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Novel Insights Into Macrophage Diversity During the Course of Pancreatitis

See "Novel circulating and tissue monocytes as well as macrophages in pancreatitis and recovery," by Manohar M, Jones EK, Rubin SJS, et al, on page 2014.

Acute pancreatitis (AP) accounts for the highest number of hospital admissions for a benign gastrointestinal disease. Although most of the cases are mild and patients recover without specific treatment, 20% of cases follow a moderate to severe course with risk for multiple organ dysfunction syndrome and death and no medical treatment is available.[1,2] Pancreatitis-related mortality depends to a large extent on the presence and duration of systemic inflammatory response syndrome,[3] which, if persistent, will result in multiple organ dysfunction syndrome.[4] Intrapancreatic zymogen activation causes acinar cell injury and tissue necrosis,[5,6] subsequently leading to recruitment of inflammatory cells to the pancreas.[7,8] Inflammatory cells determine disease severity via inducing and perpetuating local pancreatic tissue

damage and subsequent organ failure. Local pancreatic damage is significantly enhanced by infiltrating or tissue-derived leukocytes, which affect trypsinogen activation.[9–12]

During the early phase of pancreatitis, macrophages are the prevailing type of immune cells in the pancreas,[13] and the proportion of neutrophil granulocytes is considerably smaller.[7] Cells of the adaptive immune system are only scarcely detected in the pancreas, but do play a role in regulation of the systemic immune response.[5,12] Unlike neutrophils, macrophages are highly plastic and can therefore better adapt to the local environment. Macrophage polarization depends on the stage of the disease. During AP, macrophage differentiation is driven by damage-associated molecular patterns released by the destructed acinar cells, as well as by pro-inflammatory cytokines. In addition to damage-associated molecular patterns, trypsinogen activation can also enhance pro-inflammatory macrophage (M1) polarization. Intracellular cathepsin B–mediated trypsinogen activation is not necessarily restricted to acinar cells. Macrophages phagocytose necrotic areas within the pancreas and during this process take up zymogen granules, which contain trypsinogen. Phagocytosed material is degraded via the lysosomal pathway, resulting in co-localization of trypsinogen and cathepsin B in phagolysosomes of macrophages.[7] Active trypsin destabilizes the membrane of vesicles and ultimately leads to the release of contents into the cytosol.[14] This mechanism has been described for acinar cells, but also applies to macrophages. In addition to trypsin, cathepsin B also enters the cytosol and is a potent inducer of inflammasome activation, which results in pyroptotic cell death of macrophages and further drives inflammation. The release of mature interleukin (IL)-1β fosters local inflammation, which enhances pancreatic damage.[13] The IL-1β signaling pathway acts via the IL-1 receptor/MyD88, which finally results in NFκB activation and therefore drives pro-inflammation and M1-like polarization of macrophages.[7,12] Tumor necrosis factor-α (TNFα) is a cytokine released mainly by M1 macrophages and it is known that acinar cells react to TNFα.[9] In addition to acinar cell necrosis, TNFα is able to induce intra-acinar trypsinogen activation.[9] The link between necrosis and trypsinogen activation and TNFα is the death receptor–mediated necroptosis cell death pathway, which is the predominant form of cell death in case of AP.[15] The main source of TNFα is proinflammatory macrophages. In line, specific macrophage subtypes, in conjunction with other inflammatory and parenchymal cells, regulate both the initiation and resolution of inflammation during pancreatitis, which makes them an attractive target to ameliorate pancreatitis and related systemic complications.[7,12,16] Based on function, surface marker expression, and cytokine release, macrophages are classified into M1 macrophages—associated with systemic inflammatory response syndrome–related pro-inflammation, as described above[13]—and M2 macrophages—their anti-inflammatory counterpart—with further subtypes mediating type II inflammation, allergic responses, and defense against parasites (M2a); Th2 activation and immune-regulation (M2b); or tissue repair, matrix remodeling, and fibrosis (M2c)[17,18] (Figure 1). Furthermore, the polarization of macrophage subtypes in specific tissues is dynamically shaped by the surrounding microenvironment. Given their pivotal role in pancreatitis,[19] identification of macrophage subtypes as prognostic markers reflecting disease stage or as therapeutic targets could be a promising approach to developing specific therapies for the medical management of AP and other sterile inflammatory diseases—a clearly unmet clinical need.

