Macrophages

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Specialization determines outcomes in inflammation and cancer

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The functional heterogeneity of macrophages has ontological and microenvironmental bases, and differentially affects pathology. In pancreatitis, tissue-resident macrophages promote protective fibrosis that favors the maintenance of pancreatic homeostasis. In pancreatic ductal adenocarcinoma, they promote tumor progression by facilitating stromal desmoplasia.

During organogenesis, macrophages originating during fetal development from embryonic progenitors differentiate into self-regenerating subsets of tissue-resident macrophages (TRMs) that seed into all tissues and become essential for tissue growth, homeostasis and repair[1]. In adults, meanwhile, blood monocytes are derived primarily from the bone marrow, with some contribution from splenic hematopoiesis, and orchestrate defense effector and repair functions, also acquiring phenotypic traits of TRMs. Hence, physiological events (such as ontogenesis) and pathological conditions (including allergic and chronic inflammation, tissue repair, infection and cancer) concur to dictate phenotypic heterogeneity and the functional skewing of macrophage populations in vivo[2]. In this issue of *Nature Immunology*, Baer et al.[3] study the role of TRMs and monocyte-derived macrophages in inflammation (cerulein-induced pancreatitis) and cancer (pancreatic ductal adenocarcinoma, PDAC), and propose that TRMs have opposite roles in these two pathological contexts — protective in pancreatitis and protumoral in PDAC (Fig. 1). This divergence is implemented through the activation of specific functional programs in mesenchymal cells (fibroblasts). The authors' conclusions support the centrality of ontogeny in the specialization of macrophage functions, which necessarily integrates with a variety of physiological, pathological and aging-related variables[4].

Macrophage functions can also evolve dynamically under the control of cell-intrinsic mechanisms, as observed during the spontaneous recovery of homeostatic functions in macrophages undergoing lipopolysaccharide-mediated tolerance[5]. During tumor development, dynamic adaptation of macrophage functions may also occur in conjunction with the remodeling of tumor microphysiology (with changing oxygen availability, pH, glucose levels, amino acids, lipid metabolism and inflammatory signals), and as a result of hematopoietic changes

