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REVIEW

Intestinal macrophages: differentiation and involvement in intestinal immunopathologies

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Abstract Intestinal macrophages, preferentially located in the subepithelial lamina propria, represent the largest pool of tissue macrophages in humans. As an adaptation to the local antigen- and bacteria-rich environment, intestinal macrophages exhibit several distinct phenotypic and functional characteristics. Notably, microbe-associated molecular pattern receptors, including the lipopolysaccharide (LPS) receptors CD14 and TLR4, and also the Fc receptors for IgA and IgG are absent on most intestinal macrophages under homeostatic conditions. Moreover, while macrophages in the intestinal mucosa are refractory to the induction of proinflammatory cytokine secretion, they still display potent phagocytic activity. These adaptations allow intestinal macrophages to comply with their main task, i.e., the efficient removal of microbes while maintaining local tissue homeostasis. In this paper, we review recent findings on the functional differentiation of monocyte subsets into distinct macrophage populations and on the phenotypic and functional adaptations that have evolved in intestinal macrophages in response to their antigen-rich environment. Furthermore, the involvement of intestinal macrophages in the pathogenesis of celiac disease and inflammatory bowel diseases is discussed.

Keywords Monocytes · Macrophages · Intestinal mucosa · Commensal flora

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Differentiation of monocyte/macrophage subsets

Macrophages represent a functionally and phenotypically highly diverse cell population which is not only crucially involved in innate and adaptive immune reactions, but also in embryonic development, wound repair, and maintenance of local tissue homeostasis [1–3]. Macrophages, monocytes, and their lineage-committed bone marrow (BM) precursors constitute the mononuclear phagocyte system (MPS), which represents a complex cellular system across different organs.

Monocyte progenitors are continuously formed in the BM. The myeloid differentiation process is associated with a progressive loss of differentiation potential. Multipotent hematopoietic precursors, such as lineage-committed precursors $(Lin)^-$, Sca-1^+, and c-kit $(CD117)^+$ [4, 5], are precursors for the common myeloid progenitor cells [6] and the common lymphoid progenitor cells [7]. In CX3CR1-EGFP transgenic mice [8], a subset of myeloid BM cells ($CD115^+$, $CX3CR1^+$, $CD117^+$, and Lin^-) was identified as precursors for macrophages and dendritic cells (DC) but not for neutrophils [9]. These common progenitor cells of the MPS are, therefore, called myeloid lineage macrophage-DC progenitors (MDP). Upon adoptive transfer into the BM of recipient mice, MDPs differentiate into circulating monocytes [9, 10]. After leaving the BM, monocytes circulate in the vasculature for several days before they enter their target tissue to complete their differentiation into macrophages (Fig. 1). The selective recruitment of blood monocyte subsets to peripheral sites of the body is crucial for maintaining the distinct populations of differentiated tissue macrophages [11]. Nevertheless, at least under steady-state conditions, local cell proliferation may also contribute to the replenishment at least of some tissue macrophage subsets [12]. As an example, Kupffer cells