In this issue of *Gastroenterology*, Manohar et al,[20] introduce novel subsets of monocytes and macrophages and indicate their potential pathologic roles in mild and severe mouse models of pancreatitis (caerulein-induced mild AP and choline-deficient-ethionine supplemented diet-induced severe AP). The authors dissect the heterogeneity of monocytes and macrophages during the different stages of pancreatitis and recovery using up-to-date cytometry by time of flight, validated by fluorescence-activated cell sorting analysis. In addition, they transfer their findings into human peripheral blood mononuclear cells derived from patients with pancreatitis. The authors observed remodeling and reshaping of $CD206^{+}$ M2 macrophages and monocytes, deciphering 6–7 different subsets of macrophages and monocytes based on the differential expression of MHCII, Ly6Gc, and CD44 or CD45RB, respectively. However, absolute abundance, functional characterization, and disease-specific modulation are missing.

For example, during the recovery phase from mild AP, the authors detected a macrophage subset characterized by $MHCII^{hi}Ly6Gc^{hi}$ expression, and potential mediators possibly secreted from this subset are interferon-gamma, IL-4, IL-10, and IL-17A, along with decreased Foxp3 expression. In contrast, a $MHCII^{hi}CD54^{hi}LAP\text{-}TGF^{hi}$ subset is associated with ongoing injury from severe AP, secreting IL-4 and IL-17 and showing increased expression of Foxp3 (Figure 1). The altered expression of Foxp3 points toward tight regulation by TGF-β and its involvement in disease progression in this murine model of severe AP. The $MHCII^{lo}Ly6Gc^{hi}CD44^{lo}$ subset might cause induction of IL-10 and TGF-β at 12 hours and 24 hours, which could indicate its role in regeneration. In line with a previous study[21] that showed the association of IL-22 with pancreatic acinar regeneration, Manohar et al,[20] report a novel macrophage subset, $MHCII^{hi}Ly6Gc^{lo}CD44^{hi}$, linked to increased IL-22 expression during recovery from pancreatitis, indicating a regenerative function. However, also in severe AP, 4 macrophage subtypes (ie, $MHCII^{lo}CD54^{hi}$, $MHCII^{hi}CD54^{hi}$-$LAP\text{-}TGF\text{-}\beta^{hi}$, $MHCII^{hi}CD54^{lo}LAP\text{-}TGF\text{-}\beta^{hi}$, and $MHCII^{hi}CD54^{lo}LAP\text{-}TGF\text{-}\beta^{lo}$) expressed IL-4 and IL-22 at 72 hours, but failed to alter the definitive course of AP in the models used.

Although the identified novel subtypes of $CD206^{+}$ macrophages could play an important role during different phases of pancreatitis and be involved in determining the severity of AP, there is an urgent need to investigate associated downstream signaling cascades and disease outcomes after manipulation of these macrophage subtypes. This

