Microbiota driven macrophage mediators in pathogenesis and treatment of gut leakage: current strategies and future perspectives

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Abstract

Macrophages play key roles in tissue homeostasis, defense, disease and repair. Macrophages are highly plastic and exhibit distinct functional phenotypes based on micro-environmental stimuli. Despite several advancements in understanding macrophage biology and their different functional phenotypes in various physiological and pathological conditions, currently available treatment strategies targeting macrophages are limited. Macrophages' high plasticity and diverse functional roles – including tissue injury and wound healing mechanisms – mark them as potential targets to mine for efficient therapeutics to treat diseases. Despite mounting evidence on association of gut leakage with several extra-intestinal diseases, there is no targeted standard therapy to treat gut leakage. Therefore, there is an urgent need to develop therapeutic strategies to treat this condition. Macrophages are the cells that play the largest role in interacting with the gut microbiota in the intestinal compartment and exert their intended functions in injury and repair mechanisms. In this review, we have summarized the current knowledge on the origins and phenotypes of macrophages. The specific role of macrophages in intestinal barrier function, their role in tissue repair mechanisms and their association with gut microbiota are discussed. In addition, currently available therapies and the putative tissue repair mediators of macrophages for treating microbiota dysbiosis induced gut leakage are also discussed. The overall aim of this review is to convey the intense need to screen for microbiota induced macrophage-released pro-repair mediators, which could lead to the identification of potential candidates that could be developed for treating the leaky gut and associated diseases.

Microbiota driven macrophage mediators in pathogenesis and treatment of gut leakage: current strategies and future perspectives

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Key words: Macrophages, Microbiota, Gut leakage, Pathogenesis, Barrier damage**,** Tissue-repair

Abbreviations:

CCL2/ - C-C motif chemokine ligand

CCR2 - C-C chemokine receptor type 2

CCR2-KO - CCR2 Knockout

CX_3CR1 - C-X3-C Motif Chemokine Receptor 1

CSF-1 - colony-stimulating factor

CD – Cluster of differentiation

COX2 - cyclooxygenase-2

EV – Extracellular Vesicle

FOXP3+ T - forkhead box P3

FODMAP - fermentable oligosaccharides, disaccharides, monosaccharides and polyols