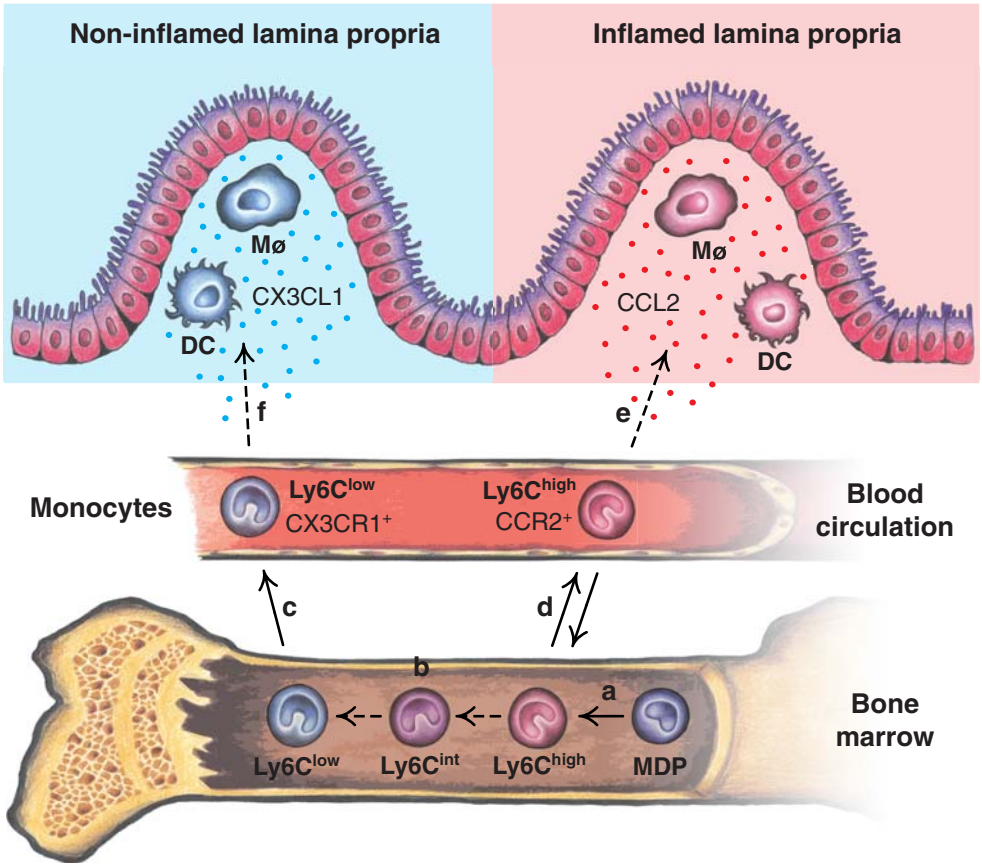

Fig. 1 Origin of monocytes and their differentiation into macrophages (*Mø*) and dendritic cells (*DC*) in the intestinal lamina propria in mice. **a** MDP give rise to Ly6C^{high} BM monocytes [9]. **b** The conversion from Ly6C^{high} to Ly6C^{low} monocytes possibly takes place over an intermediate cell type (*Ly6C^{int}*) in the BM [10, 38]. **c** BM monocytes are released into the peripheral blood circulation, while under homeostatic conditions (**d**), the majority of Ly6C^{high} blood monocytes home back to the BM [10]. **e** Ly6C^{high} monocytes express CC-chemokine receptor 2 (*CCR2*) and may be recruited to sites of inflammation by responding to CC-chemokine ligand 2 (*CCL2*) [10, 20]. **f** Ly6C^{low} monocytes express CX3C-chemokine receptor 1 (*CX3CR1*) and possibly replenish the tissue-resident Mø and DC populations by responding to cleaved CX3C-chemokine ligand 1 (*CX3CL1*). Note that CX3CL1 (fractalkine) is expressed by intestinal epithelial and endothelial cells [101–104], while CCL2 is mainly produced at sites of mucosal inflammation [105]. *Solid arrows* represent pathways that are supported by published data, while *dashed arrows* indicate proposed, still hypothetical pathways. *Blue dots* proteolytically cleaved CX3CL1, *red dots* CCL2. *BM* bone marrow, *MDP* Mø–DC progenitor

[13], microglial cells [14], Langerhans cells [15], and alveolar macrophages [16, 17] were shown to also proliferate locally. In contrast to those tissue macrophages, intestinal macrophages were reported to be nonproliferating [18]. Remarkably, in contrast to effector cells of the adaptive immune system, the proliferative capacity of BM-derived macrophages is blocked after activation with interferon-γ (IFNγ) by a cyclin-dependent kinase (Cdk) inhibitor [19].

Research on human cells has been restricted to in vitro studies and, thus, most data on the functional differentiation of monocyte/macrophage subsets were obtained in animal models. Monocyte heterogeneity is conserved between different species, and the functional commonality of these systems led to the identification of corresponding monocyte subsets in human, mouse, rat, and pig [12]. Adoptive cell transfer experiments and the availability of chemokine receptor-deficient mouse lines were instrumental to obtain insight into the biological heterogeneity of monocytes and macrophages. Although the precise lineage relationships are not yet firmly established in detail, it is generally accepted that distinct subsets of BM and blood monocytes are the precursors of DCs, resident macrophages (i.e., macrophages that are located in the tissue under homeostatic conditions), and inflammatory macrophages (also named exudate macrophages), which are recruited to the tissues during inflammatory conditions. In mice, the "inflammatory" macrophages seem to be derived from a recruited, short-lived inflammatory subset of blood monocytes. These "inflammatory" monocytes are characterized by the high expression of CCR2 and Ly6C and intermediate expression of CX3CR1, while noninflammatory monocytes (CCR2^{-}, Ly6C^{-}, and CX3CR1^{hi}) may differentiate into long-lived resident myeloid cells, including resident tissue macro-

phages [20]. Jung et al. have generated a reporter mouse line in which the gene coding for the fractalkine receptor (CX3CR1) was replaced by a green fluorescent protein reporter gene [8]. Hence, in these CX3CR1-GFP mice, cells of the MPS compartment can be visualized and functionally distinct monocyte subsets can be distinguished (and fractionated) based on the differential expression of CX3CR1-GFP.

It is becoming increasingly evident that macrophages are functionally highly promiscuous and that the exerted functions are crucially determined by the local tissue microenvironment. Hence, the broad functional diversity of mononuclear phagocytes and the wide range of activating signals that may affect their functions is a characteristic attribute of this cell population [12, 21, 22]. This is reflected not only by the plasticity of the functional phenotypes of monocyte/macrophage subsets in vitro, but also by the diversity of functions exerted by macrophage subsets isolated ex vivo from tissues under physiological and inflammatory conditions. These functional and phenotypic adaptations are most prominent in resident macrophages of the intestinal mucosa and have evolved during the coevolution of the local immune system with the microbial flora of the gut lumen.

Heterogeneity of the mononuclear phagocyte system

Cells of the MPS show a remarkably dynamic nature of the functions they may exert when cultured and investigated in vitro. This is reflected in vivo by the high specialization of tissue macrophages at different anatomical locations (e.g., Langerhans cells in the epidermis, osteoclasts in the bone, microglial cells in the brain, splenic macrophages in the red and white pulp and in the marginal zone of the spleen, and the resident macrophages of the intestinal mucosa [12]). The functional heterogeneity and plasticity of macrophages is already evident in their progenitor cells, the monocytes.

In humans, monocytes are generally divided into two distinct subsets based on their size and granularity and also their differential expression of cell surface receptors (pattern recognition receptors [PRRs], Fc receptors [FcRs], and chemokine receptors). These two main monocyte subsets may exert distinct functions and are believed to represent precursors of distinct macrophage subsets. The so-called inflammatory or classical monocytes are bigger (18 μm in diameter), highly phagocytic, and represent the larger subset (80–90% of circulating monocytes in humans). Inflammatory monocytes are $CD14^{hi}$, $CCR1^{+}$, $CCR2^{+}$, $CXCR2^{+}$, and $CX3CR1^{lo}$ and are termed "inflammatory" due to their inflammatory chemokine receptor expression pattern. The so-called resident monocytes are smaller (14 μm in diameter) and represent only 10% of the peripheral blood monocytes. In contrast to the inflammatory monocytes, resident monocytes are generally negative for CD14 and CCR2 but express CD16, CCR5, and CX3CR1 at high levels [23]. The classical (inflammatory) monocytes are believed to migrate chemotactically to sites of inflammation where the production of proinflammatory chemokines such as MCP-1 (CCR2 ligand) and IL-8 (CXCR2 ligand) is upregulated. Resident monocytes, on the other hand, are recruited under homeostatic conditions to noninflamed tissues by virtue of their pronounced expression of CX3CR1 [24]. The classification into inflammatory and resident populations is solely based on the different migration behavior of these two subsets under inflammatory and homeostatic conditions. At present, it is not yet entirely clear to what extent this classification also correlates with possible inflammatory vs. resident functional properties of the respective cell populations. As an example, during infections, even the resident-type monocyte subset ($CD16^{+}$) may increase [25, 26] and these cells produce substantial amounts of tumor necrosis factor (TNF) upon stimulation with Toll-like receptor (TLR) agonists [27]. Hence, macrophages with a "resident" phenotype may also exert proinflammatory functions. Both resident and inflammatory monocyte subsets can differentiate into DCs in vitro by the addition of IL-4 and GM-CSF [28, 29]. An additional, though smaller, third population of blood monocytes was described in humans. This monocyte subset simultaneously expresses CD14, CD16, and CD64. Because of their high phagocytic activity (shared with the inflammatory subset) and the high expression of major histocompatibility complex (MHC) class II (shared with the resident subset), these cells were called transitional monocytes [30, 31].

