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Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells

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Summary

Blockade of colony-stimulating factor-1 (CSF-1) limits macrophage infiltration and improves response of mammary carcinomas to chemotherapy. Herein we identify interleukin (IL)-10 expression by macrophages as the critical mediator of this phenotype. Infiltrating macrophages were the primary source of IL-10 within tumors, and therapeutic blockade of IL-10 receptor (IL-10R) was equivalent to CSF-1 neutralization in enhancing primary tumor response to paclitaxel and carboplatin. Improved response to chemotherapy was $CD8^+$ T cell-dependent, however IL-10 did not directly suppress $CD8^+$ T cells or alter macrophage polarization. Instead, IL-10R blockade increased intratumoral dendritic cell expression of IL-12, which was necessary for improved outcomes. In human breast cancer, expression of *IL12A* and cytotoxic effector molecules were predictive of pathological complete response rates to paclitaxel.

Keywords

chemotherapy; paclitaxel; interleukin-10; interleukin-12; macrophages; dendritic cells; mammary carcinoma; breast cancer

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Introduction

Tumor-associated macrophages are associated with poor clinical outcome in most carcinomas, with clinical and pre-clinical data indicating this is largely due to macrophage promotion of angiogenesis, local invasion and increased metastatic capacity (Bingle et al., 2002; Ruffell et al., 2012a). As macrophages are highly dependent on the colony-stimulating factor-1 (CSF-1) and CSF-1 receptor (CSF-1R) pathway for survival in most tissues (Pollard, 2009), there is significant interest in therapeutically targeting this pathway. In murine models, small molecule inhibitors of CSF-1R induce tumor regression in glioblastoma multiforme (Pyonteck et al., 2013), prevent tumor growth in cervical cancer (Strachan et al., 2013), and partially delay growth of implanted melanoma cell lines (Mok et al., 2014). An αCSF-1R monoclonal antibody (mAb) has also demonstrated clinical efficacy in diffuse-type giant tumors, a disease driven by overexpression of CSF-1 and recruitment of myeloid cells (Ries et al., 2014).

In the MMTV-PyMT transgenic model of luminal B-type mammary carcinoma, tumor infiltration by F4/80$^+$ macrophages parallels disease progression (DeNardo et al., 2009); MMTV-PyMT mice harboring homozygous null mutations in the CSF-1R gene exhibit limited tumor angiogenesis, slowed tumor progression kinetics and inhibited pulmonary metastasis (Lin et al., 2001). Consistent with these genetic studies, therapeutic depletion of macrophages from tumors with mAbs or small molecule inhibitors targeting CSF-1/CSF-1R respectively, exhibit no efficacy as single agents (DeNardo et al., 2011; Strachan et al., 2013), however, when administered in combination with standard-of-care chemotherapy (CTX), significantly slow primary tumor growth kinetics and diminish pulmonary metastasis (DeNardo et al., 2011). Based on this data, clinical studies combining the CSF-1R small molecule inhibitor PLX3397 and chemotherapy are currently underway (NCT01596751, NCT01525602).

Although macrophages have been found to directly promote survival of mammary carcinoma cells in a cathepsin-dependent manner in vitro (Shree et al., 2011), our previous studies reported that enhanced chemotherapeutic efficacy associated with CSF-1R-inhibition was dependent on cytotoxic CD8$^+$ T cells (DeNardo et al., 2011), thus indicating a role for macrophages in suppressing a T cell responses during CTX. Several studies have demonstrated that tumor-associated macrophages directly suppress CD8$^+$ T cell activation in vitro (DeNardo et al., 2011; Doedens et al., 2010; Movahedi et al., 2010), and CSF-1R inhibition has been found to enhance adoptive cell transfer therapy in melanoma models (Mok et al., 2014). However, the mechanism(s) by which macrophages suppress anti-tumor responses, either directly or indirectly in vivo, remains unclear. Here we sought to inform ongoing clinical trials by delineating functionally significant mechanisms whereby tumor-infiltrating macrophages suppress CD8$^+$ T cell responses during chemotherapy.

Results

Macrophages are the primary source of IL-10 in mammary carcinomas

To identify the molecular mechanism(s) by which macrophages limited response to CTX in mammary carcinomas, we effectively depleted macrophages through administration of αCSF-1 mAb in combination with paclitaxel (PTX) in MMTV-PyMT tumor-bearing mice (Fig. 1A), and examined the status of tumor vasculature (Fig. S1A–B), as well as expression of immune-related genes in whole tumor tissue (Fig. 1B). Although macrophages promote formation of abnormal tumor vasculature (Stockmann et al., 2008), we did not observe changes in density of $CD31^+$ vessels, ratio of pericytes to endothelial cells, or extravasation of either doxorubicin or PTX into mammary tumors, as quantitatively evaluated by immunofluorescence approaches or liquid chromatography-mass spectrometry, respectively (Fig. S1C–D), thus indicating that improved responses to CTX were unlikely linked to improved tumor hemodynamics. Instead, gene expression data revealed decreases in several myeloid-associated genes relative to control tissue, including *Il1a*, *Il10*, *Nos2*, *Cxcl10*, *Ccl3*, *Ccr2*, and inversely, increased expression of *Gzmb*, *Ccl5*, *Cd8a*, and *Il12a*, consistent with our previous report of a role for $CD8^+$ T cells in mediating response to CTX following CSF-1R inhibition (DeNardo et al., 2011). We found no change in genes reflecting a $CD4/T_H1$ response (*Ifng*, *Tbx21*, *Cd4*).

Of the mRNAs exhibiting reduced expression following αCSF-1 mAb therapy, *Il10* is the most strongly associated with an established immunosuppressive role in vivo (Moore et al., 2001). We confirmed macrophages as the primary source of IL-10 in untreated mammary carcinomas by evaluating FACS-sorted epithelial versus stromal cell populations (Fig S1E–F). *Il10* expression was limited to $CD45^+$ leukocytes, with expression observed in $Ly6C^+$ monocytes, $CD11b^+$ DCs, $CD4^+$ T cells and $F4/80^+$ macrophages (Fig. 1C). Macrophage expression of *Il10* was approximately 10-fold higher than other leukocyte populations, with an additional ~1.5-fold average increase in expression by $MHCII^{LO}$ versus $MHCII^{HI}$ macrophages. We further characterized the $MHCII^{HI}$ and $MHCII^{LO}$ macrophage subsets and found that both were effectively depleted by αCSF-1 mAb treatment (Fig. S1G–I), as well as exhibiting similar nuclear morphology in cytospins (Fig. S1J); however, $MHCII^{LO}$ macrophages displayed increased expression of several markers associated with T_H2/M2-type programming at both the protein (MSR1, MRC1, IL4Rα) (Fig. S1K) and mRNA level (*Cd163*, *Msr1*, *Mrc1*, *Il4ra*, *Arg1*, *Ptgs2*) (Fig. S1L).

Based on high expression of *Il10* by macrophages, and its partial correlation with $M2/T_H2$-type biomarkers in mammary carcinomas, we evaluated expression of *IL10* in human breast cancer samples from the TCGA dataset against genes associated with presence of myeloid cells (*CSF1R*, *CD14*, *CD68*) or macrophage polarization (*MSR1*, *MRC1*, *CD163*). We identified significant correlation between these mRNAs, with the most significant correlation occurring for *MSR1* and *CD163* (Fig. 1D). *IL10* expression did not correlate with *FOXP3* expression (data not shown), despite reports of regulatory T cells (T_{Reg}) being a critical source of IL-10 in some murine tumor models (Stewart et al., 2013). As the association between *IL10* expression and macrophages markers was relatively weak ($R < 0.23$), we also evaluated the presence of IL-10 protein by immunohistochemistry in human

breast cancer samples. In accordance with the gene expression correlations, we observed high expression within stromal cells, including $CD163^+$ cells with a myeloid morphology (Fig. 1E–F). In contrast to murine tumor tissue however, we also observed variable expression within tumor epithelial cells. Thus while macrophages, in particular T_H2/M2-type macrophages, are associated with expression of IL-10 in both murine mammary carcinomas and human breast cancer, IL-10 production within human breast tumors displays increased variability and complexity.

Blockade of the IL-10 receptor improves response to PTX

To examine whether IL-10 was functionally relevant for regulating response to CTX, we treated late-stage tumor-bearing MMTV-PyMT mice with an IL-10 receptor-blocking mAb (αIL-10R; clone 1B1.3A) prior to and throughout a chemotherapeutic regimen of 10 mg/kg PTX administered every 5 days (Fig. 2A). While neither αIL-10R nor αCSF-1 mAb alone altered tumor growth kinetics relative to control mice over this time period, combinatorial PTX with either mAb significantly slowed tumor growth more effectively than PTX alone, with significant growth differences occurring following the 2^{nd} dose of PTX. Using a syngeneic orthotopic implantable PyMT explant model to evaluate survival, we observed greater than 2-fold increased survival with either αCSF-1 mAb/PTX or αIL-10R mAb/PTX (10 days) following initiation of treatment at approximately 1.0 cm in average tumor diameter, as compared to mice treated with PTX alone (4.5 days), with no additional improvement by combining αCSF-1 and αIL-10R mAbs plus PTX (Fig. 2A–B). MMTV-PyMT mice treated with αIL-10R mAb also displayed an enhanced response to 50 mg/kg carboplatin (CBDCA), with tumors regressing approximately 50% over the course of treatment (Fig. 2C). Using the C3(1)-TAg model of triple negative mammary carcinogenesis (Deeb et al., 2007; Maroulakou et al., 1994) we also found significant diminution of primary tumor growth when combining PTX with either αCSF-1 or αIL-10R mAbs (Fig 2D, S2A–C). Thus the ability of IL-10 to limit chemotherapeutic efficacy was not limited by the type of chemotherapeutic or the subtype of mammary carcinoma being examined.