FMT – Fecal Microbial Transplant

FGF-10 - fibroblast growth factor 10

GM-CSF - Granulocyte macrophage colony-stimulating factor

HGF - Hepatocyte growth factor

ICAM-1 - intercellular adhesion molecule

IEC - intestinal epithelial cells

IGF-1- Insulin-like Growth Factor 1

IGF-1 - Insulin-like Growth Factor

IL – Interleukin

IBD – Inflammatory Bowel Disease

IBS – Inflammatory Bowel Syndrome

Ly6C - lymphocyte antigen 6 complex

LPS – Lipopolysaccharides

MHCII - major histocompatibility complex II

MyD88 - myeloid differentiation factor 88

MIF - macrophage migration inhibitory factor

MMP-10 - Matrix metalloproteinase 10

miRNAs – Micro RNAs

NO - nitrogen monoxide

PDGF - platelet-derived growth factor

PD-L1 and PD-L2 - Programmed death ligand 1 and 2

Plet1- Placenta Expressed Transcript 1

SCFAs – Short Chain Fatty Acids

SIBO - Small intestinal bacterial overgrowth

STAT3 - Signal transducer and activator of transcription 3

sTREM2 - Soluble triggering receptor expressed on myeloid cells 2

TNFα - Tumor necrosis factor alpha

TGF- β - Transforming growth factor-beta

Treg – T regulatory cell

TLR-2 - Toll-like receptor

UC – Ulcerative colitis

VEGF-α - Vascular endothelial growth factor

Introduction

Macrophages were first described as phagocytes in 1882 by Élie Metchnikoff, this cell type has been found residing in almost every tissue of the body as large, tissue-resident myeloid cells, characterized as having pseudopodia and phagocytic granules and by distinct functional profiles. As a central part of the innate immune system, they serve a crucial host defense function, but also contribute to the maintenance of tissue homeostasis by clearing apoptotic and damaged cells. Macrophages also play an essential role during organogenesis in embryonic development, where they are highly concentrated at sites of high cell death, such as the developing limb buds [1, 2]. These tissue re-modeling functions are maintained in adults and support wound healing and tissue repair/remodeling processes after infection and injury. Macrophages can also acquire tissue-specific phenotypes and functions in different organs. Although they exert tissue-specific functions, all such tissue-specific macrophages also release common soluble mediators including enzymes, cytokines, chemokines, and arachidonic acid derivatives, as well as glycoproteins such as fibronectin, that help with the maintenance of homeostasis and tissue repair [3, 4].

Origin, differentiation and plasticity

After a decade of studies, the role of monocyte/macrophage functions was found to be dependent on the state of macrophage differentiation [5, 6]. Re-established concepts in macrophage biology have led to a new understanding of the origins, biology and phenotypes of lung macrophages. Mounting evidence in recent years has revealed the plasticity of macrophages and has indicated that macrophages may arise through

differentiation through a pre-cursor cell type (Fig. 1). Macrophages originate during the prenatal stage from the yolk sac and fetal liver, and during the postnatal stage from the bone marrow [7, 8]. Tissue-resident macrophages were shown to arise from embryonic progenitors that seed in the intestine and mature locally before and shortly after birth. They are maintained by proliferative self-renewal mechanisms throughout life, largely independent of replenishment by blood monocytes in the steady state [9-11]. However, during inflammation, blood monocytes are recruited from the bone marrow to inflamed intestinal tissues where they differentiate into macrophage populations. These cells with distinct ontogenetic and proliferative histories are exposed to regional signals in inflamed intestinal tissue, but their distinct responses and further programming are largely unexplored.

Tissue-resident macrophages originate from both the yolk sac and fetal liver in the pre-natal stage. During inflammation/injury, an additional subset of macrophages originate from the bone marrow and migrate into inflamed intestinal tissues [12]. These infiltrated macrophages require predominantly CCL2/CCR2 axis, which was evidenced in CCR2-KO mice that showed deficit monocyte infiltration into gut tissues [13, 14]. These infiltrated macrophages are exposed to the micro-environmental stimuli and correspondingly adapt their functional repertoire, and differentiate into tissue resident macrophages, if these are depleted, by inflammatory stimuli or by infection [15].

In mice, two blood monocyte subsets have been distinguished based on the differential expression of Ly6C and CX_3CR1 [16]. Monocytes that express high Ly6C levels, intermediate CX_3CR1levels and high CCR2 levels are termed $Ly6C^{hi}$ monocytes. They are also known as inflammatory monocytes due to their ability to migrate to sites of inflammation and produce pro-inflammatory cytokines during infection or tissue damage [16] [17]. The second major monocyte subset in mice characterized by low Ly6C expression, high CX_3CR1expression and low CCR2expression is termed the $Ly6C^{low}$subset, which patrols for monocytes, acting to maintain capillary integrity [18]. After extravasation, $Ly6C^{hi}$ monocytes differentiate into macrophages and monocyte-derived dendritic cells (Mo-DCs). Despite resident macrophages longevity and self-renewing property during homeostasis [19], infiltrated macrophages ($CD11c^{low}CD11b^{hi}$) from circulating blood $Ly6C^{hi}$ monocytes can complement the prenatally established macrophage compartment, especially under severe inflammatory conditions such as irradiation and infection that cause severe depletion of the resident macrophage population [20]. During later stages of injury long-lived self-renewing resident macrophages can also help in replenishing the resident pool. Thus, resident macrophages may have a chimeric origin, being derived from both yolk sac/fetal liver as well as from bone marrow monocytes,[21, 22] [23]. In addition, the distinct functions of resident and recruited macrophages suggests these were well classified based on their origins [24]. Moreover, several experiments as far back as the early 1970s established that the influx of macrophages plays a crucial role in tissue repair [25, 26]. Peripheral blood monocytes can also replenish intestinal macrophages through mechanisms dependent on granulocyte-macrophage colony-stimulating factor (GM-CSF) and CSF-1 signaling in a stimulus-specific manner [27]. Later, these macrophage subsets were classified as alternatively activated macrophages [28], which were found to be involved in development, repair, and tissue homeostasis processes [29].