In the mouse, all circulating monocytes are $CD115^{+}$, $CD11b^{+}$, and $F4/80^{lo}$. Similar to their human counterparts, murine monocytes may be further subdivided into two major subsets, defined mainly by the differential expression of CX3CR1, CD62L, and CCR2. Murine inflammatory monocytes express CCR2, CD62L, and Ly6C at high levels and express CX3CR1 at intermediate levels, while the murine resident monocyte subset is characterized by the high expression of CX3CR1 and the absence of CCR2 and CD62L on the cell surface [20]. In humans as well as in the mouse, inflammatory monocytes appear to be more granular and, with a diameter of 10–14 μm, larger than the resident monocytes (8–12 μm). Using Ly6C, which shares an epitope with Gr-1, as an additional marker of the inflammatory monocyte subset, Geissmann and collaborators demonstrated that the inflammatory subset ($Ly6C^{+}$) corresponds directly to the human classical $CD14^{+}$ monocyte subset, thus confirming the conserved relationship of the MPS between humans and mice. While this relevant finding is in full support of using mouse models also for

defining monocyte/macrophage functions in humans, there are still differences between the mouse and human monocyte/macrophage system as shown in Fig. 2. The ratio of the two main monocyte subsets is generally stable in mice with a small excess of Ly6C$^+$ monocytes under homeostatic conditions [32]. Cells of the short-lived inflammatory subset were shown to migrate to sites of experimentally induced inflammation in a CCR2-dependent manner. By virtue of their recognition of MCP-1 (CCL2) and the CD62L-mediated interaction with high endothelial venules, inflammatory monocytes are recruited to the lymph nodes that drain the sites of inflammation [33]. The CCR2-dependent recruitment of inflammatory monocytes is essential for the defense against bacterial (*L. monocytogenes*) and protozoal (*T. gondii*) infections [34]. The exit of Ly6C$^+$ BM monocytes from the BM may also

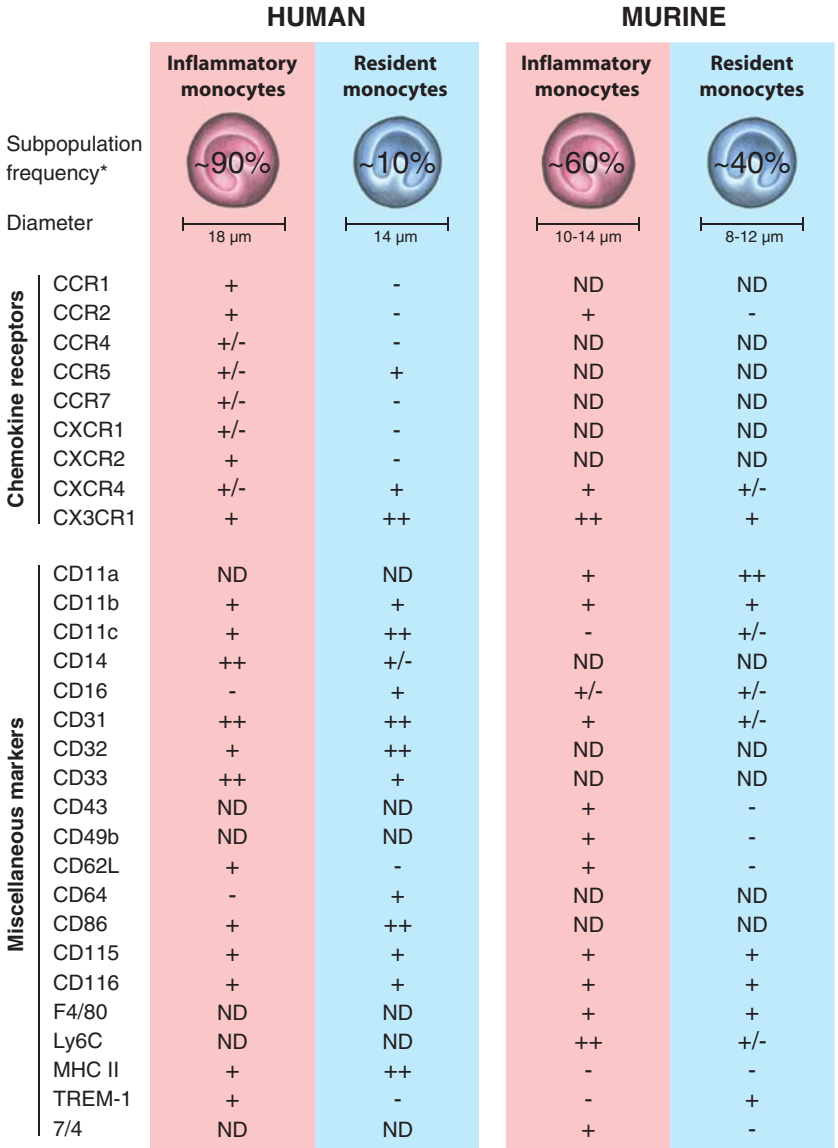

Fig. 2 Frequency and phenotype of the two main circulating monocyte subsets in humans and mice. Human inflammatory monocytes refer to the classical CD14^{hi}CD16$^-$ monocytes (corresponding population in mice: CX3CR1^{lo}CCR2$^+$), and human resident monocytes refer to the CD14^{lo}CD16$^+$ monocytes (corresponding population in mice: CX3CR1^{high}CCR2$^-$). Expression levels of the particular markers are semiquantitatively indicated as *minus sign* absent, *plus/minus sign* variable, *plus sign* positive, *double plus sign* high expression, and *ND* not determined. *Asterisk* frequencies of the respective blood monocyte subsets are indicated as percentages relative to the total circulating monocytes. Data are derived from refs. [20, 23, 24, 31–34, 38, 106–111] and the authors' own unpublished data. *7/4* an unidentified mouse antigen recognized by the monoclonal antibody 7/4, *F4/80* a monoclonal antibody recognizing the mouse homolog of the human glycoprotein EMR1 (*EMR1* epidermal growth factor module-containing mucin-like hormone receptor 1)

