}$*Mer*$^{-/-}$ (black bar) mice before (day 0), during the course of DSS treatment (days 3 and 7), and 2 d after the completion of DSS treatment (day 14) as detected by qPCR. Lamina propria macrophages isolated from *Axl*$^{-/-}$*Mer*$^{-/-}$ mice were used as internal negative controls. (*E*) Expression of the indicated genes in sorted macrophages from the lamina propria of WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice during DSS treatment, as detected by qPCR. Shown are mRNA levels for Nos2, IL-17, TGF-β, and IL-10 in the F4/80^{high}CD11b$^+$ population after 7 d of DSS treatment, along with mRNA levels for IL-6, TNF-α, IL-12, and RELM-α on F480$^+$CD11b$^+$ population at 2 d after the completion of DSS treatment (day 14). Data are presented as representative images or as mean ± SEM of at least four independent samples per group. $*P < 0.05$; $**P < 0.01$.

molecule ATP-competitive inhibitors and biologics, various Axl and Mer inhibitors are currently in preclinical development (4, 5, 16). Our results indicate that the systemic targeting of Axl and Mer entails potential pitfalls and may actually increase the risk of inflammation-associated cancer.

The role of Axl and Mer in preventing inflammation-associated colorectal cancer is consistent with their role in limiting inflammation (26). Other genes are known to exert paradoxical effects in tumorigenesis and tumor progression; for example, the deubiquitylation and ubiquitin editing enzyme A20 functions as an oncogene in a number of solid tumors, yet A20 loss of function is associated with increased incidence of lymphoid malignancies (37). The potent capacity of A20 to inhibit NF-κB signaling has been suggested to account for its function as a tumor suppressor (37). On the other hand, the function of A20 as an antiapoptotic gene promotes tumorigenesis (37).

Axl and Mer function in a radioresistant macrophage population in the colon appears to be essential in preventing enhanced colitis. Remarkably, Mer has been recently defined as a distinctive and universal marker of mature, tissue-resident macrophages (38). It would be interesting to explore whether the immunoregulatory properties of TAM signaling described here for lamina propria macrophages extend to other tissue-resident macrophage populations. Similar to intestinal Axl$^+$Mer$^+$ macrophages, tissue macrophages in the liver, epidermis, and microglia have been shown to express high levels of F4/80 (38). In addition, F4/80^{high} tissue macrophages originate from the yolk sac and arise independently of the monocytic lineage (39, 40). Recent data reported by Merad et al. (41) show a substantial turnover of tissue-resident macrophages in the lung, peritoneum, and BM, suggesting that these macrophages locally self-maintain in the steady state with only a minimal contribution from monocytes. Furthermore, expansion of pleural resident macrophages in situ, rather than the recruitment from the blood, has been proposed as a signature of innate mechanisms of inflammation in pathological settings, such as *Litomosoides sigmodontis* infection (42). The investigation of whether Axl$^+$Mer$^+$ lamina propria macrophages originate from yolk sac precursors and the identification of mechanisms that control their induction represent exciting areas of future research.

Our data also suggest that Axl and Mer are not required for the differentiation or proliferation of intestinal macrophages, as demonstrated by the lack of significant differences in the frequency of lamina propria macrophages or the expression of F4/80 and CD11b between WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice (Fig. 5*D*). How Axl and Mer are activated in this macrophage population in the context of an inflammatory response remains to be determined, however. The TAM agonists Pros1 and Gas6 are expressed by murine and human macrophages (43, 44); thus, it is possible that TAM signaling in macrophages is triggered in an autocrine manner. In addition, we have recently demonstrated that T-cell–derived Pros1 constitutes a relevant in vivo source of this anti-inflammatory TAM ligand (45). In this context, T cell–macrophage interaction could possibly provide a mechanistic model for the activation of TAM signaling in intestinal tissue homeostasis. This local function of Axl and Mer, along with their well-described role in circulating innate immune cells, underscores the complexity of Axl and Mer anti-inflammatory signaling in vivo. Furthermore, a similar anti-inflammatory function of Axl and Mer in other radioresistant cell types of the colon also may play a role in preventing colitis. The conditional ablation of Axl and Mer in specific cell types will further reveal the function of these genes in intestinal mucosal homeostasis.

Targeting of RTKs may be associated with "on-target toxicity." For example, the anticancer use of the ERBB2 inhibitor trastuzumab can result in cardiotoxicity (46). However, recently described approaches allow active targeting of kinase inhibitors to specific organs and cells by conjugating these molecules to targeting ligands. For example, conjugation of the VEGF inhibitor to a cyclic arginine-glycine-aspartic acid peptide targets

this compound to the tumor vasculature (47). Emphasis on developing therapeutic approaches that specifically target Axl and Mer signaling within tumor cells and spare the protective anti-inflammatory function of these RTKs may improve Axl and Mer efficacy as an anticancer target. Future studies focused on unraveling the divergent molecular functions of Axl and Mer signaling pathways in specific cell types are of fundamental importance for determining the suitability of therapeutic targeting of these kinases in cancer models.

Materials and Methods

Experimental Animals. $Axl^{-/-}$ and $Mer^{-/-}$ mice have been described previously (48). B6.SJL-$Ptprc^{a}Pepc^{b}$/BoyJ (CD45.1) mice were obtained from Jackson Laboratory. All mice were bred at Yale University's animal facility, were specific pathogen-free, maintained under a strict 12-h light cycle (lights on at 7:00 AM and off at 7:00 PM), and given a regular chow diet. In all of the experiments, age-matched WT and KO mice at age 4–6 wk were cohoused for a minimum of 2 wk. All experimental procedures were approved by Yale University's Institutional Animal Use and Care Committee.

AOM/DSS-Induced Tumorigenesis. Age- and sex-matched, cohoused, 8- to 12-wk-old mice were i.p. injected with AOM (Sigma-Aldrich) at a dose of 12.5 mg/kg body weight. After 5 d, the mice were treated with 1.5% DSS (MP Biomedicals) in the drinking water for 7 d, followed by 14 d of regular water. This cycle was repeated three times.

DSS-Induced Colitis. To induce acute colitis, 8- to 12-wk-old mice were treated with 1.5% DSS in the drinking water for 12 d, followed by regular access to water for 2 d. In the chronic colitis experiments, DSS was administered for 12 d, followed by normal water for 7 d; this treatment was repeated three times.

Detailed descriptions of the materials and methods used in this study are provided in *SI Materials and Methods*.

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