However, gut-resident macrophages do not fit readily into this 'M1-M2 paradigm', having some of hallmarks of both M1 and M2 macrophages [28]. For instance, they express high levels of MHCII and produce TNFα constitutively, features normally associated with M1 or 'classically activated' macrophages. However, they also express CD206, CD163, and produce interleukin-10 (IL-10), features associated with M2 or M2-like macrophages [2]. However, they fail to express arginase, which is a key feature of M2 macrophages. Thus, like most tissue macrophages in vivo, those resident in the gut wall adapt to their local environment in complex and specific ways that may not be reflected by the rigid classification of the M1-M2 paradigm.

Figure 1. Macrophage origin, differentiation and plasticity

Gut leakage

Emerging studies have showed different causes of gut leakage**(Figure 2),** however, altered gut microbiota (dysbiosis) is the underlying fundamental cause in all conditions. The intestinal epithelium, which is mainly

made up of a single layer of intestinal cells, is tightly connected with adjacent cells to form a critical, continuous physical barrier, which regulates the selective permeability of luminal content [30] [31]. Disruption of this epithelial barrier increases intestinal permeability, resulting in leaky gut syndrome: an acute inflammatory condition characterized by functional and structural losses and disruption of epithelial integrity the basement membrane, which lead to the efflux of bacterial toxins and other pro-inflammatory mediators into systemic circulation [32]. During intestinal epithelial cell injury, loss of tight junction integrity and apoptosis drive increased permeability and, in severe cases, eventually to a denuded basement membrane. During microbial dysbiosis, unfavorable inflammatory cascades are activated in the gut lumen that trigger the proinflammatory cytokine storm leading to intestinal epithelial membrane damage. Despite increasing insights into disease pathogenesis from experimental and clinical studies, dysbiosis remains a leading cause of several diseases among patients in critical care, with multi-organ failure and increasing mortality rates [33]. Increasingly, the body of evidence implies a link between dysbiosis-induced intestinal barrier dysfunction and development of several pathogenic conditions such as inflammatory bowel disease[34], obesity [35], bone diseases [34], liver diseases [36], autoimmune diseases [37], and neuroinflammation [32]. Intestinal pathobionts are observed to be increased in patients with inflammatory disorders, where they accelerate systemic inflammation by translocating across the epithelial barrier to reach extraintestinal tissue **(Figure 2).** Gut microbiota can regulate host immune cells including intestinal macrophages [38]. Other periodontopathic bacteria's like *Porphyromonas gingivalis* and*Fusobacterium nucleatum* can induce gut dysbiosis and damage epithelial cells [39, 40]. To date, no satisfactory, specific treatments have been developed to treat leaky gut syndrome. Moreover, several probiotic agents from successful trials, showing promise in both experimental and preclinical studies, failed to show an overall improvement in mortality during clinical trials, due to adverse effects and unfavorable alterations in the gut dysbiosis [41]. Therefore, there is an urgent medical need for the development of novel therapies to further improve clinical outcomes.