depend on CCR2-mediated chemotaxis since $CCR2^{-/-}$ mice under homeostatic and inflammatory conditions have reduced numbers of circulating inflammatory blood monocytes in the peripheral blood compared to $CCR2^{+/+}$ mice, while the resident $Ly6C^{-}$ monocyte subset remains unaltered [34, 35]. In CX3CR1-GFP transgenic reporter mice [8], circulating monocytes are distinguished based on their differential CX3CR1-GFP expression. The inflammatory monocyte subset is GFP^{lo} while resident-type monocytes are GFP^{hi}. Inflammatory GFP^{lo} monocytes upregulate MHC class II and CD11c after migration to the site of inflammation. Thereafter, they move to the draining lymph node where they may further differentiate into DCs [20]. In contrast, the resident (GFP^{hi}) monocytes have a longer life span and, after adoptive transfer, they reconstitute tissue macrophage populations and DCs also under homeostatic conditions [20], while GFP^{lo} cells may migrate back to the BM without entering the peripheral tissues in the absence of inflammation [10] (Fig. 1). Similar to human monocytes, the two main mouse monocyte subsets can differentiate into DCs in vitro in the presence of IL-4 and GM-CSF [20].

One of the central questions of monocyte differentiation is the time and site of differentiation into inflammatory vs. resident monocyte subsets and to which extent monocytes of one subset may later also acquire phenotypic and functional properties of the other subset. In adoptive transfer experiments where both subsets were introduced in recipient mice under homeostatic conditions, inflammatory ($Ly6C^{+}$) monocytes disappeared rapidly from the circulation, while the resident monocytes ($Ly6C^{-}$) persisted. The disappearance of the $Ly6C^{+}$ inflammatory monocytes was mostly due to a conversion of the inflammatory subset into the resident $Ly6C^{-}$ subset. $Ly6C^{+}$ monocytes shuttled back to the BM, converted into $Ly6C^{-}$ monocytes, and contributed further to the generation of mononuclear phagocytes as resident-type monocytes [10]. A similar downregulation of Ly6C was also observed in in vitro cultures when $Ly6C^{hi}$-expressing cells were differentiated into macrophages [36–38]. Repopulation studies in mice that were depleted of all circulating monocytes by treatment with clodronate-loaded liposomes, revealed in the peripheral blood a population of $Ly6C^{+}$ cells already 3–4 days after depletion, while first $Ly6C^{-}$ monocytes only appeared after more than 1 week after depletion. Together with previous observations that $Ly6C^{+}$ monocytes downregulate Ly6C expression and may convert into $Ly6C^{-}$ monocytes in vivo, this study suggests that the inflammatory $Ly6C^{+}$ monocyte subset contains the precursors of the $Ly6C^{-}$ resident-type monocytes. During this phenotypic and functional conversion, cells with an intermediate phenotype are detected (approximately 5% of the circulating monocytes), characterized by the intermediate expression of Ly6C, which may correspond to the $CD14^{+}$, $CD16^{+}$, and $CD64^{+}$ transitional monocytes found in humans [38]. $Ly6C^{int}$ monocytes express a broader spectrum of chemokine receptors and may preferentially migrate to draining lymph nodes due to their expression of CCR7 and CCR8 [39]. The conversion from $Ly6C^{+}$ to $Ly6C^{-}$ monocytes was significantly altered in models of acute (*L. monocytogenes*) and chronic (*L. major*) infection, resulting in a significant increase of the immature $Ly6C^{+}$ monocyte population [38]. The correlation of a resident vs. an inflammatory phenotype with a defined functional profile remains poorly characterized also for mouse monocyte subsets. Resident ($Ly6C^{-}$) blood monocytes were described as cells with a patrolling behavior that allowed them to rapidly invade tissues in case of tissue damage or infection. These $Ly6C^{-}$ cells initiated an early innate immune response at sites of inflammation, mainly by producing TNF, and then differentiated into macrophages [40]. This is in contrast to the inflammatory ($Ly6C^{+}$) monocytes, which arrive later at sites of infection and show a more DC-like phenotype [41].

The limited insight into the biological and functional properties of distinct monocyte subsets can be attributed to the absence of specific markers to distinguish the various differentiation and also activation stages of the MPS. The phenotypic and functional heterogeneity of the monocyte/macrophage system may be attributed to the remarkably dynamic adaption of macrophage subsets to their microenvironment. This is in part also reflected by the different macrophage subsets that can be generated in vitro with distinct sets of cell-derived and environmental stimuli [21, 22, 42]: Classical activation can be induced by in vitro culture of macrophages with IFNγ and LPS. This results in increased antigen presentation, production of proinflammatory cytokines, and an enhanced microbicidal activity. In vitro activation of monocytes in the presence of Th2-type cytokines (IL-4 or IL-13) yields the so-called alternatively activated macrophages characterized by an increased endocytic activity, cell growth, tissue repair, and parasite killing. The culture of monocytes/macrophages in the presence of IL-10 and transforming growth factor-β (TGFβ) is associated with an anti-inflammatory (or regulatory) phenotype with increased production of IL-10, TGFβ, and PGE_2. However, it is still unclear whether corresponding macrophage subsets also exist in vivo and to which extent the differentiation of macrophage lineages is predetermined. It appears likely that in vivo macrophages are exposed to a broad range of soluble and cell surface-expressed mediators that have the potential to activate and differentiate macrophages in a much more elaborate manner than can be mimicked by an in vitro

culture and, consequently, may result in a highly versatile macrophage phenotype.