Regarding pulmonary metastasis, whereas αCSF-1 mAb/PTX diminished both the number and size of metastastic foci in MMTV-PyMT mice (Fig. 2E–G), inclusion of αIL-10R mAb to this regimen yielded no additional benefit. Neither αCSF-1 nor αIL-10R mAbs caused changes to proliferating cells (BrDU-positivity) or cell death (cleaved caspase-3 positivity) in metastatic foci beyond that observed with PTX alone (Fig. S2D). Our interpretation of this data was that whereas CSF-1R-activated macrophages produce epidermal growth factor (EGF) that in turn fosters neoplastic mammary epithelial cell invasion and metastasis (DeNardo et al., 2009; Lin et al., 2001), macrophage-derived IL-10 was not involved in programs regulating metastasis, and instead represented a macrophage-dependent pathway within primary tumors regulating response to chemotherapy.

Since a significant clinical issue theoretically limiting efficacy of CSF-1/CSF-1R-targeted therapy is perceived liver toxicity due to reduced density of liver Kupffer cells (Strachan et al., 2013; Wei et al., 2005), we evaluated overall health of mice enrolled in these studies by several criteria. As expected, based upon a role for Kupffer cells in clearance of serum enzymes (Radi et al., 2011), we observed increased serum alkaline phosphatase (ALKP),

alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Fig. S2E) following αCSF-1 mAb therapy. Similar to what has been reported in non-human primates and rodents (Radi et al., 2011) however, neutralizing CSF-1 alone or in combination yielded no adverse effects on animal health with respect to liver pathology, weight loss, or renal toxicity (Fig. S2F–H). No changes in animal health were noted with αIL-10R mAb alone or in combination. Thus, targeting of either IL-10R or CSF-1 improved efficacy of PTX without evidence of acute toxicity.

Improved response to PTX is CD8$^+$ T cell-dependent

To reveal mechanisms whereby macrophage IL-10 regulated response to CTX, we examined the presence of T cell populations in mammary tumors either 2- or 5-days following the final dose of PTX. 5-days post PTX, αCSF-1 mAb or αCSF-1 mAb/PTX resulted in a small increase in the presence of CD4$^+$FoxP3$^+$ T_{Reg} cells, with no significant changes observed in CD4$^+$FoxP3$^-$ T_H cells or CD8$^+$ T cells (Fig. S3A). In contrast, in mammary tumors evaluated 2-days post PTX, αCSF-1 mAb and αCSF-1 mAb/PTX therapy resulted in a significant increase in the relative frequency of CD8$^+$ T cells (Fig. 3A). As this change may have resulted from depletion of the major leukocyte population, we also evaluated the relative frequency of CD4$^+$ T_H cells and found no significant alteration in most groups (Fig. 3B), resulting in an increased CD8/CD4 T cell ratio within tumors from αCSF-1 treated animals (Fig. 3C). No changes were observed in CD4$^+$ or CD8$^+$ T cell infiltration following αIL-10R mAb or αIL-10R mAb/PTX therapy at either time point. Since increased CD8$^+$ T cell infiltration was observed with αCSF-1 mAb even in the absence of CTX, and αCSF-1 mAb/PTX treatment increased mRNA expression of *Gzmb* in whole tissue (Fig 1B), we evaluated whether any of the combinatorial therapies increased the density of granzyme B (GZMB)-expressing cells, indicative of perhaps a “better” CTL response. Indeed, both αCSF-1 mAb/PTX and αIL-10R mAb/PTX therapy increased tumor density of GZMB-positive cells 2-days post PTX by approximately 1.5-fold (Fig. 3D). Increased presence of GZMB-positive cells was significant as CD8-depletion prior to PTX ablated improved outcomes for tumor-bearing mice treated with αIL-10R mAb/PTX therapy (Fig. 3E), similar to our previous finding utilizing a CSF-1R antagonist (DeNardo et al., 2011).

IL-10 does not influence macrophage recruitment, polarization or function

Since macrophages are known to express IL-10R, we examined whether IL-10R-blockade was directly influencing macrophage presence or function in mammary tumors. Whereas αCSF-1 mAb led to reduced presence of the two predominant populations of macrophages, i.e., MHCII^{HI} or MHCII^{LO}, in mammary carcinomas (Fig. 4A, 4B), blockade of IL-10R exerted no similar impact (Fig. 4A, 4B), as well as having no effect on the presence of monocytes or neutrophils in tumors (Fig. 4C–D).

As macrophages isolated from MMTV-PyMT mammary tumors have been reported to exert immunosuppressive activity ex vivo (DeNardo et al., 2011; Doedens et al., 2010), we next evaluated the role of IL-10 in mediating this activity. Notably, the ability of tumor-infiltrating macrophages to suppress splenic CD8$^+$ T cell proliferation in vitro was largely isolated to the MHCII^{LO} population (Fig. 4E–F), correlating with presence of MHCII^{LO} macrophages in areas of tumor hypoxia in situ (Fig. 4G) and higher expression of *Arg1* (Fig.

S1L), similar to models of lung carcinogenesis (Laoui et al., 2014; Movahedi et al., 2010). That said, neither αIL-10 neutralizing mAb nor αIL-10R blocking mAb altered the immunosuppressive capacity of either the $MHCII^{HI}$ or $MHCII^{LO}$ macrophage subset ex vivo (Fig. 4H), and conversely, addition of IL-10 did not suppress proliferation, or expression of GZMB, interferon (IFN)-γ or tumor necrosis factor (TNF)-α by $CD8^+$ splenocytes during in vitro stimulation (Fig. S3B–E). Based on these data, and the fact that IL-10 can promote IL-2-induced proliferation of human (Groux et al., 1998) and mouse $CD8^+$ T cells (Chen and Zlotnik, 1991) when used at high concentrations (100 U/ml), we concluded that macrophage-derived IL-10 was likely regulating $CD8^+$ T cell functionality indirectly. This conclusion was bolstered by the fact that gene expression programs in macrophages isolated from tumor-bearing mice treated with αIL-10R mAb were only modestly altered (Fig. S4) with the exception of reduced expression of *Socs3*, a downstream target gene of IL-10R signaling (Fig. 4I).

Dendritic cells express high levels of IL-10R

To address this hypothesis and identify cells infiltrating mammary tumors that potentially were being regulated by macrophage-derived IL-10, we evaluated expression patterns of IL-10R by flow cytometry. Although IL-10R expression has been observed on colonic epithelium (Denning et al., 2000), the IL-10 binding subunit of IL-10R (IL-10Rα/IL-10R1) was restricted to $CD45^+$ leukocytes in mammary tumors, with the exception of low expression by platelet-derived growth factor receptor α $(PDGFR\alpha)^+$ fibroblasts (Fig. 5A). To identify select lineages contributing to $CD45^+$ expression, we isolated leukocyte subsets from mammary tumors as compared to equivalent populations from spleens (where possible) of non-tumor bearing mice (Fig 5B). These data revealed significantly increased expression of IL-10R on $CD4^+$ T cells, $Ly6C^{HI}$ monocytes, macrophages and DCs, specifically $CD103^-CD11b^+$ and $CD103^+CD11b^-$ DC subsets, in tumors, with no significant increased expression on $CD8^+$ T cells as compared to normal spleen (Fig 5B).

Based on these findings, we hypothesized that increased expression of IL-10R on select myeloid cells indicated their potential role in regulating functional $CD8^+$ T cell responses following CTX in response to macrophage-derived IL-10. Since αIL-10R mAb therapy did not alter presence of macrophages or monocytes in tumors (Fig. 4A–C), and did not significantly alter macrophage gene expression programs (Fig. S4), we evaluated the relative percentage of DCs infiltrating tumors from the various experimental groups. As shown in Fig. 5C, there was an ~1.5-fold increase in $CD103^-CD11b^+$ DCs in tumors from both αCSF-1 mAb/PTX and αIL-10R mAb/PTX treatment groups, with a further ~3-fold increased presence in tumors from αCSF-1 mAb/αIL-10R mAb/PTX-treated mice, as compared to PTX alone. $CD103^+CD11b^-$ DCs were also increased by either αCSF-1 mAb/PTX or αIL-10R mAb/PTX treatment, although the effects of αCSF-1 mAb/PTX were more significant (Fig. 5C). Immunofluorescent staining of carcinomas revealed that $CD103^+$ cells (reflecting populations of DCs and $CD8^+$ T cells) were dispersed throughout tumor stroma in proximity to $F4/80^+$ macrophages, with limited invasion of the tumor parenchyma (Fig. 5D). Although the density of $CD103^+$ cells was increased by αCSF-1 mAb/PTX treatment, their localization appeared unchanged (Fig. 5D).

As tumor macrophages and tumor DCs both express CD11c, albeit with higher surface expression by DCs (Fig. S1K), we FACS-sorted tumor macrophages, DCs, monocytes, and neutrophils to affirm the correct populations had been isolated by FACS (Fig. S5). Confirming our gating strategy, $CD11b^+$ and $CD103^+$ DCs displayed preferential expression of DC transcription factors (e.g. *Zbtb46*, *Ciita*, *Pvrl1*) and surface markers (e.g. *Ccr7*, *Flt3*, *Notch2*, *Cd26*) as compared to either $MHCII^{HI}$ or $MHCII^{LO}$ macrophage populations.