Macrophages in regulating intestinal barrier function

Intestinal macrophages play a key role in the gut immune system and the regulation of gastrointestinal physiology, including gut motility and secretion. In homeostatic conditions, macrophages are one of the most abundant intestinal immune cells. Intestinal macrophages inhabit the lamina propria and are involved in a variety of biological processes, including removal and degradation of microorganisms and tissue repair [42, 43]. Intestinal macrophages also limit inflammation, facilitate the survival of local FOXP3+ T regulatory cells and maintain epithelial integrity [44, 45]. Mouse intestinal macrophages express CD64, Mertk, CD11c, MHCII and F4/80 while human macrophages express CD64. Moreover, while in mice they are characterized by different subsets in the intestine, such as $CD14^{hi}$HLA-$DR^{lo}CD209^{lo}CD163^{lo}$, $CD14^{hi/lo}$HLA-$DR^{hi}CD209^{lo}CD163^{lo}CD11c^{+}$, $CD14^{lo}$HLA-$DR^{hi}CD209^{hi}CD163^{hi}$and $CD14^{hi}$HLA-$DR^{hi}CD209^{hi}CD163^{hi}$, no significant functional differences have been observed [21, 23]. These populations are distinguished from dendritic cells by the expression of CD14 [23]. Intestinal macrophages localize closely with epithelial cells and disruption of this association results in loss of intestinal barrier function, as reported in inflammatory bowel diseases such as ulcerative colitis (UC) and Crohn's disease [46]. Both epithelial and macrophage protein tyrosine phosphatase non-receptor type 2 is found to have an important role in facilitating the association of intestinal epithelial cells with macrophages to maintain intestinal barrier function [47]. However, the mechanisms by which macrophages regulate intestinal epithelial barrier function need further validations. A pro-inflammatory cytokine storm drives the recruitment of monocytes that polarize to M1 macrophages in intestinal tissues and release pro-inflammatory cytokines like TNF-α, interleukin (IL)-6, IL-1β and NO, which disrupt intestinal barrier function [48]. Pathogens cross the damaged intestinal epithelial cell barrier and stimulate macrophages to produce more pro-inflammatory cytokines, such as IL-1, IL-6, IL-18, transforming growth factor-α (TGF- β) and tumor necrosis factor-β (TNF-α) are released. These act on intestinal epithelial cells directly or indirectly, leading to the injury or necrosis or apoptosis of intestinal epithelial cells, disruption of the epithelial barrier and deregulation of tight junction proteins, as observed in IBD [49]. Similarly, macrophage exposure to lipids from a high fat diet restricted the uptake of apoptotic cells and induction of IL-10 [50]. This results in poor intestinal barrier repair and continued intestinal injury leading to gut leakage. Despite there being few available studies on the role of macrophages in upregulating tight

junction proteins, macrophage released mediators like macrophage inhibitory factor and its receptor cluster of differentiation 74 (CD74) have been reported to play a major role in the maintenance of the intestinal barrier function [51, 52]. In a recent study, the sub-epithelial macrophages protected the distal colon epithelial cells from hazardous luminal fungal metabolites, thereby preventing gut leakage and maintaining the barrier function. In the absence of these macrophages, dysregulated intake of fungal products by the epithelial cells lead to apoptosis and subsequent loss of epithelial barrier integrity [44]. This study speculated that intestinal macrophages play a significant role in maintaining the barrier function. Patients with chronic ulcerative colitis (UC) who survive the acute stage, enter a proliferative response phase which is characterized by the presence of hyperplastic intestinal epithelial cells and fibroblasts. The intestinal epithelial stem cells migrate, proliferate and differentiate into absorptive enterocytes, mucus-secreting goblet cells, hormone-secreting enteroendocrine cells, and tuft cells in order to reconstitute the integrity of the junctional epithelium integrity. Epithelial repair during the proliferative phase may result in the complete restoration of gut barrier function. A proper re-epithelialization is frequently affected which can result in progression to the fibrotic phase of UC. However, there is more to explore to improve our understanding regarding the co-ordination of microbiota with macrophages and epithelial cells.

Figure 2. Macrophage derived factors induce gut leakage and mediators in intestinal epithelial barrier repair mechanisms.