The intestine as a major reservoir of macrophages

The gastrointestinal tract represents the most complex and extensive compartment of the immune system. Due to the sophisticated modifications of the small intestinal mucosa, including folds, villi, and microvilli, the total intestinal surface (in humans) amounts up to 300–400 m^2 and the number of colonizing luminal bacteria (up to 10^{14}), representing approximately 1,000 different species, even exceeds the number of cells of the human body [43, 44]. A monolayer consisting of epithelial cells serves as a physical barrier that restricts the penetration of luminal antigens and microbial products and helps to retain the commensal bacteria in the gut lumen. At the same time, the intestinal lamina propria contains an enormous number of immune cells with potent effector functions. About 70% of all lymphocytes are located within the mucosal immune system, and approximately 10% of all intestinal lamina propria mononuclear cells are found to be macrophages [45]. The luminal bacterial density increases from the small intestine (SI) to the ileum (distal portion of SI) and the large bowel. Accordingly, intestinal macrophages are most abundant in the colon with slightly lower frequencies in the SI. Hence, the mucosa of the small and the large

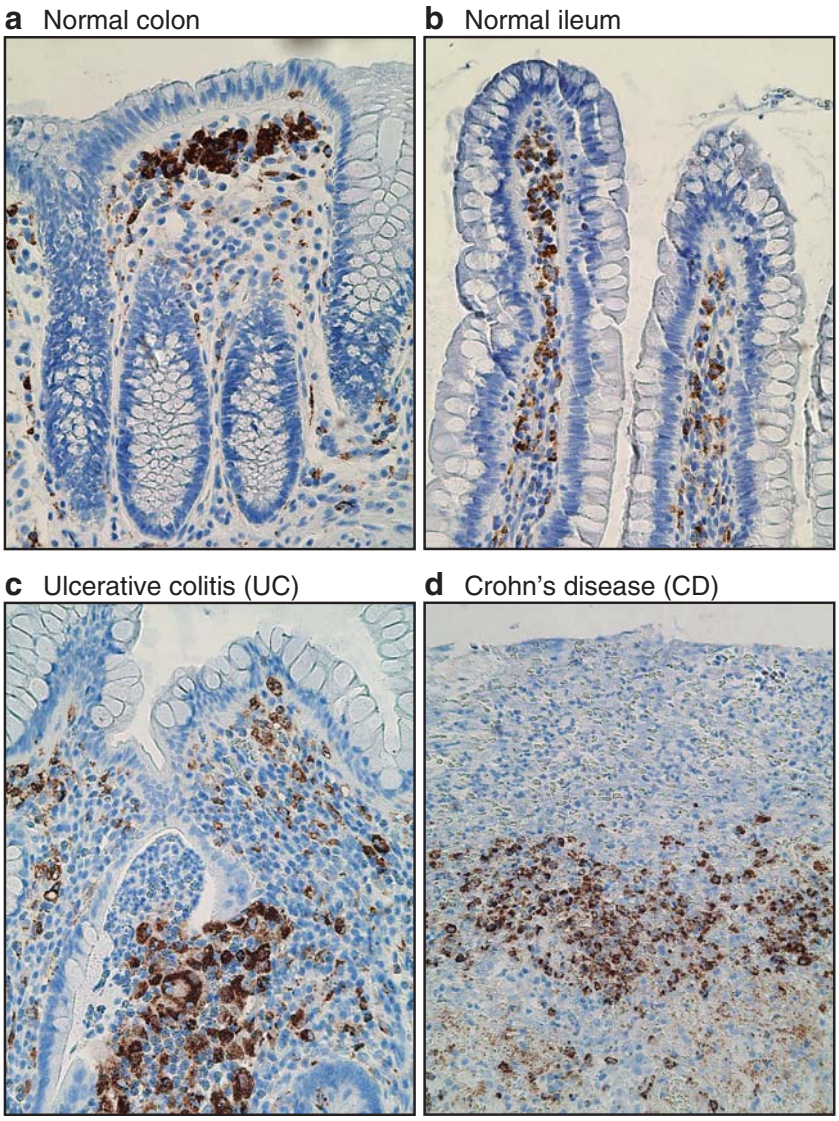

Fig. 3 Immunostaining for the macrophage-specific cytoplasmic protein CD68 on human intestinal tissue sections. Under homeostatic conditions (**a**, **b**), most macrophages (*brown*) are strategically located in the subepithelial mucosa, while during active flares of IBD (**c**, **d**), the tissue architecture is massively distorted and the numerous macrophages are dispersed in the affected intestinal segments

intestine represents the largest reservoir of tissue macrophages in humans [45] and mice [46]. Under homeostatic conditions, most of the intestinal macrophages are located in the subepithelial mucosa, i.e., at the preferential site of antigen entry (Fig. 3a, b). At these strategic positions, they are instrumental for the rapid elimination of gut lumen-derived bacteria that have breached the epithelial barrier. The highly efficient phagocytic and bactericidal activity of intestinal macrophages prevents the dispersal and proliferation of possibly harmful bacteria. Hence, resident intestinal macrophages exert critical functions for maintaining local tissue homeostasis including the efficient removal of apoptotic cells and foreign debris [47]. Despite the proximity of the large number of intestinal macrophages to the antigen- and bacteria-rich environment, only minimal signs of inflammation are observed in the gut [48]. Under homeostatic conditions, resident intestinal macrophages, together with regulatory T cells, inhibitory DCs, and possibly even endothelial cells, produce immunomodulatory cytokines that prevent excessive inflammation [49].

