Overview

The emerging roles of intestinal macrophages in sickness and in health

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Abbreviations:
CD = cluster of differentiation
IL = interleukin
TLR = Toll-like receptor
TNF = tumour necrosis factor
IBD = inflammatory bowel disease

Abstract

The immune system in the intestine represents a unique environment that can quickly respond to harmful pathogens, but it remains tolerant to antigens from food and commensal bacteria. This balance between protective immunity and tolerance is largely dependent on the mononuclear phagocytes in the intestine, such as macrophages. Intestinal macrophages, unlike other macrophage populations in the body, are hypo-responsive to stimuli although they originate from fully responsive blood monocytes. The intestinal environment seems to instruct monocytes to mute their function upon arrival to the gut in order to adapt to the antigen-rich environment. While their main role in the healthy gut is to maintain homeostasis, in disease macrophage phenotype and function is changed and these cells become the drivers of inflammation and disease progression.

INTRODUCTION

Immediately after birth, the intestine is colonised by a vast microbial community that reaches 10^{13}-10^{14} bacteria per gram of luminal content *(1, 2)*. Alongside the exposure to these commensal bacteria the intestine is also exposed to a variety of food proteins and pathogenic organisms, which means that it constantly encounters more antigens that any other part of the body *(1)*. In order to protect against infection but at the same time avoid the unnecessary inflammatory responses to beneficial microbiota and food, the immune system in the intestine has evolved into the largest and most complex part of the host immune system. The homeostasis here is balanced through a strict hierarchy of mechanisms that include many immune and non-immune cells. One of the key regulators of homeostasis in the intestine are macrophages. This review describes the adaptations of intestinal macrophages to their antigen-rich environment and a different role they play in a healthy and diseased state.

THE UNIQUE PROPERTIES OF INTESTINAL MACROPHAGES

Most of the macrophages in the body are effector cells which get activated by molecular patterns expressed on different pathogens through Toll-like receptors (TLR). Upon activation these cells increase the secretion of cytokines and other pro-inflammatory mediators, increase the expression of co-stimulatory molecules (such as CD80, CD86, CD40) and exhibit enhanced killing of microorganisms *(3)*. The intesti-

nal macrophages, however, display somewhat different function and phenotype than classically described macrophages. They are highly unresponsive to any TLR stimulation *(4-6)* and do not produce pro-inflammatory cytokines *(7)* or other pro-inflammatory mediators, such as nitric-oxide (NO) or reactive oxygen species (ROS) *(8, 9)*. Furthermore, they express only low levels of co-stimulatory molecules, such as CD80, CD86 and CD40 *(10)*. They do produce anti-inflammatory cytokine IL-10, constitutively and in response to TLR ligation *(11)* and, as shown recently, they produce pro-inflammatory TNF-α even in a steady state, but this does not lead to inflammation *(12)*. Despite this anergy, intestinal macrophages exhibit strong phagocytic and bacteriocidal activity which allows them to efficiently clear bacteria, cellular debris and foreign material *(13)*. The differences between intestinal macrophages and other macrophages are summarised in Table 1. Thanks to these adaptations intestinal macrophages are able to survey the gut without mounting an immune response to beneficial antigens coming from commensal bacteria and food.

TABLE 1
Phenotypic comparison of macrophage populations
Difference between intestinal macrophages and conventional inflammatory macrophages.

Property	Intestinal macrophage	Inflammatory macrophage
Co-stimulatory molecules (CD80, CD86, CD40)	–	+
Responsiveness to TLR stimulation	–	+
Cytokine production (IL-12, IL-6, IL-23)	–	+
Phagocytosis	++	+
NO production	–	+
ROS production	–	+
IL-10 production	+	+
TNF-α production	+	+

HOW DO INTESTINAL MACROPHAGES GET THEIR UNIQUE PROPERTIES?

All tissue macrophages are derived from bone marrow stem cells through a highly regulated cascade of events. In the bone-marrow, a combination of cytokines that include IL-1, IL-3 and IL-6 together with granulocyte/macrophage colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor (M-SCF) induce proliferation of a monocyte precursor into a monoblast, then a promonocyte and finally a monocyte *(4)*. After leaving the

bone marrow, monocytes enter the blood, where they circulate for days before migrating into tissue *(14)*. It is believed that there are two different populations of monocytes; one that migrates and replenishes macrophages in the resting tissue and one that is recruited during inflammation *(15)*. The first population was termed „resident" monocytes. „Resident" monocytes are non-inflammatory and inert to stimuli *(15)*. The second population are termed „inflammatory" monocytes and unlike „resident" monocytes they are fully responsive to stimulation and home to inflamed tissue *(15)*. Because of their distinctive, non-inflammatory features, intestinal macrophages were thought to derive from „resident" monocytes. Surprisingly, experiments combining resident cell ablation with precursor cell transfer have shown that resident intestinal macrophages, actually, derive from „inflammatory" monocytes *(16, 17)*. Once they arrive in the gut these „inflammatory" monocytes then adapt to the gut environment by acquiring a non-inflammatory gene expression signature *(12)*. In other words, it seems that the intestinal environment is educating monocytes upon their arrival to the gut, making them match the dynamic nature in which they have arrived.