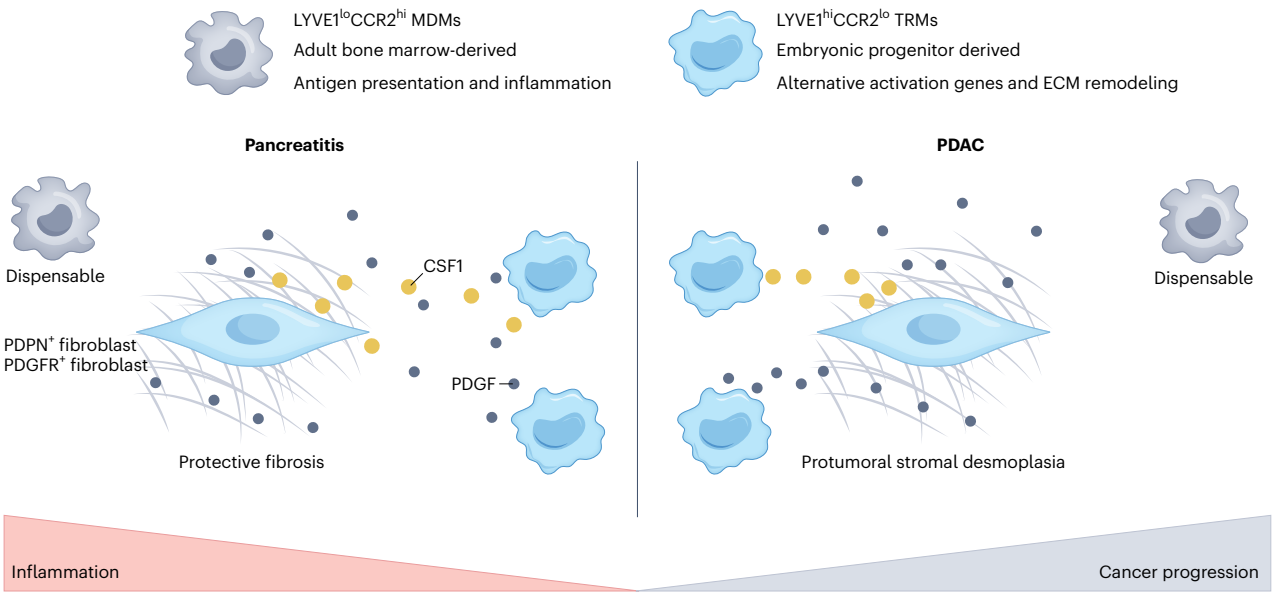

Fig. 1 | Beneficial versus detrimental roles of tissue-resident macrophages in different disease contexts. Baer et al.[3] have identified opposing roles of macrophages in inflammation and cancer. Left, in pancreatitis, TRMs have a protective role, activating a PDGF-dependent fibrotic response through a paracrine communication loop (PDGF–CSF1) with fibroblasts. The role of LYVE1loCCR2hi MDMs in pancreatitis and PDAC is considered dispensable by the authors, as these cells do not have a protective role in pancreatitis or a protumor role in PDAC. Right, in PDAC, TRM-mediated activation of fibroblasts results in protumoral stromal desmoplasia.

(for example, emergency hematopoiesis) that influence the differentiation and availability of myeloid progenitors[6]. Growing interest in the mechanisms that drive this functional heterogeneity underscores its relevance in both physiology and pathology, fueling efforts to therapeutically re-educate macrophage functions[7].

Baer et al.[3] found that, in both pathological settings (pancreatitis and PDAC), there was a substantial increase in the number of pancreatic CD163$^+$ macrophages and in acinar-to-ductal metaplasia, correlating with a greater number of fibroblasts and collagen deposition. The authors carried out lineage-tracing analysis using FLT3-Cre mice crossed to R26-LSL-eYFP mice: this mouse model makes it possible to mark all cells derived from hematopoietic stem cells with yellow fluorescent protein (YFP), while embryonic-derived macrophages are unlabeled. Their results confirmed that the expanded TRM population was of both adult bone marrow (FTL3–YFP$^+$) and embryonic (FTL3–YFP$^-$) origin, with the latter preferentially expanding through in situ proliferation. Differential gene-expression analysis showed that part of the embryonic Ftl3–YFP$^-$ macrophages fell in a LYVE^{hi} cluster, which is endowed with protumoral activity[8], and largely expressed alternative activation genes and genes involved in remodeling of the extracellular matrix (ECM) such as *Mmp9*, *Adam33* and *Ecm1*. By contrast, the FTL3–YFP$^+$ macrophage population colocalized with a LYVE1^{lo} CCR2^{hi} cluster and showed high expression of antigen-processing and -presentation genes (MHC class II genes), costimulatory molecules, inflammatory mediators and NF-κB signaling molecules. The LYVE1^{hi} macrophages had a distinct distribution compared with LYVE1^{lo} macrophages and were localized mainly in the stromal areas between pancreatic lobules. Parallel analysis in human pancreatitis and PDAC tissues confirmed a high degree of conservation between mouse and human LYVE1$^+$ macrophages, in terms of both gene-expression profiles and stromal localization.

The authors then used a combination of neutralizing antibodies to the macrophage-stimulating cytokine CSF1 plus clodronate-loaded liposomes to deplete TRMs during cerulein-induced pancreatitis in mice. In this setting, the animals presented a loss of more than 90% of amylase-expressing acinar cells, a substantial loss of CK19$^+$ ductal cells, increased expression of cleaved caspase-3, a loss of body weight and a high mortality rate, indicating that F4/80$^+$LYVE1$^+$CD163$^+$ TRMs are crucial in maintaining pancreas homeostasis following tissue damage by pancreatitis. In contrast, CCR2-deficient mice — which have a reduction in blood Ly6C^{hi} monocytes and monocyte-derived macrophages — did not show differences in survival, body weight or pancreas weight compared with wild-type controls, suggesting that these cells are largely dispensable for tissue homeostasis.