Distinct properties of intestinal lamina propria macrophages

The local tissue microenvironment substantially affects the functional and phenotypic differentiation of tissue macrophages. Hence, during the coevolution of the host immune system with the intestinal flora, the macrophage compartment in the intestine developed several functional adaptations to maintain local tissue homeostasis (Fig. 4). In contrast to their progenitor cells, the resident monocytes, intestinal macrophages do not serve as professional antigen-presenting cells (APC) due to their low, or even absent, cell surface expression of CD40, CD80, and CD86 [50]. Intestinal macrophages are deficient in several innate immune recognition mechanisms and activating receptors [51, 52] that make them refractory to LPS and other microbe-associated molecular patterns (MAMP), which are present abundantly in the intestinal microflora. They also lack the Fc receptors for IgA (CD89) and for IgG (CD16, CD32, and CD64) [52] and the complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18), and most intestinal macrophages also lack the integrin $\alpha 2\beta 1$ (LFA-1 and CD11a/CD18) [53]. These Fc and complement receptors on phagocytes usually mediate cellular activation, secretion of proinflammatory cytokines (such as TNF, IL-1β, IL-6, IL-8, and IL-12), and induction of potent adaptive immune responses [53]. These adaptations, however, do not impair the phagocytic activity which is exceptionally potent in intestinal macrophages [53].

Normal intestinal lamina propria macrophages are generally not only refractory to the induction of proinflammatory cytokine production by MAMP (e.g., LPS, or heat-killed *S. aureus*, muramyl dipeptides), but also by cytokines (e.g., TNF, IFNγ), or upon phagocytosis of necrotic cells [18, 53]. This is in sharp contrast to most other tissue macrophages and blood monocytes. Intestinal macrophages also mostly lack the triggering receptor expressed on myeloid cells-1 (TREM-1), an efficient amplifier of acute and chronic inflammatory reactions that is generally expressed on peripheral blood neutrophils and most monocytes and macrophages of secondary lymphoid organs [54]. Engagement of TREM-1 on TREM-1-positive myeloid cells leads to enhanced secretion of proinflammatory mediators (e.g., TNF, IL-1β, MCP-1, IL-6, and IL-8) by monocytes, upregulates several cell surface molecules (e.g., CD40, CD86, and CD32) on monocytes, and prolongs the survival of TREM-1-positive inflammatory cells [54, 55].

Such a selective inflammatory anergy of intestinal macrophages in the presence of avid host defense and scavenger functions represents an optimized functional adaptation of these effector cells located in close proximity to the extraordinary load of mostly commensal bacteria-derived, immunostimulatory molecules in the gut lumen. These adaptations efficiently contribute to limiting the dissemination of luminal bacteria to the local mucosa and are also a prerequisite for the tight regulation of the immune responses of the adaptive immune system in the intestinal mucosa. As a further illustration of such a perfect functional adaptation, murine colonic macrophages become triggered to produce large amounts of IL-10 after encountering whole commensal bacteria [56].

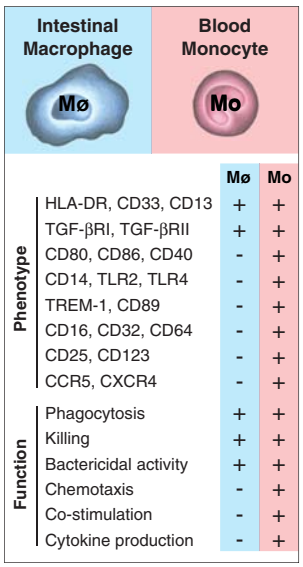

Fig. 4 Distinct phenotypic and functional properties of intestinal macrophages and blood monocytes. The presence, or absence, of a particular marker or functional feature is indicated as *plus sign* and *minus sign*, respectively. Data are collected from refs. [50–54, 86, 98, 112–114] and the authors' own unpublished results. *Mø* macrophage, *Mo* monocyte

Regulation of phenotype and functions of intestinal macrophages

A variety of intestinal cell types including epithelial cells, subepithelial myofibroblasts, fibroblasts, lamina propria lymphocytes, and intraepithelial lymphocytes are all able to produce soluble factors, particularly, TGFβ and IL-10, which may affect the phenotypic and functional properties of intestinal macrophages [57–60]. The importance of stromal cell-derived factors in shaping the functional capacities of intestinal macrophages is illustrated by the effects induced in blood monocytes upon exposure to intestinal stromal cell-conditioned media. This treatment reduced the expression of several innate response receptors, induced inflammatory anergy, and increased their phagocytic activity, similar to normal intestinal macrophages. These effects were efficiently blocked by anti-TGFβ antibodies, thus confirming the central role for TGFβ in this process [53]. IL-10, another relevant immunomodulatory cytokine produced under physiological conditions in the intestinal lamina propria, might also influence the functional properties of intestinal macrophages either by directly dampening monocyte/macrophage functions or indirectly by upregulating TGFβ production in distinct cell types, including immature DCs and macrophages [61–63]. Intriguingly, monocytes cultured in the presence of recombinant TGFβ alone or together with IL-10 did not gain an intestinal macrophages-like phenotype such as absence of CD14, whereas IL-10 and TGFβ synergistically downregulate CD89 and TREM-1 surface expression on blood monocytes and prevented the upregulation of these markers by proinflammatory stimuli such as LPS or TNF [54]. This indicates that, in addition to IL-10 and TGFβ, other intestinal mucosa-derived factors and cognate cell–cell interactions may be required for the full differentiation of recruited monocytes into resident intestinal macrophages. Indications for the additional requirement of cognate interactions between macrophages and intestinal epithelial cells for the complete differentiation into resident intestinal tissue macrophages was indeed obtained in cocultures of monocytes with multicellular spheroids of intestinal epithelial cells. In these in vitro cocultures, the monocytes acquired an intestinal macrophage-like phenotype, characterized by reduced CD14, CD16, CD11b, and CD11c expression and reduced LPS-stimulated IL-1β mRNA expression [64]. This effect, however, was not seen in transwell coculture of monocytes with the same epithelial cells or epithelial cell-conditioned medium and, hence, is likely to require direct cell–cell contacts between macrophages and intestinal epithelial cells [64]. Intestinal lamina propria macrophages are separated from the epithelial cells by the basement membrane [65]. However, subepithelial macrophages may directly interact with intestinal epithelial cells by sending cell protrusions through pores present in the basement membrane [66]. Components of the basement membrane and extracellular matrix were indeed also found to affect macrophage differentiation [67].