IL-10 represses IL-12 expression by DCs

Along with the increase in DC infiltration observed following either αIL-10R mAb/PTX or αCSF-1 mAb/PTX therapy (Fig. 5C), we noted an almost 4-fold increase in *Il12a* expression in tumors from αCSF-1 mAb/PTX-treated mice (Fig. 1B). As $CD8^+$ T cells in MMTV-PyMT mammary tumors expressed both subunits of the IL-12 receptor (Fig. S6A–B), and IL-12 is known to enhance $CD8^+$ T cell proliferation and effector function (Trinchieri, 2003), we investigated a possible role for IL-10 in regulating IL-12 production by DCs and thereby influencing $CD8^+$ T cell responses during CTX. To evaluate this, we first examined mRNA expression of the IL-12 subunits in sorted leukocyte populations. In mammary tumors from untreated mice, expression of *Il12a* mRNA was observed in most populations; however, mRNA expression of the p40 subunit of IL-12 (*Il12b*) was limited to DCs and macrophages (Fig 6A). Thus, although multiple leukocytes expressed *Il12a* mRNA, only DCs and macrophages appeared primed to produce IL-12. Indeed, IL-12p40 protein was only detectable by intracellular flow cytometry in a small population of tumor-associated $CD103^+$ DCs (Fig. 6B), the cell type expressing the highest levels of *Il12b* mRNA.

IL-10 regulates IL-12 production by macrophages and DCs in vitro, depending on the type and kinetics of stimulation (D'Andrea et al., 1995; Koch et al., 1996). We thus affirmed that pre-incubation with IL-10 prevented IFN-γ/lipopolysaccharide (LPS)- and αCD40/LPS-induced production of IL-12p70 by FLT3-ligand matured bone marrow-derived DCs (Fig. 6C). This regulation occurred at the level of mRNA expression, as IL-10 prevented upregulation of *Il12a* mRNA following αCD40/LPS stimulation (Fig. 6D), and upregulation of *Il12b* mRNA (Fig. 6E) and the corresponding IL-12p40 protein (Fig. S6C) following stimulation by any combination of IFN-γ, LPS or αCD40. Although IL-10 can induce downregulation of some toll-like receptor (TLR) signaling components (Knodler et al., 2009), IL-10s capacity to suppress IL-12 expression was not dependent on suppressed DC activation, as CD86 expression was only mildly reduced by IL-10 during stimulation with IFN-γ/α CD40 or αCD40/LPS stimulation (Fig. S6D).

Since these data indicated that IL-10 regulated IL-12 expression at the mRNA level in vitro, we isolated macrophages and DCs from mammary tumors 2-days following the 2^{nd} dose of PTX (day 7) to determine whether IL-10 was functionally regulating IL-12 expression in myeloid cells in vivo. Consistent with our previous findings (Fig. S4), αIL-10R mAb/PTX therapy did not alter expression of either gene in isolated macrophages (Fig. 6F). In both $CD103^-CD11b^+$ and $CD103^+CD11b^-$ DC populations however, αIL-10R mAb/PTX therapy increased expression of *Il12a*, with *Il12b* expression also enhanced in $CD103^+CD11b^-$ DCs (Fig. 6F). The upregulation of *Il12b* and detection of IL-12p40 at the protein level in $CD103^+CD11b^-$ DCs indicates that these may be the critical source of IL-12

within mammary tumors; however as IL-12p40 is produced in excess of IL-12p35, this is not definitive.

To evaluate whether IL-12 was functionally significant with regards to the enhanced CD8-dependent responses observed in either αIL-10R mAb/PTX or αCSF-1 mAb/PTX-treated experimental groups, late-stage MMTV-PyMT mice were treated with a neutralizing antibody against either IL-12p40 (recognizing IL-12 and IL-23) or IL-12p70 (recognizing only IL-12) prior to administration of PTX. While neither αIL-12p40 mAb, nor αIL-12p70 mAb altered response of tumors to PTX alone (Fig. 6G), both neutralizing mAbs blocked the improved response to PTX observed following αIL-10R mAb therapy (Fig. 6G). Furthermore, consistent with our finding that macrophages were the primary source of IL-10 in mammary tumors (Fig. 1), both IL-12 neutralizing antibodies also reversed the improved response to PTX observed following treatment with αCSF-1 mAb (Fig. 6G).

IL12A expression correlates with increased pathologic complete response in breast cancer patients

Based on these data indicating a significant role for IL-12 in mediating $CD8^+$ T cells responses to PTX in mammary carcinomas, we next evaluated whether *IL12A* or *IL12B* mRNA levels correlated with presence of DCs (Fig. 7A) in human breast cancers utilizing the TCGA dataset (TCGA, 2012). Interestingly, only *IL12A* correlated with expression of transcription factors associated with DCs (*CIITA*) or the human equivalent of the $CD103^+$ DC subset (*BATF3*, *IRF8*). Expression of *IL12A*, but not *IL12B*, was associated with evidence of a cytotoxic T cell response in these samples, as seen by a correlation with *GZMB*, *CD8A*, and *IFNG* expression (Fig. 7B). Based on these associations, we next evaluated two published datasets annotated for pathologic complete response (pCR) following treatment with CTX (Hess et al., 2006; Tabchy et al., 2010). Here, high expression of *IL12A*, along with the DC transcription factors and cytotoxic effector molecules, all associated with an improved rate of pCR, with a ~2-fold increase in the response rate observed for many of the genes (Fig. 7C). Taken together, these results indicate an important cytokine axis in breast cancers wherein macrophage-derived IL-10 suppresses IL-12 production by DCs during CTX, thereby limiting cytotoxic $CD8^+$ T cell responses in carcinomas (Fig. 8). Blockade of either the IL-10/IL-10R pathway or CSF-1/CSF-1R pathway thus improves response to CTX and increases survival of tumor-bearing mice.

Discussion

Herein we describe an interaction between macrophages and DCs in mammary tumor microenvironments, wherein macrophage-derived IL-10 indirectly blunts $CD8^+$ T cell responses by inhibiting DC production of IL-12 following CTX. Tumor-associated macrophages have long been described to possess an immunosuppressive phenotype (Mantovani et al., 2002), but the in vivo relevance of this phenotype has largely been inferred. Direct suppression by immature myeloid cells is usually linked to metabolism of L-arginine or production of free radicals (Gabrilovich and Nagaraj, 2009). Supporting a role for these pathways in macrophage-mediated suppression, hypoxia promotes macrophage

suppressive capacity and induces expression of arginase-1 (Doedens et al., 2010), macrophages can suppress T cell proliferation through L-arginine depletion (Rodriguez et al., 2003), and, as reported herein, $MHCII^{LO}$ macrophages exhibit tropism for hypoxic regions and exhibit enhanced suppressive capacity. However, the degree to which nutrient depletion in a closed in vitro system reflects the tumor microenvironment is unclear, and other studies have found that $MHCII^{LO}$ macrophages from implanted lung carcinoma models (Movahedi et al., 2010) and macrophages from human ovarian carcinoma ascites (Kryczek et al., 2006) suppress T cell proliferation independent of arginase and NOS activity. Human macrophages have instead been found to directly suppress T cell responses through programmed death-ligand 1 (PD-L1) in hepatocellular carcinoma (Kuang et al., 2009) and B7-H4 in ovarian carcinoma (Kryczek et al., 2006), and possibly indirectly through T_{Reg} cell recruitment through CCL22 (Curiel et al., 2004). Along with these studies, data presented herein describe a functional role for macrophages in mediating immune suppression within tumors, and ascribe this role to suppression of DC function.

The drivers of *Il10* expression by tumor macrophages are unclear. In general *Il10* expression by macrophages is thought to reflect activation by TLR ligands and type I IFN (Saraiva and O'Garra, 2010). We noted modestly elevated expression of *Il10* from $MHCII^{LO}$ macrophages, but while higher IL-10 secretion has also been observed in $MHCII^{LO}$ macrophages from implanted lung carcinomas (Laoui et al., 2014), there is no evidence that hypoxia promotes *Il10* expression. $MHCII^{LO}$ macrophages also expressed higher surface levels of IL-4Rα, however, we previously reported that absence of $CD4^+$ T cells did not influence IL-10 production by macrophages despite the ability of both IL-4 and IL-13 to promote IL-10 production in vitro (DeNardo et al., 2009). Notably, mammary gland macrophages constitutively expressed *Il10* at levels equivalent to that found in tumor macrophages, indicating that the homeostatic environment of the mammary gland is sufficient to promote *Il10* expression, without a requirement for properties of the tumor microenvironment such as inflammation or hypoxia.

IL-10 has the potential to be expressed by most leukocyte populations (Saraiva and O'Garra, 2010), as well as by normal and malignant epithelial cells (O'Garra et al., 2008). Despite this extensive expression capacity, IL-10 production specifically by T_{Reg} cells is critical for maintaining tolerance in the colon (Saraiva and O'Garra, 2010), limiting T_H17 inflammation in tumors (Stewart et al., 2013), and suppressing polyp formation (Dennis et al., 2013). In contrast to the colon, we found that in mammary carcinomas, macrophages expressed the highest level of *Il10* mRNA, with 10-fold lower expression observed in other leukocytes, and no expression observed by tumor epithelia. As the dominant immune population in murine mammary tumors, macrophages are thus the critical source of IL-10.