Given that LYVE1$^+$ macrophages represent a major embryonically derived population of TRMs located within the stromal areas of the pancreas, Baer et al.[3] then crossed Lyve1-Cre mice to *Csf1r*$^{fl/fl}$ mice to specifically delete the CSF1 receptor (CSF1R) in LYVE1$^+$ cells. Although Lyve1$^{\Delta CSF1R}$ mice treated with serial injections of cerulein showed no reduction in the total number of macrophages or changes in any LYVE1$^-$ population, they did exhibit reduced expansion of fibroblasts expressing podoplanin (PDPN) or platelet-derived growth factor-α (PDGFRα). These changes were not observed in cerulein-treated CCR2-deficient mice, suggesting that LYVE1$^+$ TRMs critically coordinate the protective fibrotic response for maintenance of pancreatic function during tissue injury. To confirm this, the authors carried out transcriptional profiling (single-cell RNA sequencing) and gene-set enrichment analysis of total fibroblasts from mice challenged with cerulein and then treated either with immunoglobulin G plus PBS (as a control), or with CSF1 antibodies plus clodronate. Fibroblasts from the control mice were enriched in genes related to ECM production and organization, while fibroblasts from animals treated with CSF1 antibodies plus clodronate — which were depleted of TRMs — expressed pathways associated with pancreatitis severity, including interferon-α and -γ responses, as well as gene sets related to NF-κB signaling[9].

Going on to analyze receptor–ligand interactions, the authors found that the PDGF signaling pathway was enriched between LYVE1^{hi} TRMs and fibroblasts expressing the *Pdgfra* and *Pdgfrb* receptors, while all fibroblast populations produced CSF1, which signaled in all macrophage clusters. Consistent with this, use of a PDGFR inhibitor (imatinib mesylate) in cerulein-treated mice did not alter the number of LYVE1^{hi} or other TRMs, but did reduce the expression of fibronectin and the expansion of PDPN$^+$ and PDGFRα$^+$ fibroblasts, prevent the recovery of body weight and impair survival. So, accumulation of protective PDFGRα$^+$ fibroblasts during pancreatic injury relies on the PDFG–CSF1 axis, which serves as a paracrine communication loop between TRMs and fibroblasts.

Finally, Baer et al.[3] investigated the role of TRMs in spontaneous models of PDAC. Using CSF1 antibodies plus clodronate to deplete TRMs in KPC mice — which carry a pancreas-specific Cre recombinase that activates a KRAS mutation (KRAS^{G12D}) and causes loss of p53 expression — induced an 80% reduction in the number of F4/80$^+$LYVE1$^+$CD163$^+$ TRMs in the tumor tissue and lowered the burden of high-grade tumors. Similar results were obtained in a more aggressive (KPPC) and an inducible (iKRAS*) model of PDAC. By contrast, orthotopic tumor implantation in CCR2-deficient mice resulted in similar tumor weights and sizes and fibroblast numbers as in wild-type control recipients. So, TRMs are likely driving PDAC progression in part by facilitating stromal desmoplasia.

The work by Baer et al.[3] addresses the relevance of macrophage specialization in inflammation and cancer, providing evidence that the protective (pancreatitis) and protumoral (PDAC) effects of TRMs are guided through the regulation of stromatogenesis (Fig. 1). Because chronic inflammation leads to pronounced fibrosis, with destruction of organ architecture and loss of function associated with a high mortality rate[10], and because therapeutic antifibrotic options are limited, analysis of macrophage subsets during the transition from acute to chronic inflammation and their interactions with mesenchymal stromal cells should become a goal of therapeutic development. Moreover, increased stromal gene expression predicts resistance to anticancer therapy, influencing disease recurrence and mortality[11]. Hence, the dynamic relevance of the interactions between fibroblasts and macrophages should be investigated in cancers that are characterized by pronounced desmoplastic stromal reactions, such as hepatocellular carcinoma, cholangiocarcinoma, non-small cell lung cancer and breast cancer. The crosstalk described between TRMs and fibroblasts suggests possible implications for strategies aimed at turning 'cold' tumors into 'hot' ones, in the attempt to make the tumors more immunogenic and to improve the therapeutic efficacy of immune-checkpoint inhibitors[12]. In view of the well-known role of CCR2 in inflammatory disorders and cancers, the low observed impact of CCR2$^+$ monocyte-derived macrophages in the two pathologies studied here is somewhat surprising.

Macrophage heterogeneity has ancient evolutionary roots, and although it has been the subject of numerous studies, its regulatory mechanisms remain partly in shadow. The macrophage coin has both helpful and harmful faces: it remains for us to learn how to select the face with the most benefit.

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Competing interests

The authors declare no competing interests.