The efficient phagocytosis mediated by intestinal lamina propria macrophages should not only be viewed as a mere clearing of potentially harmful agents and dying cells, but may also contribute actively to the establishment of a local immunosuppressive milieu. Phagocytosis of apoptotic cells may induce in macrophages the secretion of immunosuppressive cytokines such as IL-10, whereas the secretion of proinflammatory cytokines such as TNF, IL-12, and IL-1β is significantly reduced [68–70]. Hence, phagocytosis of apoptotic cells may actively alter the functional behavior of intestinal macrophages.

Site-specific differences in the cellular composition of the intestinal mucosa

The intestinal immune system is the largest and most complex part of the immune system and each compartment of the intestine, from the duodenum to the ileum and the cecum to the rectum, displays distinct regional specializations that may directly affect the functional properties of the local resident immune cells. Most of the intestinal macrophages accumulate beneath the epithelium covering the luminal surface of the lamina propria (Fig. 3a, b). This characteristic distribution pattern is commonly seen at all intestinal sites, including gastric, duodenal, ileal, colonic, and even fetal gastrointestinal mucosa [71]. When compared to macrophages in the deeper lamina propria, subepithelial macrophages display an abundant cytoplasm, containing prominent phagocytosed vesicles or fragments, reminiscent of their high phagocytic activity and efficient elimination of dying cells and foreign particles [72]. Unfortunately, at present, no specific markers are described to further differentiate resident macrophages of the normal intestine in humans and in mice. In particular, in contrast to DC subsets, no differences in the chemokine receptor expression patterns by intestinal macrophages from distinct anatomical sites of the intestinal tract were reported so far.

Macrophage subsets in the normal and inflamed intestinal mucosa

The intestinal epithelium represents a physical barrier that is far from being tight and, thus, luminal bacteria may breach the epithelial barrier to get access to the subepithelial lamina propria. These invading commensal bacteria are rapidly phagocytosed by the lamina propria macrophages,

which are strategically located underneath the epithelium. This is most obvious in the large intestine where the concentration of luminal bacteria is several orders of magnitude higher than in the SI and where highest frequencies of subepithelial macrophages are seen (Fig. 3a, b). The importance of the functional adaptations of the local intestinal immune system to its unique environment is best illustrated during failure of these mechanisms, e.g., during the development of chronic intestinal inflammation, either in patients with celiac disease, inflammatory bowel diseases (IBD), or in various mouse strains deficient for genes involved in the control of immune responses such as IL-10 and TGFβ [73–76]. Deregulated immune response(s) against an otherwise harmless luminal microflora in susceptible individuals is now generally recognized as a key factor in the pathogenesis of IBD.

Celiac disease

Celiac disease, or celiac sprue, is a malabsorption syndrome which is initiated in genetically predisposed persons by the ingestion of gluten, a major protein of wheat and related cereals. The disease is triggered by exposure to gliadin peptides, which are derived from protease-digested gluten and are transported across the intestinal epithelial barrier to the lamina propria where they are deamidated by the enzyme tissue transglutaminase (tTG). These deamidated, gliadin-derived neoantigens are preferentially presented by HLA-DQ2 or HLA-DQ8 molecules to pathogenic CD4 T cells in the lamina propria [77]. Histologically, the affected small intestinal mucosa in florid celiac disease reveals a hyperplasia of the crypt cells, a hypercellularity of the lamina propria with locally increased numbers of CD4 T cells and plasma cells, together with an atrophy of the villi, leading to a complete flattening of the mucosa. These morphological changes are generally reversible during a gluten-free diet. Intestinal intraepithelial lymphocytes are typically increased in florid celiac disease and particularly TCRγδ T cells are overrepresented in the affected small intestinal epithelium. The interaction of the activating receptor NKG2D on a subset of small intestinal TCRγδ T cells with the cognate NKG2D ligand on enterocytes, i.e., the class Ib molecules MICA/B, can induce cytotoxic effector functions in subsets of TCRγδ T cells and CD8 TCRαβ T cells, thus, contributing to the characteristic villous atrophy in patients with florid celiac disease [78, 79]. In addition to these lymphocyte-mediated effects, a disease-exacerbating role has been also ascribed to macrophages in response to the enhanced presence of gliadin in the small intestinal mucosa: Gliadin-derived peptides reportedly directly stimulated peritoneal macrophages for an enhanced secretion of cytokines including TNF and the monocyte recruiting chemokine CCL5 [80]. In an in vitro system, gliadin also enhanced the epithelial permeability and a MyD88-dependent increased synthesis of IL-12 and TNF in primary mouse macrophages [81].

Inflammatory bowel diseases

IBD cover two major clinical entities, i.e., ulcerative colitis (UC) and Crohn's disease (CD). In UC, the inflammatory process is restricted to the mucosa of the large bowel and only in the most active stages of the disease may lead to ulcerations. Excessive inflammation in CD may involve any part of the gastrointestinal tract with the SI and/or the colon being most often affected. In CD, all layers may be involved by the inflammatory reaction and fissures and fistula formation are a severe complication of active disease. In CD, segments of macroscopically normal bowel may be present between affected bowel segments ("skip lesions"). The breakdown of oral tolerance, i.e., loss of attenuated T cell responses against the indigenous flora, may initiate IBD in genetically susceptible individuals and can result from alteration(s) in signaling pathways of PRRs, as it is likely the case for mutations in the NOD2/CARD15 locus that represents a susceptibility locus for CD [82–84]. Therefore, deregulated MAMP signaling and the resulting defects in APCs function or in APC–T cell interactions may lead to deregulated T cell responses. Hence, aberrant activation and functions of intestinal macrophages are also likely to contribute to chronic intestinal inflammation.