Macrophages in tissue repair and regeneration

Macrophages represent the first line of defense in their resident tissues and serve as a unique leukocyte population by actively coordinating homeostasis, defense mechanisms and resolution of inflammation. Macrophages are an important source of chemokines, matrix metalloproteinases and other inflammatory mediators that drive the initial cellular response following injury [53]. After the early inflammatory phase subsides, the predominant macrophage population polarize to a wound healing phenotype that is characterized by the production of numerous growth factors including PDGF, TGF-β1, IGF-1, and VEGF-α, that promote cellular proliferation, mucosal wound healing and blood vessel development [53-55]. They also produce soluble mediators that stimulate local and recruited tissue fibroblasts to differentiate into myofibroblasts, that facilitate wound contraction and closure as well as the synthesis of extracellular matrix components [53]. The proliferation and expansion of neighbouring parenchymal and stromal cells are also regulated by macrophages, and if the injury is severe, macrophages can also activate additional stem cell and local progenitor cell populations that participate in repair. Thereafter, monocytes and/or macrophages exhibiting a mostly anti-inflammatory phenotype become the dominant population [53]. Macrophages respond to and secret IL-10, TGF-β and other inhibitory mediators, including cell surface receptors like PD-L1 and PD-L2 that play a major role in the tissue repair process [53] [56]. Both clinical and in vivo studies have shown an association of early onset inflammatory bowel disease (IBD) with IL-10 or IL-10 receptor mutations in intestinal chemokine receptor CX3CR1-expressing resident macrophage. These data demonstrate that macrophage-derived IL-10 is dispensable for gut homeostasis [45]. Moreover, several studies have observed the role of macrophages in regeneration, wound closure, angiogenesis, and clearance of dead and aged cells [54, 57]. Therefore, elucidating the mechanisms by which macrophages promote tissue regeneration at a requisite micro-environment may divulge strategies for the regeneration of injured organs.

Mounting evidence suggest different monocyte and macrophage population possess distinct and non-redundant roles in tissue repair, fibrosis, and regeneration [24]. The mechanisms that instruct macrophages to adopt pro-inflammatory, pro-wound healing, pro-fibrotic, anti-inflammatory, anti-fibrotic, pro-resolving, and tissue regenerating properties in various organ systems is not well understood [58]. Although effective wound repair and tissue regeneration is often associated with the preferential expansion of local tissue macrophages exhibiting an anti-inflammatory phenotype, when the injury is locally severe or chronic, additional inflammatory monocytes may also be required to restore normal tissue architecture. Nevertheless, the rapid conversion of these pro-inflammatory TNF-α producing macrophages to an anti-inflammatory IL-10 and TGF-β1 producing phenotype appears to be critical to the long-term survival of stem and progenitor cell populations in most tissues [59]. Thus, to facilitate effective organ regeneration and prevent fibrosis, the

monocyte and macrophage response must be finely tuned.

Macrophage secreted tissue repair mediators, like TGF-β, IL-10, IL-23 which are essential for the remodelling phase of wound healing, can be directed to treat gut leakage. Further IL-33, IL-4, IL-13 from macrophages are found to be capable of activating wound healing macrophages [60]. In addition, emerging pro-repair mediators like Granulin [61] and Plet1 [62], a wound repair mediator, have been recently shown to be expressed by resident alveolar macrophages and intestinal dendritic cells [63, 64]. Moreover, a deficiency of intestinal macrophages may increase susceptibility to infection and inhibit the activity of tissue repair. These studies suggest a promising role of these proteins as therapeutic agents to treat gut inflammation and gut leakage. However, the mode of delivery and mechanism of action has to be carefully considered during investigations. Taken together, macrophages are crucially involved in many aspects of wound healing. Depending

on their polarization and the phase of wound healing, they may promote wound closure. Identifying mechanisms that support repair, and understanding how these pathways are dysregulated in disease, are key to improving resolution of intestinal tissue damage during gut leakage.