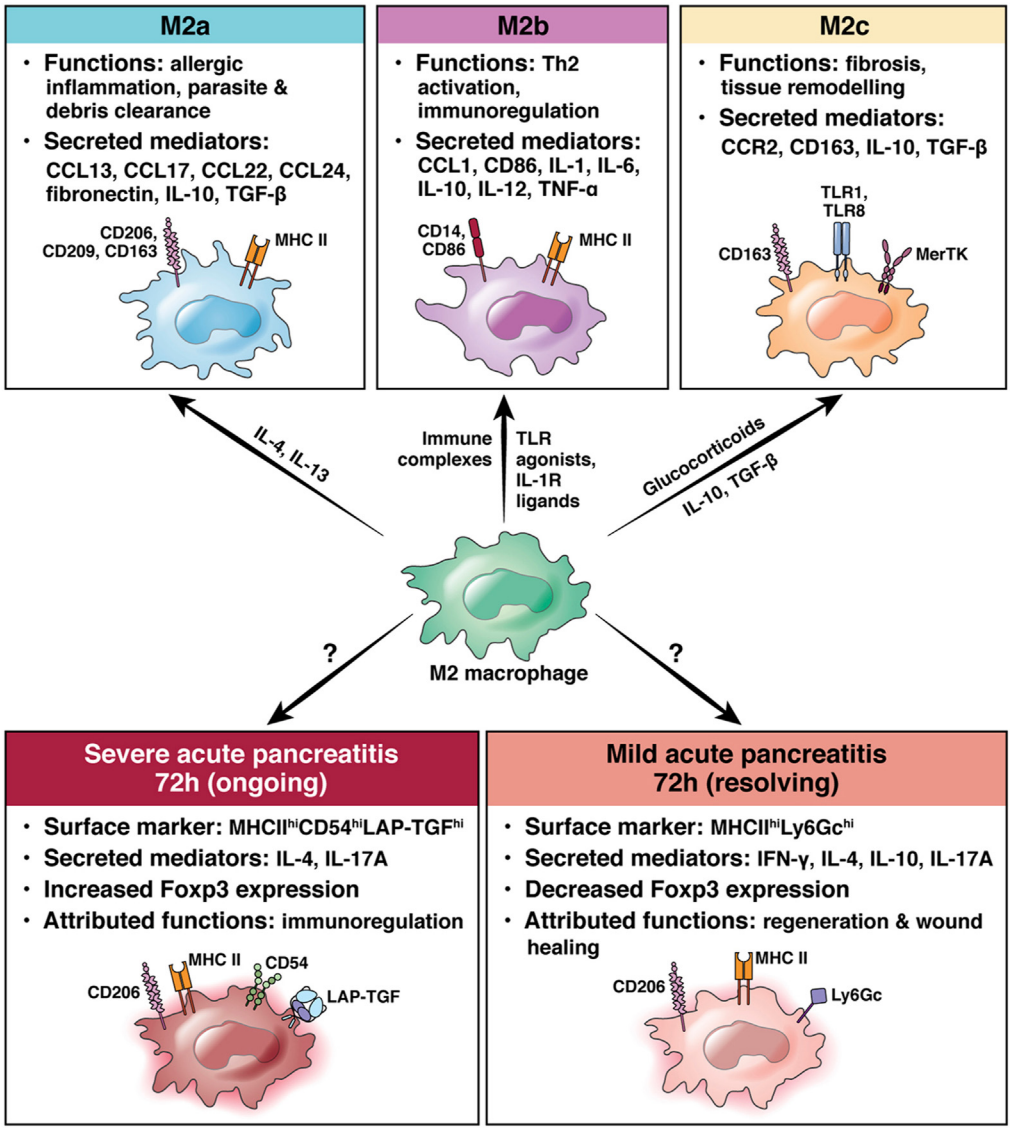

Figure 1. Novel M2 macrophage subtypes and their proposed role during AP. Depending on the severity of AP, distinct M2 macrophage subtypes that differ with respect to surface markers, expression levels of inflammatory mediators, and Foxp3 expression from previously described M2 macrophage subtypes (M2a–c) could be identified using cytometry by time of flight technology.[18,20] Further research should attempt to clarify their biological role in progression and resolution of pancreatitis and how this could be used to benefit patients.

nicely conducted study illustrates a dilemma of up-to-date research—we generate an enormous amount of data, integrate them by biostatistical methods, but only associate and not causally link them to the disease studied. The current study, although important to drive the field, has to be considered as exploratory, and no conclusion as to the relative importance of the different subtypes regarding the course of disease can be drawn. Manohar et al[20] have opened a door toward a better understanding of the origin and biologic significance of these novel $Ly6Gc^{+}/CD206^{+}$ monocytes and macrophages, but to gain further insight, ex vivo expansion of these subsets, adoptive transfer in disease-relevant models, genetic mouse models, and lineage-tracing strategies are needed. Moreover, to unravel the role of different subtypes in diverse disease states and the underlying mechanism of damage, such as necrosis, apoptosis, pyroptosis, necroptosis, and phagocytosis, dissecting the spatial distribution within the damaged pancreas as well as their absolute abundance in tissue is warranted. Of note, as macrophages are able to undergo self-renewal in vivo, they might be excellent targets for treatment[22] and, as such, the current study is an important step toward understanding the complex immune response in pancreatitis.

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