ROLE OF INTESTINAL MACROPHAGES IN A HEALTHY STATE

Because of their hypo-responsiveness to stimuli and inability to mount an inflammatory response, the only role of intestinal macrophages was thought to be phagocytosis of apoptotic cells and debris. However, they have been shown to be important for intestinal homeostasis as depletion of macrophages leads to intestinal inflammation *(18)*. One of their homeostatic-inducing roles is the regulation of epithelial cell integrity (Figure 1A). Intestinal macrophages produce prostaglandin E2 and promote survival and proliferation of epithelial progenitor cells during colonic wound healing *(19)*. Also, they produce cyclo-oxygenase 2 (COX2) which has been linked with anti-inflammatory effects, as myeloid cell specific COX2 knockout mice have increased expression of pro-inflammatory cytokines, such as IL-6, TNF-α and IFN-γ, compared to wild type *(20)*. Furthermore, intestinal macrophages are closely associated with epithelium and can extending processes across the epithelial layer into the intestinal lumen and sample bacteria *(21, 22)*. Information they gain this way can instruct and recruit other immune cells. Indeed, macrophages in the intestine have been shown to maintain regulatory T-cells which are also important for the suppression of immune response upon exposure to food antigen. Therefore, both cells work together to establish oral tolerance and promote gut homeostasis (Figure 1A) *(23)*.

A DIFFERENT FACE OF MACROPHAGES IN INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is an inflammatory disorder of the gastrointestinal tract characterised by an
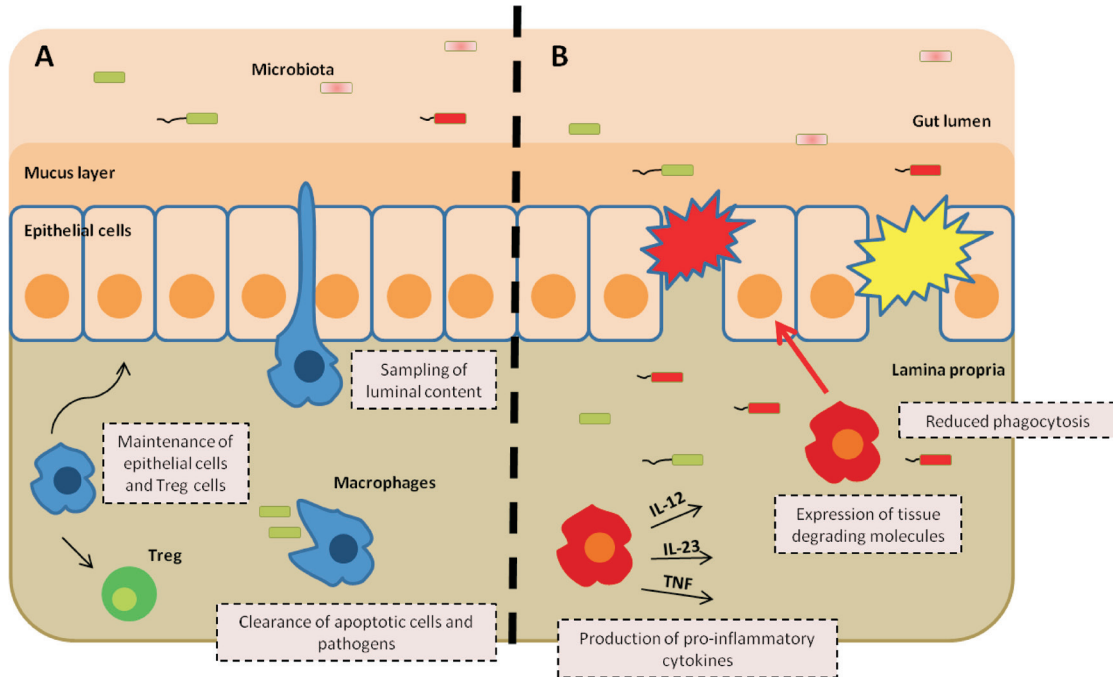
Figure 1. *Role of intestinal macrophages in a healthy state (A) and in disease (B).* In healthy intestine macrophages protect the epithelial cells barrier, support the induction of regulatory T-cells and destroy pathogens that breach the epithelial layer. In this way they maintain homeostasis and oral tolerance (A). In disease macrophages lose their homeostatic properties, show an enhanced pro-inflammatory phenotype and drive tissue injury and disease progression (B).