Interplay between intestinal macrophages and microbiota

Macrophages form a well-established cell-type of the innate immune system that probe the host or invading microbes using pattern recognition receptors and exhibit efficient phagocytic and bactericidal activity [65]. Studies using germ-free or gnotobiotic mice models reported the potential role of gut microbiota in modulating macrophage phenotypes in the colon. Emerging studies have demonstrated the functional association of macrophages and gut microbiota that might be crucial to further understand and treat several diseases associated with gut dysbiosis-induced leaky gut syndrome. Despite the association of pro-inflammatory M1 and M2 macrophage phenotype in intestinal inflammatory and repair conditions respectively [66], the role of specific bacterial species on modulating macrophage phenotype is less studied. For example, bacteria such as *Enterococcus faecalis*polarize colonic macrophages toward the proinflammatory M1 phenotype in mice [67]. In addition, few studies have demonstrated an indirect association of dysbiosis with M1 phenotypes. For example, antibiotics induced dysbiosis with reduced Firmicutes and Bacteroidetes and decreased faecal concentrations of short chain fatty acids (SCFAs) have been associated with the accumulation of pro-inflammatory macrophages [68]. In contrast, *Clostridium butyricum* directly induced IL-10 production by intestinal macrophages in inflamed mucosa and triggered polarisation of the macrophages into the anti-inflammatory phenotype through the TLR-2/MyD88 pathway [69]. In addition,*Bacteroides fragilis* and Clostridia class, *Fusobacterium nucleatum* induce M2 polarization [70]. *Lactobacillus intestinalis* and *Lactobacillus johnsonii* drive macrophage to M2 phenotype and resolve mercury induced injury and intestinal permeability in vitro [71]. *Lactobacillus johnsonii* alleviates colitis via promoting M2 macrophages [72]. *Mytilus coruscus* reduced pro-inflammatory cytokines from macrophages in vitro and increased the abundance of some probiotics like Anaerotruncus, Lactobacillus, Desulfovibrio, Alistipe, Odoribacter, and Enterorhabdus in the colon and improved intestinal barrier integrity in vivo [73]. In another study, microbiota were observed to drive macrophage polarization towards the M2 phenotype and subsequently promote stem cell differentiation [74]. Interestingly, an in vivo study demonstrated the depletion of macrophages alters bacterial gut microbiota by promoting fungal overgrowth [75]. In another study, gut microbiota was shown to drive macrophage-dependent intestinal stem cell self-renewal mechanisms [76]. Moreover, microbial metabolites also have been studied in intestinal epithelial barrier repair via modulating macrophages. For example, butyrate, docosahexaenoic acid and other SCFAs reduce inflammation and help repair the intestinal barrier by driving M2 macrophage polarization [77] [78] [79]. In addition, bacterial components such as LPS and flagellin are reported to modulate and activate M2 macrophages represented by IL-3 and TGF-b signaling [80]. Macrophages infected by F. nucleatum upregulate IDO (Indoleamina 2,3-diossigenasi) on the cell surface, suggesting an additional mechanism whereby F. nucleatum might trigger macrophage-driven immunosuppression [81]. These studies suggest an explicit association of macrophages and gut microbiota and a careful modulation of microbiota composition might trigger a favorable macrophage phenotype to treat different disease conditions. Therefore, identifying the key mechanisms of gut microbiota and their products in regulation of macrophage phenotypes in the intestine needs further investigation for the development of

an effective therapeutic approach for treating gut leakage and its associated diseases.

Current treatment strategies

Growing evidences show a clear association of gut leakage with several diseases at extra-intestinal distinct organs including liver, lung, brain and metabolic diseases like obesity, etc. Leaky gut syndrome is a theory that intestinal permeability is not only a symptom of gastrointestinal disease but an underlying cause that develops independently. Currently, the suggested treatment options are probiotics to restore gut barrier function. This treatment may help maintain the health of gut lining by preventing overgrowth of pathogenic bacteria in the gut. However, the ability of probiotics to recover intestinal barrier function needs further investigation. The other treatment option is prebiotics, usually plant fibers that might feed beneficial gut bacteria. In contrast, avoiding dietary fats and sugars, which diminish pathobionts, could support the growth of beneficial gut bacteria. Moreover, a diet format, Low FODMAP Diet (stands for fermentable oligosaccharides, disaccharides, monosaccharides, and polyols, which are short-chain carbohydrates (sugars) that the small intestine absorbs poorly) has been suggested for the treatment of irritable bowel syndrome (IBS) and/or small intestinal bacterial overgrowth (SIBO). FODMAP foods are assumed to aggravate the gut leakage, however, there are no scientific evidences available. In addition, bacteria such as aerotolerant lactobacilli or wound-associated *Akkermansia muciniphila* might be critical for wound and anastomotic healing in the gut [82]. Moreover, kinase inhibiting drugs like ruxolitinib were found to alleviate inflammation, apoptosis, and intestinal barrier leakage in UC via STAT3 [83]. Vitamin D and the amino acid L-glutamine may specifically help repair gut lining [84, 85] however, more evidence is required to conclude these experimental findings. Nevertheless, there are certain limitations in treatment with probiotics and dietary habits. For example, designed dietary habits are considered difficult to follow with a modern lifestyle and the probiotics might induce unfavorable gut dysbiosis and further exaggerate the conditions. Fecal microbiota Transfer (FMT) has shown promising effects in experimental studies [86], while a single clinical study using total gastrointestinal flora transplantation showed a success rate of 89.3% in attenuating leaky gut syndrome [87]. Therefore, there is an urgent need to search for new therapeutic options to treat gut leakage that stands as an underlying condition for several other diseases.