In human breast cancer, we similarly found no association between *FOXP3* and *IL10* expression, but rather observed a correlation between *IL10* expression and genes associated with either the presence (*CSF1R*, *CD14*, *CD68*) or polarization (*CD163*, *MSR1*) of macrophages. With no evidence of toxicity and the potential to simultaneously block multiple pro-tumorigenic macrophage pathways, targeting of the CSF-1/CSF-1R pathway continues to be an attractive therapeutic approach. That said, the increased diversity of leukocytic infiltrates in human breast cancer (Ruffell et al., 2012b), and our observation that

breast tumor epithelial cells expressed IL-10, argues that neutralizing the effects of IL-10 in human breast cancer may require more selective targeting to maximize a cytotoxic T cell response during CTX. This could take the form of a human αIL-10R blocking antibody, or kinase inhibitors against downstream IL-10R signaling components such as Janus kinase 1 (JAK1) or Signal transducer and activator of transcription 3 (STAT3). It might also be possible to enhance chemotherapeutic efficacy by directly activating myeloid cells to produce increased levels of IL-12, either alone or in combination with IL-10R antagonists. While toxicity resulting from systemic myeloid activation is a concern, it is promising to note that a relatively safe CD40 agonist is in clinical trials (Beatty et al., 2013).

IL-10 is often referred to as a pleiotropic cytokine, and its dual role in cancer likely reflects this. Both IL-10-deficiency and IL-10-overexpression can promote anti-tumor immune responses in mice (O'Garra et al., 2008). While IL-10-deficient mice are resistant to ultraviolet-induced skin carcinogenesis (Loser et al., 2007), they are also sensitive to skin and colon carcinogenesis (Mumm et al., 2011; Sturlan et al., 2001). Some of this disparity most assuredly reflects the significance of local tumor microenvironments in sculpting immune responses, but also likely reflects local variations in bioavailable IL-10 (O'Garra et al., 2008). IL-10 is a potent suppressor of DC activation, with even 1.0 U/ml largely ablating IL-12 secretion, as well as suppressing DC differentiation in vitro (Allavena et al., 1998). On the other hand, high concentrations of IL-10 can promote IL-2-dependent proliferation of $CD8^+$ T cells (Chen and Zlotnik, 1991; Groux et al., 1998), and IL-10 is involved in $CD8^+$ T cell memory formation in some, but not all, infection models (O'Garra et al., 2008). This has led to divergent approaches for targeting IL-10 as an anticancer therapeutic: blocking IL-10R to enhance myeloid cell function (Vicari et al., 2002) versus injecting exogenous IL-10 to directly promote an anti-tumor T cell response through activation of intratumoral $CD8^+$ T cells (Emmerich et al., 2012; Mumm et al., 2011). $CD8^+$ T cells in mammary tumors of MMTV-PyMT did not express IL-10R, indicating that exogenous IL-10 would likely prove ineffective in this context, but ongoing clinical studies will determine the validity of this approach in patients.

Blocking IL-10R has not been used extensively as an approach for anti-cancer therapy, but has been reported to induce tumor regression in combination with CpG oligonucleotides in various subcutaneous tumor models, ostensibly through macrophage/DC activation and increased expression of IL-12 (Guiducci et al., 2005; Vicari et al., 2002). In sharp contrast to these studies, response to CTX in subcutaneous tumor models has been found to occur independently of IL-12 and *Batf3*-dependent DCs, and is instead mediated by an immune cell/cytokine pathway involving IFN-β IL-17, and IFN-γ, with production by $CD11b^+CD103^+$ DCs, γδ T cells and $CD8^+$ T cells, respectively (Kroemer et al., 2013; Ma et al., 2013). The importance of this pathway for response to CTX has been called into question using transgenic models of mammary carcinoma, as these respond to CTX independently of adaptive immune cells, with the suggestion that this is due to use of implantable versus spontaneous tumors (Ciampricotti et al., 2012). We instead suggest that these differences are due at least partially to anatomical location. Thus, preventing macrophage infiltration via αCSF-1 mAbs (or CSF-1R antagonists) or limiting macrophage functionality via αIL-10R mAbs, enables CTX and resultant cell death to benefit from

productive $CD8^+$ T cell responses elicited in tumors. However, unlike subcutaneous models, our data indicate a critical role for IL-12 and possibly $CD103^+$ DCs, again highlighting the role of anatomical location in determining response to therapy. This concept has been elegantly demonstrated in a recent study wherein subcutaneously implanted tumors were found to be more sensitive to immunotherapy than the same cell lines implanted orthotopically (Devaud et al., 2014).

DCs in solid tumors are generally viewed as critical primers of de novo T cell responses (Chen and Mellman, 2013), and therapeutic targeting of DCs has focused on this aspect. Our data does not definitively demonstrate a role for DCs within mammary tumors, and systemic suppression of DC function through IL-10 has been observed (Yang and Lattime, 2003). At the same time however, removal of tumor-draining lymph nodes from mice bearing subcutaneous tumors did not alter response to CTX (Ma et al., 2013), tertiary lymphoid structures have been implicated in T cell activity in melanoma (Chen et al., 2013), and the presence of follicular helper T cells correlates positively with patient outcome and response to CTX in breast cancer (Bindea et al., 2013; Gu-Trantien et al., 2013). These studies align well with our finding that gene expression indicative of a $CD8^+$ T cell response (*GZMB*, *CD8A*, *IFNG*) or the presence of DCs (*IL12A*, *CIITA*, *IRF8*) coincides with improved response to neoadjuvant PTX, and together hint at the importance of favorable DC activity within the tumor microenvironment promoting an anti-tumor immune response to chemotherapy.

Experimental Procedures

Ethics statement

De-identified human tissue was received from the University of California, San Francisco (UCSF) Department of Pathology with patient consent forms obtained at the time of tissue acquisition. Authorization for the use of samples was through the UCSF Committee on Human Research (05028310) under “exempt category 4” for individuals receiving de-identified biological specimens.

Animal care and use

FVB/n strain background mice harboring the polyoma middle T (PyMT) transgene under the control of the mouse mammary tumor virus (MMTV) promoter (Guy et al., 1992), and the simian virus 40 large tumor antigen (SV40 TAg) under control of the rat prostatic steroid binding protein gene [C3(1)] (Maroulakou et al., 1994) have been previously described. Implantation of orthotopic mammary tumors was performed as described (DeNardo et al., 2011) using single-cell suspensions from mammary tumors of d80-d85 MMTV-PyMT transgenic mice combined 1:1 with matrigel (BD Biosciences). Treatment schedules were initiated as indicated in the respective figures. Monoclonal antibodies (αCSF-1/5A1, αIL-10R/1B1.3A, αCD8/2.43, IgG_1/HRPN, αIL-12p75/R2-9A5, αIL-12p40/C17.8) were obtained from BioXCell and were administered by intraperitoneal injection at 1.0 mg/mouse, with follow-up doses of 0.5 mg every 5 days. Clinical grade paclitaxel (Hospira) or carboplatin (Novaplus) was administered intravenously every 5 days at 10 mg/kg or 50 mg/kg, respectively. Prior to terminal cardiac perfusion with PBS containing 10 U/ml of

Heparin (Sigma-Aldrich), mice were intraperitoneal injected with 50 mg/kg BrdU (Roche) for 90 minutes. Resected tissues were either flash frozen in liquid nitrogen, directly embedded in optimal cutting temperature (OCT, Sakura Finetek) medium post-resection, or incubated overnight in neutral buffered formalin prior to ethanol dehydration and paraffin embedding. Mice were maintained either within the UCSF Laboratory for Animal Care barrier facility or the OHSU Department of Comparative Medicine barrier facility. All experiments involving animals were approved by the respective Institutional Animal Care and Use Committee.

Statistical Analysis

Statistical analyses were performed using Prism (GraphPad). Statistical significance was determined via an unpaired *t*-test, unpaired *t*-test with Welch's correction, 2-way ANOVA, or Mann-Whitney as indicated. Heat maps were generated GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/). All heat maps are displayed on a log scale with values normalized per row, with hierarchical clustering performed with a one minus Pearson correlation. Linear regression analysis in breast cancer was performed in Prism using the dataset from The Cancer Genome Atlas Network (TCGA, 2012). Gene expression data from fine needle aspirate obtained prior to neoadjuvant chemotherapy with paclitaxel in breast cancer patients was obtained from 2 published datasets (GSE20194, GSE20271) annotated for pathologic complete response (Hess et al., 2006; Tabchy et al., 2010).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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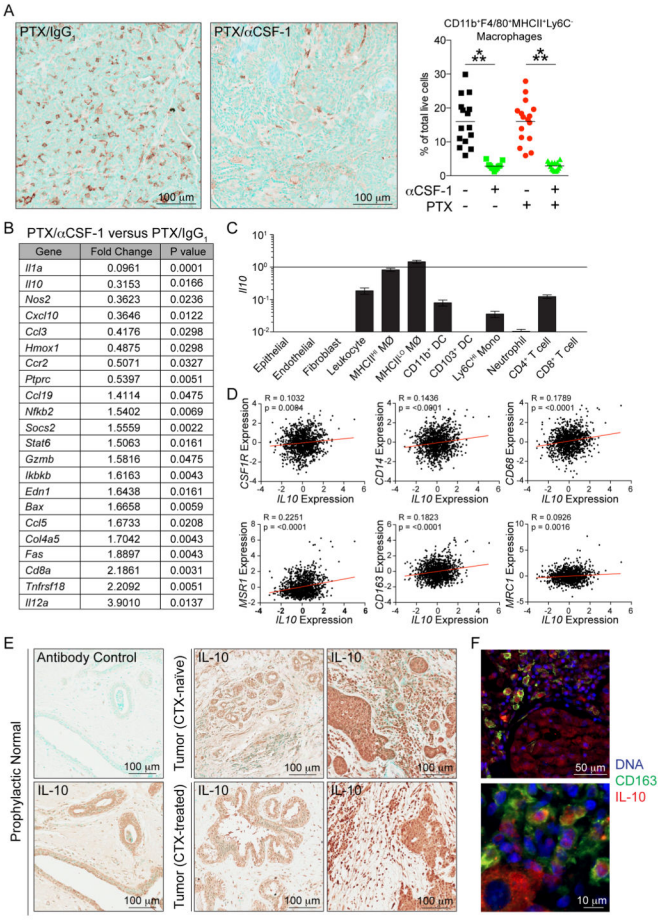
Significance