abnormal immune response to antigens of the intestinal content that leads to a persistent inflammatory state *(24)*. Two major forms of IBD are Crohn's disease and ulcerative colitis, distinguished by the area they affect. While ulcerative colitis exclusively effects the colon, Crohn's disease can affect the whole gastrointestinal tract, but is mainly localised to the colon and ileum *(25)*. The exact cause of IBD remains poorly understood, however different environmental factors are considered risk factors for IBD, such as smoking, diet, drugs, stress and enteric flora *(26)*. Both ulcerative colitis and Crohn's disease are characterised by the disruption of the epithelial cell barrier and the dysfunction of the immune system. Disruption of intestinal epithelial barrier in IBD allows the invasion of commensal bacteria. This leads to influx of inflammatory cells and their constant activation. Indeed, patients with active IBD have an increased number of macrophages in the inflamed intestinal mucosa (27) and these macrophages display a different phenotypic and functional profile than under homeostatic conditions (Figure 1B). Macrophages in IBD patients display increased levels of co-stimulatory receptors, such as CD40, CD80 and CD86 which enables the crosstalk and activation of T-cells *(28)*. This is coupled with an increased production of pro-inflammatory cytokines, such as IL-12 and IL-23 *(6)*. IL-12 drives IFN-γ production from T-cells, which then in turn increases macrophage activation and also increases epithelial cell permeability *(6)*. IL-23 promotes a development of a CD4$^+$ phenotype characterised by the

production of IL-17, called Th17 cells, which are involved in the pathogenesis of IBD *(6)*. Macrophages in IBD are also a main source of TNF-α, which is considered to be a „master regulator" of pro-inflammatory cytokine production. It has a pivotal role in orchestrating the production of a pro-inflammatory cytokine cascade and therefore drives the disease *(29)*. Furthermore, macrophages in the IBD show increased expression of pathogen-recognising receptors, such as TLR2 and TLR4 and also triggering receptor expressed on myeloid cells (TREM)-1 which triggers the synthesis and secretion of inflammatory factors *(7, 30)*. Engagement of TREM-1 leads to increased secretion of IL-1β, IL-6 and IL-8 *(31)*. This pro-inflammatory response leads to epithelial apoptosis, necrosis, formation of granuloma and fibrosis *(32)*. Expression of tissue degrading cathepsins by intestinal macrophages has also been seen in IBD *(33)*, together with an increased release of nitric oxide and oxygen radicals that further contribute to macrophage-dependent tissue damage *(34)*.

While macrophages in the inflamed intestine show an enhanced pro-inflammatory phenotype, their ability to eradicate intracellular pathogens is decreased as their phagocytosis is significantly reduced *(35)*. This probably accounts for recurrent infection in IBD patients with pathogens that directly target macrophages, such as *Mycobacterium paratuberculosis (36)*. Some of the disruptions in macrophage signalling are also reported to contribute to intestinal inflammation. Selective disruption in signal

transducer and activator of transcription (STAT)3 signalling in macrophages leads to impaired production of anti-inflammatory IL-10 and spontaneous development of colitis in mice *(37)*. The mutant form of nucleotide-binding oligomerization domain-containing protein (NOD)2, associated with Crohn's disease, is also expressed on macrophages and therefore may contribute to disease pathology *(38)*.

CONCLUSION

Intestinal macrophages show distinct phenotypic and functional features in a healthy state and in disease. In healthy state, intestinal macrophages are highly anti-inflammatory cells, capable of destroying pathogens without initiating an immune response and damaging the surrounding tissue. Thanks to these features they maintain an immunosuppressive milieu in the gut which counteracts the constant influx of antigens from the food and commensal bacteria. In disease, however, the immunosuppressive balance is disturbed. These anti-inflammatory macrophages suddenly lose their homeostatic properties and become sensitised to gut microbes. This is followed by an increased production of pro-inflammatory mediators and activation of surrounding immune cells. Previously immunologically inert macrophages now become drivers of pathogenesis and tissue destruction. It is unclear at what point this functional switch between homeostatic and inflammatory properties occurs and what is driving it. Identification of key factors that influence macrophages in a healthy state and disease, together with better understanding of macrophage plasticity in the gut, is of a great importance if we want to find new targets for the treatment of gut inflammation. Hopefully by returning macrophages into their original, homeostatic role we can restore the physiological balance and attenuate inflammation, rather than just targeting symptoms of disease as currently available therapies do.

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