In the inflamed intestinal mucosa of patients with active IBD, the numbers of macrophages are increased (Fig. 3c, d). Some of these macrophages display a different phenotypic and functional profile than under physiological conditions. In contrast to the characteristic anti-inflammatory phenotypic and functional profile of normal intestinal lamina propria macrophages, at sites of mucosal inflammation, macrophages express relevant levels of T cell costimulatory molecules such as CD40, CD80, and CD86 [50]. Furthermore, subsets of intestinal macrophages express PRRs such as TLR2 and TLR4 at the site of intestinal inflammation. These inflammatory macrophages often coexpress CD14, CD89, and TREM-1 [18, 51, 85–87]. In these inflammatory conditions, the close proximity of subepithelial macrophages to the intestinal flora thus results in a constant, excessive activation of the mucosal innate immune system and monocytes recruited during inflammatory conditions likely fail to acquire the tightly regulated and adapted intestinal macrophage phenotype. Consequently, they may react to luminal antigens, function as potent effector cells, exert high antigen-presenting capability, and

become potent producers of proinflammatory cytokine [51]. Since both costimulatory molecules and "danger signals" are present, these macrophages trigger the adaptive immune system to react against luminal antigens. Under inflammatory conditions, the epithelial barrier function is usually disturbed and, as a result, the influx of luminal microbes and microbial products to the lamina propria is increased. This further enhances the activation and recruitment of additional inflammatory cells to sustain the inflammatory process. The DAP-12-associated activating receptor TREM-1 triggers the synthesis and secretion of proinflammatory cytokines, and consequently, the local tissue destruction. In the affected areas of patients with active UC or CD, TREM-1-expressing macrophages are significantly increased when compared to normal intestinal tissues. Engagement of TREM-1 on positive macrophages in patients with active IBD leads to a significant, several-fold increased secretion of TNF and other proinflammatory mediators such as IL-1β, MCP-1, IL-6, and IL-8 [87]. Hence, deregulated macrophage functions at distinct levels likely contribute to the development and perpetuation of intestinal inflammation characteristically seen in patients with IBD [88].

The relevance of appropriate macrophage regulation for maintaining local tissue homeostasis in the gut has been also nicely demonstrated in mouse models. As an example, selective disruption of Stat3 signaling in macrophages leads to severely impaired production of IL-10 and consequently leads to the spontaneous development of colitis [89]. Similarly, IL-$10^{-/-}$ mice spontaneously develop colitis as a consequence of the preferential macrophage differentiation into proinflammatory subsets that produce large amounts of IL-12 and IL-23. Remarkably, the depletion of macrophages in these IL-$10^{-/-}$ mice prevents the development of colitis [56].

Several observations in patients provided additional, circumstantial evidence for a central participation of macrophages in the pathogenesis of IBD. As an example, recurrent infections with pathogens that directly target the macrophages (e.g. *M. paratuberculosis*, *M. avium*, or paramyxoviruses) were repeatedly reported in patients with IBD. Therefore, deregulated or aberrant responses of macrophages in patients with IBD to these intracellular pathogens may be involved in the disease process. This notion is further supported by the molecular characterization of the first IBD susceptibility locus (*ibd1*), i.e., NOD2/CARD15, which is also expressed in monocytes and macrophages [82, 83] and reacts with the bacterial component muramyl dipeptide [90, 91]. The mutant form of NOD2 (3020insC), associated with CD, does not appear to respond to muramyl dipeptide-mediated stimulation [91–94]. At present, it is still controversial whether NOD2 is a positive, or negative, regulator of TLR2-mediated responses that may be impaired in the presence of the mutated NOD2 [95–97]. Remarkably, another recently characterized IBD susceptibility locus is the receptor for IL-23, a proinflammatory cytokine produced predominantly by cells of the monocyte/macrophage lineage. Intriguingly, in patients with active CD, but not in patients with UC, intestinal macrophages produced significantly more IL-23 than intestinal macrophages from noninflamed mucosa. The enhanced production of IL-23 by intestinal macrophages from patients with CD upon in vitro culture was dependent on local IFNγ secretion and was further increased in the presence of commensal bacteria (*E. coli*, *E. faecalis*). In contrast to the production of IL-23, which was induced only in lamina propria macrophages from CD patients, mRNA expression and production of IL-6 was increased in lamina propria macrophages from both UC and CD patients when compared with macrophages obtained from noninflamed intestinal lamina propria of control patients [98].

The importance of macrophages in the pathogenesis of IBD is supported by reports identifying macrophages as the main source of TNF during the pathogenesis of IBD and the beneficial effects of anti-TNF treatments in patients with CD. The striking effects of this TNF-targeted therapy may in IBD also be attributed to the cell-depleting effect of some of these therapies, including the removal of TNF-expressing monocytes/macrophages [99]. TNF produced by nonlymphoid cells (mostly macrophages) is essential for colitis in an adoptive T cell model of colitis induction in lymphopenic recipients. Intriguingly, in this mouse model, TNF production by the transferred CD4 T cells was not required, nor sufficient, for disease induction when nonlymphoid cells, i.e., mostly macrophages, produced TNF in the affected intestinal mucosa [100].

Conclusions

Until recently, the research on the etiopathogenetic role of intestinal immunopathological disorders such as celiac disease and IBD mostly focused on effector mechanisms of the adaptive immune system such as pathogenic and regulatory T cell subsets, whereas the innate immune system had received relatively little attention. However, the past few years witnessed several important advances that indicated a central role for cells of the innate immune system in the pathogenesis of these disorders. The relevance of intestinal macrophages in the pathogenesis of IBD is highlighted by their functions as gatekeepers, driving adaptive immune responses to either tolerance induction or initiation of inflammatory reactions. Elucidation of the detailed mechanisms of how local macrophages and/or blood monocytes functionally differentiate and

participate in the pathogenesis of various inflammatory disorders may allow designing specific strategies to target the induction of deregulated adaptive immune responses and excessive inflammation. Detailed knowledge about monocytes/macrophage lineage relationships, differential homing capacities, and the plasticity of these cell subsets is crucial to design rational strategies to interfere with their involvement in excessive immunopathological reactions, including chronic inflammatory disorders of the gastrointestinal tract.

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