Therapeutics targeting the CSF-1/CSF-1 receptor pathway are currently being evaluated clinically in multiple cancer types, either as monotherapy or in combination with standard-of-care chemotherapy. However, mechanism(s) by which the CSF-1/CSF-1 receptor pathway and macrophages sustain tumor growth and/or repress response to cytotoxic therapy is unclear. Herein we report that macrophages infiltrating mammary carcinomas are the significant source of IL-10, which in turn suppresses intratumoral dendritic cell production of IL-12, and thereby limits cytotoxic T cell responses during chemotherapy. These data reveal a role for the interaction between tumor macrophages and dendritic cells in mediating response to therapy, identify a CSF-1/IL-10/IL-12 cytokine axis for targetable intervention, and reveal possible risk stratification biomarkers for patient selection.



Gene	Fold Change	P value
Il1a	0.0961	0.0001
Il10	0.3153	0.0166
Nos2	0.3623	0.0236
Cxcl10	0.3646	0.0122
Ccl3	0.4176	0.0298
Hmox1	0.4875	0.0298
Ccr2	0.5071	0.0327
Ptprc	0.5397	0.0051
Ccl19	1.4114	0.0475
Nfkb2	1.5402	0.0069
Socs2	1.5559	0.0022
Stat6	1.5063	0.0161
Gzmb	1.5816	0.0475
Ikbkb	1.6163	0.0043
Edn1	1.6438	0.0161
Bax	1.6658	0.0059
Ccl5	1.6733	0.0208
Col4a5	1.7042	0.0043
Fas	1.8897	0.0043
Cd8a	2.1861	0.0031
Tnfrsf18	2.2092	0.0051
Il12a	3.9010	0.0137

Figure 1. Macrophages are the primary source of interleukin-10 in mammary tumors

(A) Representative images of F4/80 immunoreactivity (left panels) in mammary tumors from MMTV-PyMT mice treated with IgG_1 or αCSF-1 mAb in combination with paclitaxel (PTX). Quantitation of $CD11b^+F4/80^+MHCII^+Ly6C^-$ macrophages by flow cytometry is shown to the right as a percent of total live cells. Significance was determined by an unpaired *t*-test with Welch's correction with ***p < 0.001. (B) Fold change in whole tissue gene expression in tumors from PTX/αCSF-1 mAb treated mice determined by real-time PCR using a 96-gene immune array. Only significant (p<0.05; Mann-Whitney) changes are shown. n≥12 mice per group. (C) *Il10* mRNA expression levels from FACS-sorted stromal populations isolated from untreated mice as determined by real time PCR. Data is normalized to *Tbp* expression and displayed as mean ± SEM with n=8 per cell type. MØ, macrophage; mono, monocyte; DC, dendritic cell. (D) Correlation between *IL10* expression and various myeloid-associated genes in human breast cancer samples from the TCGA dataset (n=1161). (E) Detection of IL-10 in human breast cancer by immunohistochemistry. 14 CTX-naïve and 9 CTX-treated patient samples were evaluated. Representative images reflecting low and high staining are displayed. (F) Immunofluorescent staining for IL-10, CD163, and DNA using Hoescht 33342 in human breast cancer. Representative images from 1 of 3 patient samples are displayed. See also Figure S1.

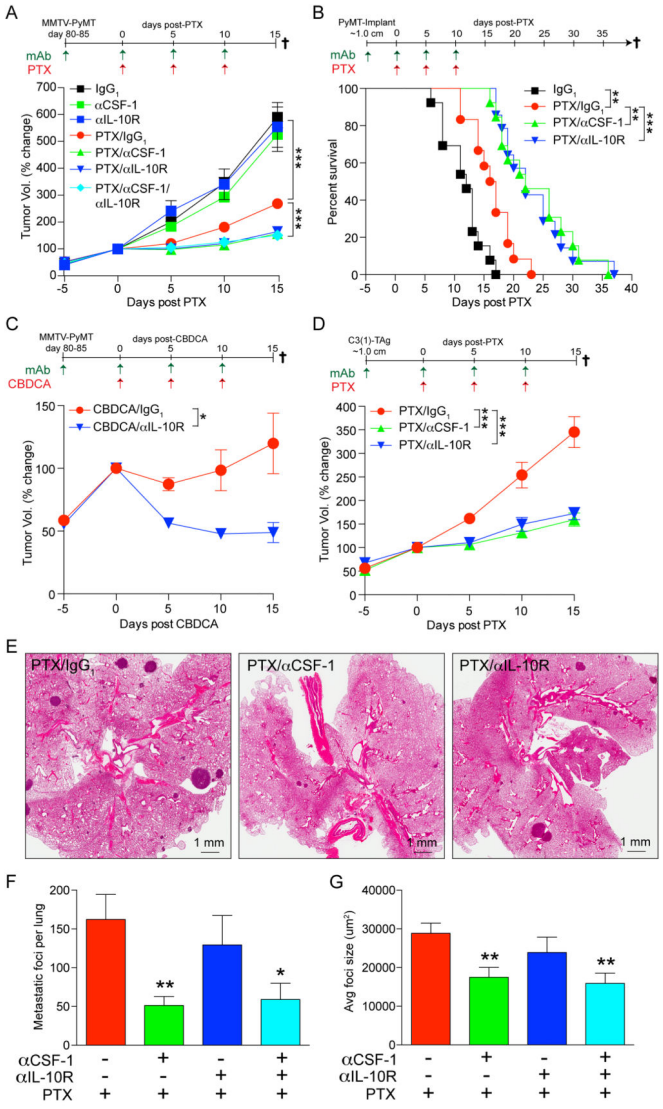


Figure 2. IL-10 receptor blockade improves response to paclitaxel

(A) Relative tumor volume in MMTV-PyMT mice following treatment with IgG_1, αCSF-1 mAb, αIL-10R mAb, PTX, or a combination thereof. Dosing strategy is displayed at top. Data is displayed as mean ± SEM with n≥9 mice per group. Significance was determined by two-way ANOVA with ***$p < 0.001$. (B) Survival of mice bearing orthotopic PyMT-derived tumor explant tumors following treatment with IgG_1, αCSF-1 mAb, αIL-10R mAb, PTX, or a combination thereof. Significance was determined by log-rank with **$p < 0.01$, ***$p < 0.001$ with n≥12 mice per group. (C) Relative tumor volume in MMTV-PyMT mice following treatment with IgG_1 or αIL-10R mAb in combination with carboplatin (CBDCA). Dosing strategy is displayed at top. Data is displayed as mean ± SEM with n≥5 mice per group. Significance was determined by two-way ANOVA with *$p < 0.05$. (D) Relative tumor volume in C3(1)-TAg mice following treatment with IgG_1, αCSF-1 mAb, or αIL-10R mAb in combination with PTX. Dosing strategy is displayed at top. Data is displayed as mean ± SEM with n≥8 mice per group. Significance was determined by two-way ANOVA

with ***$p < 0.001$. (E) Representative H&E sections of lungs taken from animals treated with PTX alone or in combination with αCSF-1 mAb or αIL-10R mAb. (F) The number of metastastic foci per lung in each treatment group from A. Data is displayed as mean ± SEM with n≥9 mice per group. Significance was determined by an unpaired *t*-test with Welch's correction with *$p < 0.05$, **$p < 0.01$. (G) The average size of metastatic foci in each treatment group. Data is displayed as mean ± SEM with n≥9 mice per group. Significance was determined by an unpaired *t*-test with Welch's correction with **$p < 0.01$. See also Figure S2.