Future perspectives

In the decades after their discovery, macrophages have been suggested as promising targets in almost all areas of biomedical research. Developments in the field of macrophage research in the past decade have led to a better understanding of the maturation and differentiation dynamics of this cell type. Accumulating evidence suggests that, apart from their well-known role in phagocytosis and foreign-antigen recognition, macrophages are endowed with high functional plasticity allowing them to acquire pro- or anti-inflammatory and tissue-reparative phenotypes during the course of inflammation, dependent on the signals they receive from the surrounding cells or from the pathogen itself. Given that the injury specific signals derived from the local microenvironment are integrated to generate specific macrophage polarization patterns, it is hypothesized by us that different compositions of microbiota dysbiosis generate unique macrophage polarization patterns at defined time points during inflammation or infection, to serve the needs of the infected/inflamed intestinal niche. A better understanding of these processes would allow the selective targeting of the macrophage pool for better host defences and accelerated intestinal epithelial barrier repair. Despite there being studies showing a relation between microbiota and macrophage phenotype, their released specific mediators that are involved in tissue repair are not well studied. Probiotics and fecal microbiota transplantation have been investigated in clinical trials for the treatment of gut leakage associated diseases including type 1 diabetes, multiple sclerosis, and rheumatoid arthritis [88]. Therefore, there is an urgent need to conduct experimental studies that focus more on those specific macrophage subsets with distinct functions and their relation to different compositions of microbiota to investigate their specific targets and released mediators, which would help explain those events instrumental in mediating pro- and anti-inflammatory mechanisms. This would help us to understand the microbiota induced macrophage-derived mediators and their interactions in an inflammatory microenvironment. Understanding these mechanisms would enable innovative therapeutic approaches like in-situ repolarization towards a regulatory or tissue-reparative phenotype, and ex-vivo gener-

ation of regulatory macrophages as a cell-based therapy to target host defense, termination of inflammation and tissue repair, to reduce intestinal epithelial barrier damage.

In addition, other possibilities of modulating microbiota and their metabolites, and so in turn the functional phenotype of the intestinal macrophages, must be considered. In one study, microbes like *Lacticaseibacillus casei* Strain Shirota were shown to modulate intestinal epithelial cell barrier integrity in vitro, via macrophages through their bacterial sensing ability and cytokine production [89]. Interestingly, the microbial metabolite butyrate was observed to be a potential regulator of epithelial barrier integrity in both in vivo and in vitro studies, by driving macrophages to an M2 phenotype, and was proposed as a candidate therapeutic target for UC [77, 78]. However, in future, the evidence for employing microbiota-derived metabolites to target macrophage plasticity, regulating the release of pro-repair mediators including EVs, method to generate the reprogrammed/reengineered macrophages and association of pro-repair macrophages and microbiota must be explored in detail.

Furthermore, the underlying pathological mechanisms of gut leakage associated diseases remain mostly unknown. Moreover, the precise part of the intestine (proximal or distal) where epithelial barrier dysfunction initially occurs has yet to be determined. Furthermore, there need to be established gut leakage animal models to enable further investigations and proof-of-concept for developing promising therapies. Finally, the role of non-bacterial microbiota like the virome, mycobiome, etc. must also be carefully considered in future investigations.

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Competing interests

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Authors' contributions

BS and RS planned and structured the manuscript. BS drafted the first draft and generated figures. PS wrote a section and revised the manuscript. RS critically reviewed and submitted the manuscript. All authors contributed to manuscript revision and approved the submitted version.

Table1. Putative macrophage tissue repair mediators to treat gut leakage

Mediators	Functions
TGF-β	Reduced inflammation, pathological structural changes, , and induce
PDGF	Enhanced the cascade of tissue repair processes required for a wound
IGF-1	Promoted the intestinal regenerative response after irradiation injury
FGF-10	Enhanced signaling through Fgfr2b receptor accelerated the repair pr
Maresin and, resolvin-1	Secretory proteins pro-resolving lipid mediators and pathways are inv
MIF and its receptor CD74	Enhanced intestinal epithelial cell regeneration, healing, and maintai
ICAM-1	Role in macrophage efferocytosis and wound healing
MMP-10	Extracellular matrix-degrading enzymes, moderating scar formation
IL-33*	Enhanced activation of wound healing macrophages
IL-1ra	Enhance the mRNA expression of COX-2, iNOS, CINC-1, HGF, and
IL-10*	Reduced inflammation, pathological structural changes, and induce a
miRNAs let-7c, miR-124 and miR-223	Reported to promote M2 macrophage polarization and suppresses M
miR-155	Central role in alternative M2 skewing in cardiac injury and colitis

Mediators	Functions
M2 macrophage-derived exosome miR-590–3p	Reduced colonic inflammation, strengthening mucosal healing, elevat
sTREM2	Enhanced M2 phenotype and preserve macrophage pool after inflamm
Lysophosphatidic acid and Sphingosine 1-phosphate	Role in monocyte–macrophage system during wound healing and forn
COX2	Potentiates efferocytosis and facilitates macrophage intestinal epithel
Exosomes TGF-β, IGF-I and VEGF	Reduced intestinal inflammation and enhance tissue repair, which rep

*Stimulated macrophage to excert the mentioned functions.

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Figure legends

Figure 1 Macrophage origin, differentiation and plasticity. Macrophages may originate both at the prenatal stage from the yolk sac and fetal liver, and during the postnatal stage from the bone marrow via CCL2/CCR2 axis. In specific tissue contexts, macrophages are programmed by local factors. Here they may be both long-lived self-renewing cells or replenished from the blood monocyte pool. The macrophage activation states in tissues can be loosely equated to macrophages in disease tissues, but they are heterogeneous in origin and phenotypically plastic, with variable contributions to disease progression. They show an alternatively activated M2 phenotype in the repair phase and could substitute the tissue resident macrophages.

Figure 2. Role of macrophages in association with microbiota in maintaining intestinal barrier function. During homeostasis in the lamina propria, cross-talk between resident intestinal macrophages and regulatory T cells (Treg) results in IL-10 and TGF-β production that helps to maintain the intact intestinal epithelial cell (IEC) barrier with tight junction (TJ) proteins. Intestinal lumen is mainly populated with healthy microbiota in homeostasis. In diseases conditions, intestinal recruited macrophages show an pro-inflammatory phenotype with release of TNF-α, iNOS, IL-1β , IL-6 and IL-18 which further damages the IEC barrier. However, if this acquired phenotype is due to phagocytosis of specific dysbiotic microbial products, is yet to be studied. In addition, macrophages that phagocytose apoptotic IEC release chemokines like CL2, CCL7, CXCL1 and 2, which recruits further neutrophils and monocytes into intestinal tissues. At this phase, the intestinal lumen observed with dysbiosis, apoptotic IEC and damaged IEC barrier with loss of TJ proteins, leading to translocation of microbial products and pro-inflammatory mediators into lamina propria and eventually to systemic circulation promoting systemic inflammation and distinct organ damage. During repair phase, macrophage acquire a pro-repair phenotype and release various tissue repair mediators including TGF-β, PDGF, IGF, VEGF and IL-10 along with proposed repair mediators like Granulin and Plet1. These mediators resolve the inflammation and repair IEC barrier by expression of TJ proteins and regulate the intestinal barrier function. However, if this acquired phenotype is due to phagocytosis of specific pro-repair microbial products, is yet to be studied.