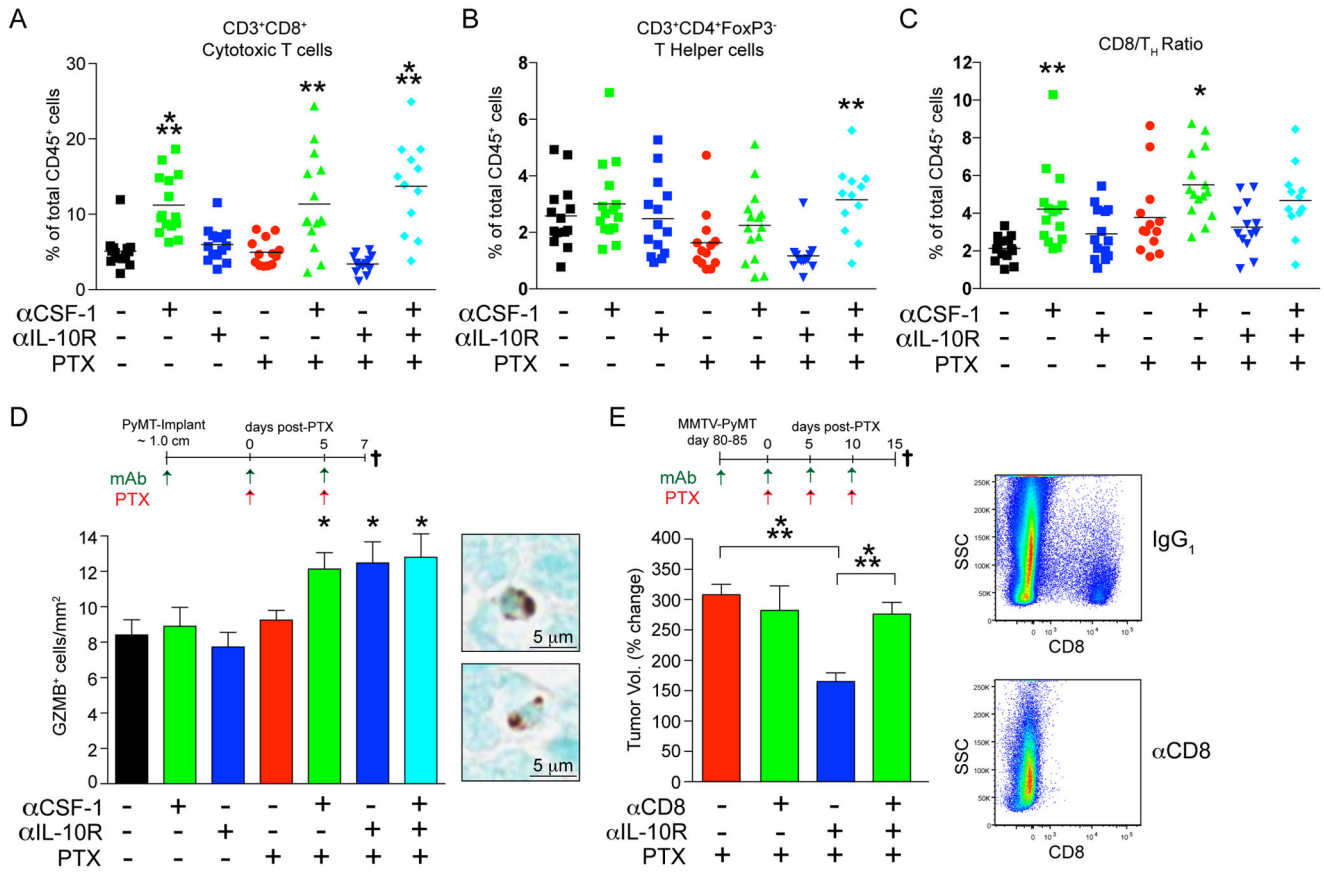


Figure 3. Response to αIL-10R/PTX is CD8$^+$ T cell-dependent

(A) Flow cytometric quantitation of CD3$^+$CD8$^+$ cytotoxic T cells (A), CD3$^+$CD4$^+$FoxP3$^-$ T_H cells (B), and ratio of CD8$^+$ to CD4$^+$ T_H cells (C) in orthotopic PyMT-derived tumors 2-days following the 2nd dose of PTX (day 7). Significance was determined by unpaired *t*-test with Welch's correction relative to IgG_1 or IgG_1/PTX control groups, with *p < 0.05, **p < 0.01, ***p < 0.001. (D) Density of granzyme B (GZMB)-expressing cells in orthotopic PyMT-derived tumors on day 7 as determined by immunohistochemistry. Data is displayed as mean ± SEM with n≥12 mice per group. Significance was determined by an unpaired *t*-test with Welch's correction relative to IgG_1 or IgG_1/PTX control groups, with *p < 0.05. Representative images are shown to the right. (E) Relative mammary tumor volume after 3 rounds of PTX in MMTV-PyMT transgenic mice following treatment with αIL-10R mAb and PTX. CD8$^+$ T cells were depleted 5-days prior to the first dose of PTX. Data is displayed as mean ± SEM with n≥5 mice per group. Significance was determined by an unpaired *t*-test with ***p < 0.001. Polychromatic dot plots displaying CD8$^+$ T cell depletion are shown to the right. See also Figure S3.

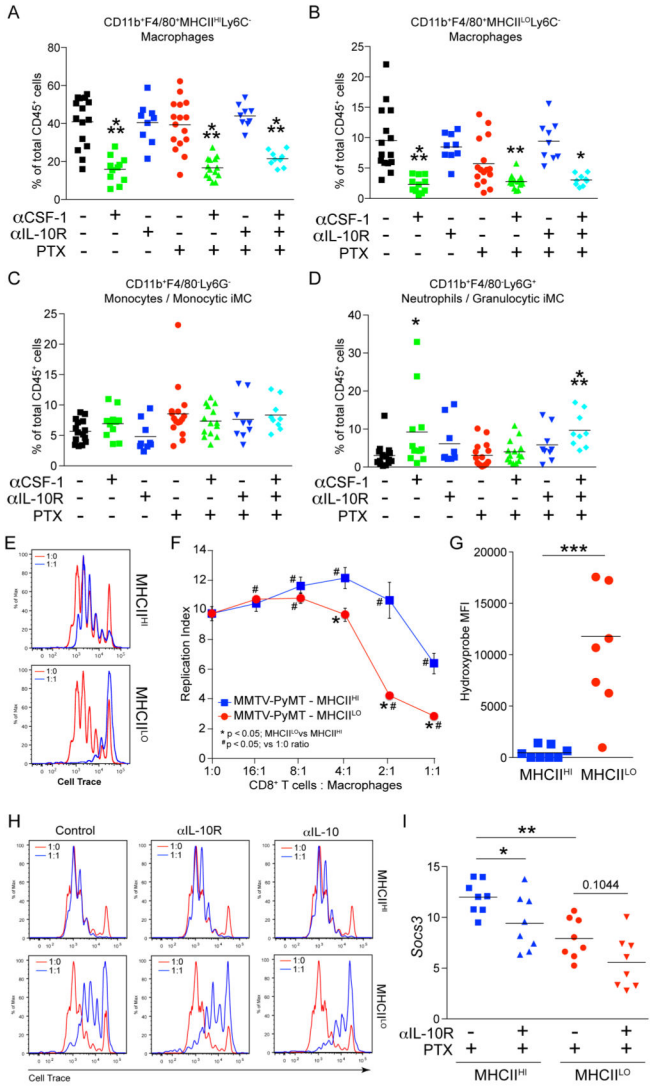


Figure 4. IL-10 does not influence macrophage recruitment, polarization or function

(A–D) Percent of total $CD45^+$ cells in mammary tumors from A, comprised of $CD11b^+F4/80^+MHCII^{HI}Ly6C^-$ macrophages (A), $CD11b^+F4/80^+MHCII^{HI}Ly6C^-$ macrophages (B), $CD11b^+F480^-Ly6G^-$ monocytes (C), and $CD11b^+F4/80^-LyG^+$ neutrophils (D). Data generated by flow cytometry. Significance determined by an unpaired t-test with Welch's correction relative to IgG_1 or IgG_1/PTX control groups, with *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (E) Representative histograms displaying cell trace fluorescence of $CD8^+$ splenic T cells stimulated with αCD3/αCD28 for 60 hrs either alone (red) or co-cultured (blue) at a 1:1 ratio with FACS-sorted $MHCII^{HI}$ or $MHCII^{LO}$ macrophages from mammary carcinomas. (F) Fold expansion of dividing $CD8^+$ T cells (replication index) is displayed at various ratios of $CD8^+$ T cells to macrophages as described in E. One of two experiments is shown, with samples assayed in triplicate. Data is displayed as mean ± SEM. (G) Detection of hydroxyprobe (pimonidazole) by intracellular staining of tumor-associated macrophages. Significance determined by unpaired *t*-test with Welch's correction with ***$p < 0.001$. (H) Representative histograms displaying cell trace fluorescence of $CD8^+$ splenic T

cells as described in E, with addition of 10 μg/ml αIL-10R or αIL-10 mAb added at the start of the incubation period. One of two experiments is shown. (I) *Socs3* expression in $MHCII^{HI}$ and $MHCII^{LO}$ tumor macrophage subsets determined by real time PCR, with data analyzed by comparative threshold cycle method using *Tbp* as a reference gene. Significance determined by Mann-Whitney with $*p < 0.05$, $**p < 0.01$. See also Figure S4.

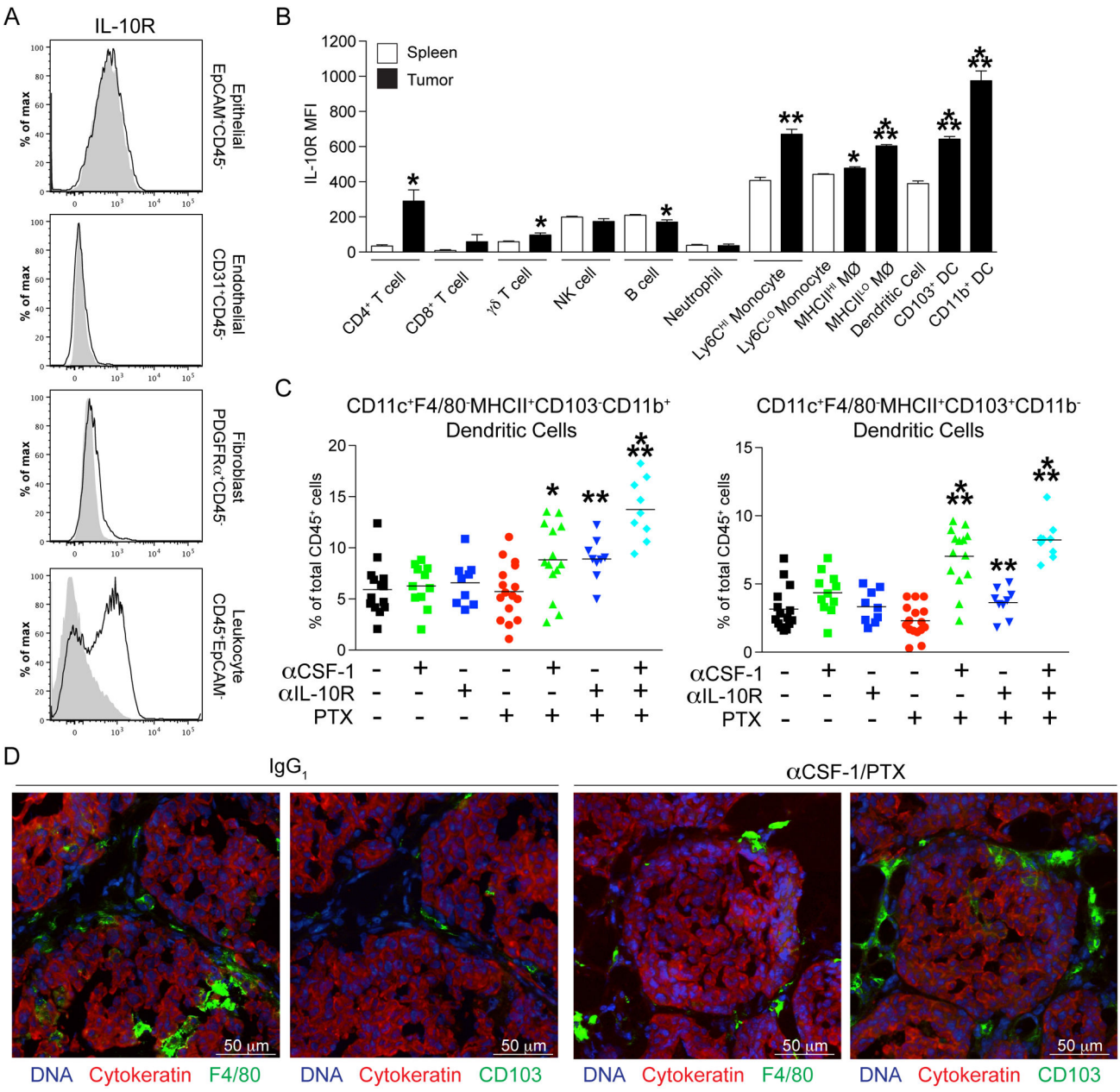


Figure 5. Expression of IL-10 receptor by tumor dendritic cells

(A) Flow cytometric analysis of IL-10R surface expression for EpCAM$^+$ epithelial cells, CD31$^+$ endothelial cells, PDGFRα$^+$ fibroblasts, or CD45$^+$ leukocytes in untreated MMTV-PyMT mice at end stage (>100 days). Fluorescence minus one (FMO) controls are shown in gray for each histogram. Representative data from 1 of 3 animals is shown. (B) Surface expression of IL-10R measured by mean fluorescence intensity (MFI) minus background in various leukocyte populations from mammary tumors from MMTV-PyMT mice as compared to similar populations in the spleens of non-tumor bearing animals. Data is displayed as mean ± SEM with n=3 mice per group. Significance determined by an unpaired *t*-test compared to the spleen, with *p < 0.05, **p < 0.01, ***p < 0.001. MØ, macrophage;

mono, monocyte; DC, dendritic cell. (C) Flow cytometric analysis of $CD11c^+F4/80^-MHCII^+CD103^-CD11b^+$ DCs (left panel) or $CD11c^+F4/80^-MHCII^+CD11b^-CD103^+$ DCs (right panel) in tumors from the MMTV-PyMT animals shown in 2A. Significance determined by unpaired *t*-test relative to IgG_1 or IgG_1/PTX control groups, with $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (D) Localization of $F4/80^+$ macrophages and $CD103^+$ leukocytes within the tumor stroma of MMTV-PyMT tumors via immunofluorescent staining of serial sections. Representative images from 1 of 3 animals are displayed for each group. See also Figure S5.

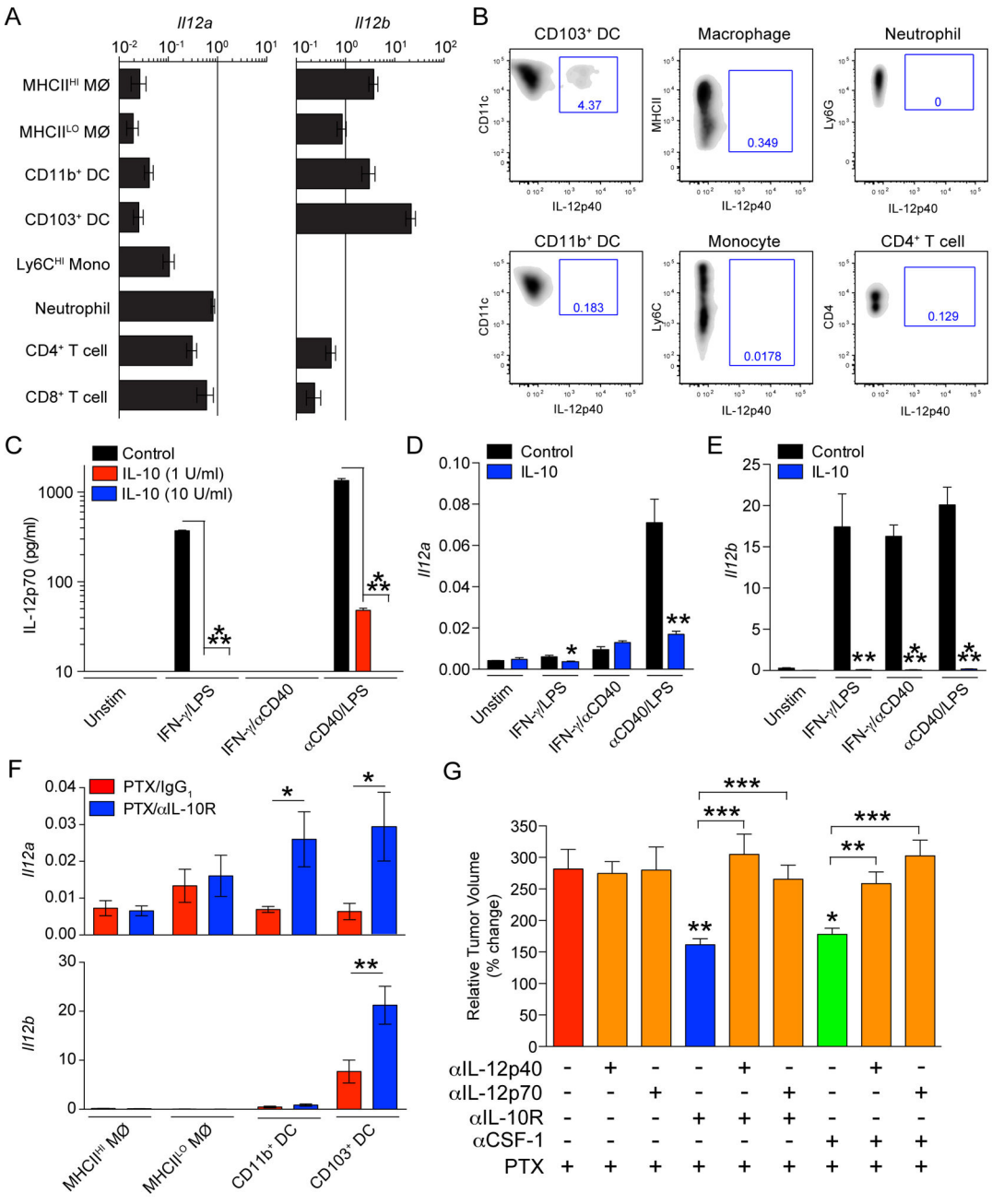


Figure 6. Response to PTX is dependent on IL-12 expression by dendritic cells

(A) *Il12a* or *Il12b* mRNA expression levels in FACS-sorted leukocytes from mammary tumors of MMTV-PyMT mice as determined by real time PCR. Data is normalized to *Tbp* expression and displayed as mean ± SEM with n=8 per cell type. MØ, macrophage; mono, monocyte; DC, dendritic cell. (B) Detection of IL-12p40 by intracellular flow cytometry in tumor leukocytes following in vivo administration of brefeldin A. Representative data from 1 of 2 animals is shown. (C) Production of IL-12p70 by BMDCs as measured by ELISA following 24-hour stimulation with IFN-γLPS, IFN-γ/αCD40 or αCD40/LPS. Cells were pre-treated for 24 hours with 1 to 10 U/ml of IL-10 prior to stimulation. (D–E) Expression of

Il12a (D) or *Il12b* (E) mRNA by BMDCs as measured by RT-PCR. Cells were pre-treated for 24 hours with 10 U/ml of IL-10 prior to stimulation. For C–E, data is displayed as mean ± SEM and samples were assayed in quadruplicate and one of two representative experiments is shown. Significance determined by unpaired *t*-test relative the control group, with *p < 0.05, **p < 0.01, ***p < 0.001. (F) *Il12a* or *Il12b* mRNA expression levels in FACS-sorted macrophage (MØ) and DC populations from mammary tumors of MMTV-PyMT mice 2-days following the 2nd dose of PTX (day 7) in combination with αIL-10R mAb. Data is normalized to *Tbp* expression and displayed as mean ± SEM with n≥4 mice per cell type. Significance determined by Mann-Whitney with *p < 0.05, **p < 0.01. (G) Relative tumor volume after 3 rounds of PTX in MMTV-PyMT transgenic mice following combination therapy with αCSF-1 mAb or αIL-10R mAb. IL-12 neutralizing mAb (αIL-12p40 or αIL-12p70) administered concurrently every 5 days. Data is displayed as mean ± SEM with n≥5 mice per group. Significance determined by unpaired *t*-test with *p < 0.05, **p < 0.01, ***p < 0.001. See also Figure S6.

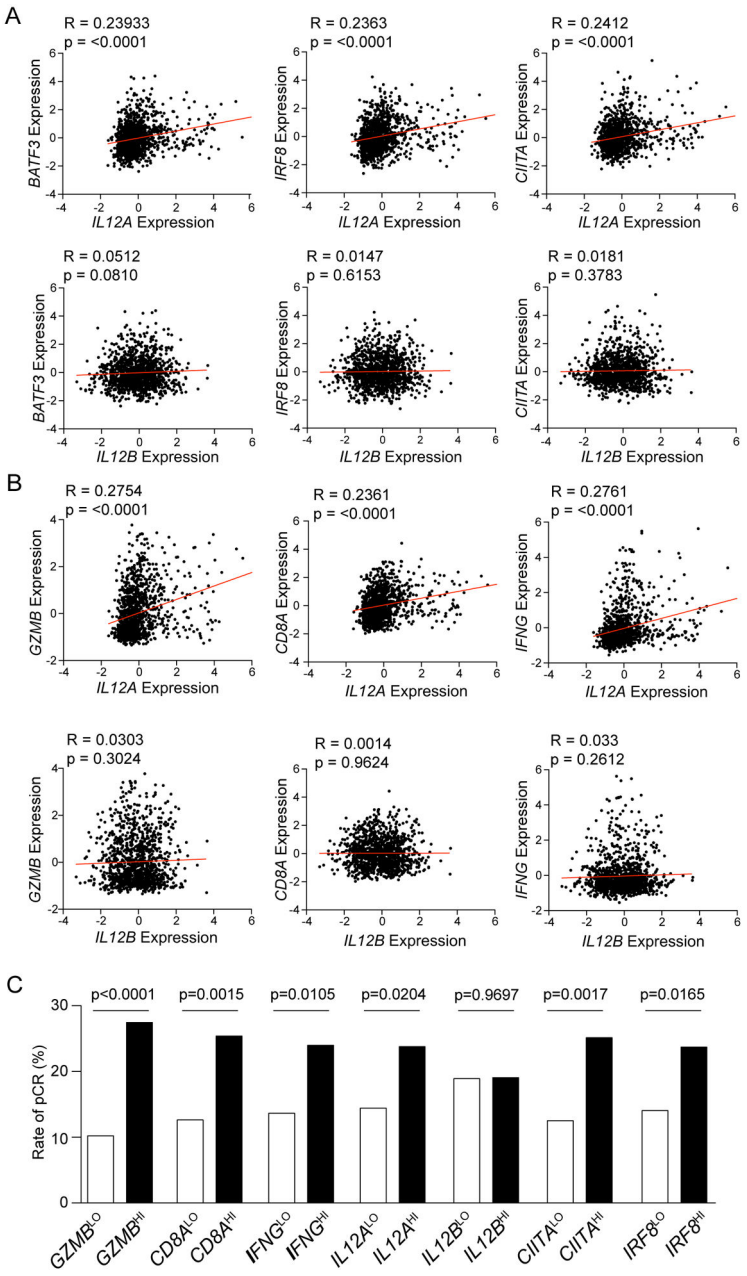


Figure 7. *IL12A* expression correlates with pathologic complete response rates in breast cancer

(A) Linear regression analysis between *IL12A* or *IL12B* expression and various DC-associated genes (*CCR7*, *IRF8*, *CIITA*) in human breast cancer samples from the TCGA dataset (n=1161). (B) Linear regression analysis between *IL12A* or *IL12B* expression and various cytotoxic lymphocyte-associated genes (*GZMB*, *CD8A*, *IFNG*) in human breast cancer samples from the TCGA dataset. (C) Frequency of pathologic complete response (pCR) in patients separated by median expression for genes associated with a cytotoxic T cell response (*GZMB*, *CD8A*, *IFNG*) or DCs (*IL12A*, *CIITA*, *CCR7*, *IRF8*). Data reflects a cohort of 379 patients constructed from 2 independent datasets. Significance was determined by chi-square.

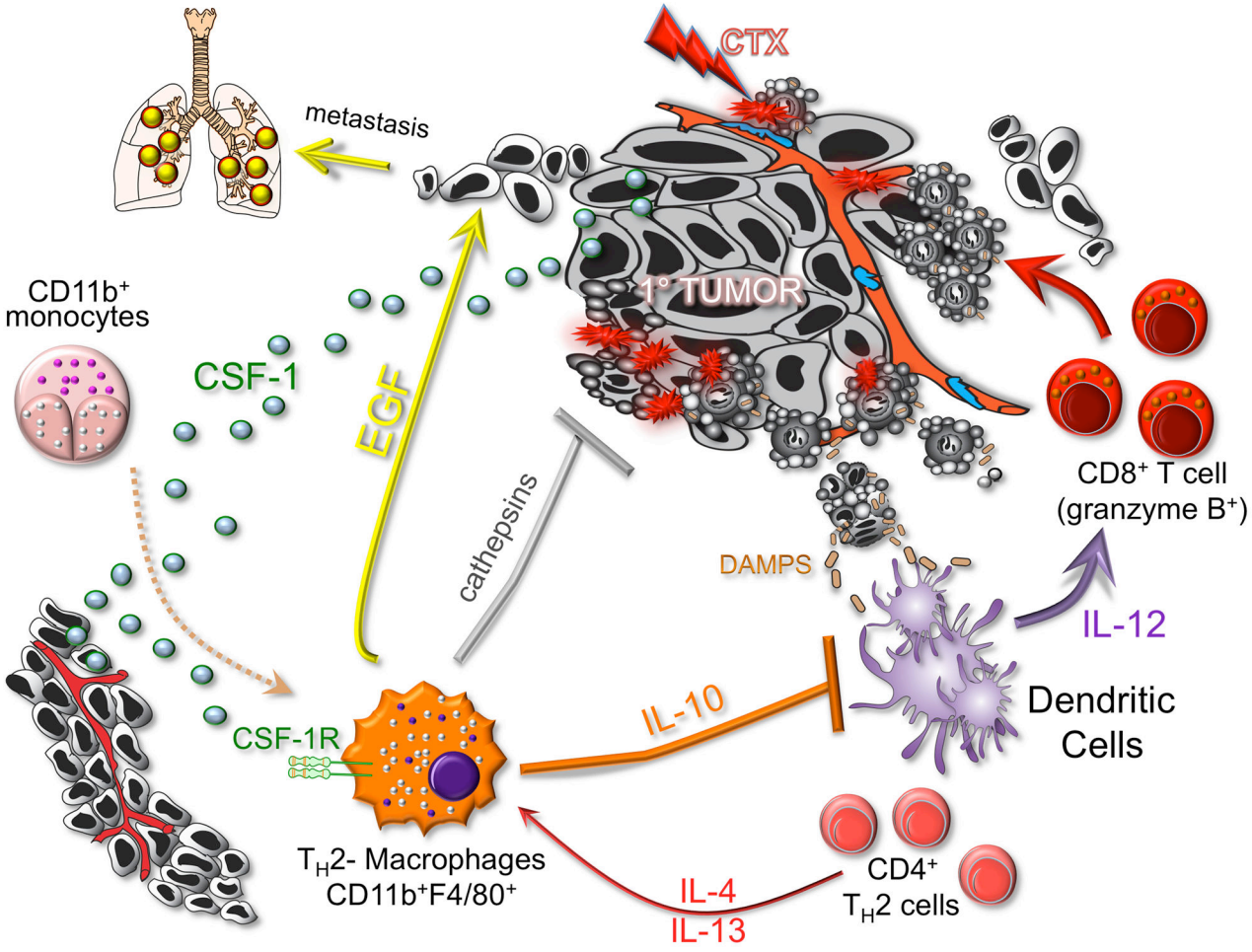


Figure 8. Model of IL-10 suppressing IL-12 production by dendritic cells and limiting the CD8$^+$ T cell-dependent response to CTX in mammary carcinomas

CD11b$^+$ monocytes are recruited into mammary tumors through increased CSF-1 gradients largely derived from epithelial cells. Once in tissue/tumor parenchyma, differentiated macrophages stimulate invasion and metastasis through EGF, which is induced in response to CSF-1, in combination with IL-4/IL-13 derived from CD4$^+$ T cells. IL-4/IL-13 also induces expression and/or activity of cathepsin proteases, directly promoting resistance to CTX-induced cell death. Macrophages further suppress CTX efficacy by indirectly limiting a cytotoxic T cell response. Macrophage-derived IL-10 suppresses the ability of dendritic cells to produce IL-12 during CTX, likely in response to damage associated molecular patterns (DAMPs). In the absence of macrophages or IL-10, IL-12 is able to promote a productive CD8$^+$ T cell response, thereby enhancing response to chemotherapy.