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Macrophages in diabetic gastroparesis– the missing link?

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Abstract

Background—Diabetic gastroparesis results in significant morbidity for patients and major economic burden for society. Treatment options for diabetic gastroparesis are currently directed at symptom control rather than the underlying disease and are limited. The pathophysiology of diabetic gastroparesis includes damage to intrinsic and extrinsic neurons, smooth muscle and interstitial cells of Cajal (ICC). Oxidative damage in diabetes appears to be one of the primary insults involved in the pathogenesis of several complications of diabetes, including gastroparesis. Recent studies have highlighted the potential role of macrophages as key cellular elements in the pathogenesis of diabetic gastroparesis. Macrophages are important for both homeostasis and defense against a variety of pathogens. Heme oxygenase 1 (HO1), an enzyme expressed in a subset of macrophages has emerged as a major protective mechanism against oxidative stress. Activation of macrophages with high levels of HO1 expression protects against development of delayed gastric emptying in animal models of diabetes, while activation of macrophages that do not express HO1 are linked to neuromuscular cell injury. Targeting macrophages and HO1 may therefore be a therapeutic option in diabetic gastroparesis.

Purpose—This report briefly reviews the pathophysiology of diabetic gastroparesis with a focus on oxidative damage and how activation and polarization of different subtypes of macrophages in the muscularis propria determines development of delay in gastric emptying or protects against its development.

Keywords

Diabetic gastroparesis; Oxidative stress; Macrophage; Macrophage polarization; Heme oxygenase 1

Introduction

Gastroparesis is defined by delayed emptying of gastric contents in the absence of mechanical obstruction. Nausea, vomiting, bloating, early satiety and abdominal pain are the major symptoms of gastroparesis.^{1–3}

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Idiopathic and diabetic gastroparesis are the most common causes of gastroparesis, each accounting for about one third of the patients with gastroparesis. Gastroparesis can also be iatrogenic due to medications or post-surgery, or be secondary to neurological, metabolic, infectious, and infiltrative disorders.⁴ With the global rise in obesity-related diabetes, gastroparesis is being increasingly recognized in type 2 diabetics.

The incidence and prevalence of gastroparesis is not well understood. The increased awareness and recognition of gastroparesis has resulted in increased health care utilization over the past 2 decades.⁵ There is a poor correlation between the degree of delay in gastric emptying and gastroparesis symptoms.^{6–10} Also in patients with longstanding diabetes, hyperglycemia may cause changes in CNS that alters visceral sensation,¹¹ making symptom-based studies unreliable. The prevalence of gastroparesis has been reported to be as high as 50% in patients with type I diabetes and 30% in patients with Type II diabetes^{7, 8} but this is an overestimate. A study from Olmsted County reported the incidence and prevalence of gastroparesis in the community. In this population-based study, prevalence of gastroparesis was 37.8 in women and 9.6 in men per 100,000 people. Diabetes increased the risk of developing gastroparesis by 30 and 8 fold in type I and II diabetes, respectively, with the 10 year cumulative incidence of gastroparesis 5.2% in patients with type I diabetes, 1.0% in Type II diabetics and 0.2% in controls. The risk was estimated to be 4 fold higher in patients with type I diabetes compared to age and sex matched type II diabetic gastroparesis.¹²

The role of hyperglycemia in gastric emptying and pathogenesis of diabetic gastroparesis has been a subject of longstanding controversy. Multiple studies have shown that acute hyperglycemia causes a delay in gastric emptying through decreased tone in the fundus and decreased contractility in the body and antrum as well as increased contraction of the pylorus, in animals and both normal and diabetic subjects.^{13–15} Acute hyperglycemia may directly modulate vagal afferent activity. Vagal afferents from rat nodose ganglia are glucose-sensitive and closure of ATP-sensitive potassium channels by glucose increases their excitability.¹⁶ Glucose also modulates excitatory synaptic transmission in vagal afferent fibers by increasing the number of presynaptic 5-HT₃ receptors as well as their response to 5HT.¹⁷ However, the effect of chronic hyperglycemia on gastric emptying is not clear, and may be different for patients with type I and II diabetes.

The control of gastric emptying is complex and requires coordinated activity of several cell types with multiple feedback loops.^{13, 18, 19} In the past 15 years several key cell types have been implicated in pathogenesis of diabetic gastroparesis. These include extrinsic and intrinsic enteric nerves, smooth muscle cells and interstitial cells of Cajal (ICC). More recently, evidence for involvement of immune cells, in particular macrophages, as a potentially unifying cell type underlying the pathophysiology of diabetic gastroparesis has emerged.^{20–22}

In this review we focus on diabetic gastroparesis and briefly overview the role of key cell types involved in pathogenesis of diabetic gastroparesis. We review the increasing body of evidence highlighting a role for muscularis propria macrophages in the development of diabetic gastroparesis. We discuss how the polarization and plasticity of muscularis propria macrophages determine the distinct function of these immunomodulatory cells and may lead

to the observed pathology in other cell types essential in the pathology of diabetic gastroparesis. Finally we propose potential therapeutic targeting opportunities.

Extrinsic neuropathy

The sympathetic and parasympathetic nervous systems innervate the stomach and aid in the control of motor, sensory and secretory responses. Autonomic neuropathy is a well-established complication of diabetes. Autonomic neuropathy was the first pathology associated with the development of diabetic gastroparesis in an animal model.^{23, 24} Postmortem studies of patients with insulin-dependent diabetes have shown severe loss of myelinated fibers in the sympathetic trunks as well as markedly enlarged dystrophic axons and nerve terminals in prevertebral sympathetic ganglia.²⁵ Oxidative stress and subsequent metabolic insult is thought to be the major mechanism in the pathogenesis of extrinsic diabetic neuropathy.

Intrinsic neuropathy

Intrinsic neuropathy with involvement of both excitatory and inhibitory nerves has been described in animal models and in humans with diabetic gastroparesis.²⁶ Electron microscopy analysis of enteric nerves from full thickness stomach biopsies from patients with diabetic gastroparesis showed evidence for neuronal abnormalities such as empty nerve terminals in most patients with diabetic gastroparesis and the encircling of nerves by a thick basal lamina and/or a thick collagen fibril sheaths.²⁷ On the other hand, using PGP9.5 as a neuronal marker in this study showed that there was little loss of PGP9.5 expression. Since nerve numbers are preserved, this suggests that while there are distinct neuronal changes, there may be a potential for reversibility of the underlying defects.^{18, 28} Neuronal nitric oxide synthase (nNOS) expression has been shown to be reduced in diabetic gastroparesis, both in animal models^{20, 29–33} and in some but not all human specimens.^{18, 34} Interestingly, in Non Obese Diabetic (NOD) mice, nNOS expression is decreased in both mice with diabetes and normal gastric emptying and in diabetic mice with delayed gastric emptying, suggesting diabetes itself may result in loss of nNOS expression.²⁰

The susceptibility of NOS containing neurons to damage in diabetes is postulated to be due to increased intracellular Ca^{2+} levels.³⁵ Ca^{2+} dependent NOS activation is increased as the result of stress-induced elevation of cytoplasmic Ca^{2+} , leading to excessive levels of the free-radical, NO. NO may be destructive on its own by causing abnormal protein S-nitrosylation or through lipid peroxidation in combination with reactive oxygen species.³⁵ Activation of receptors for advanced glycation end products (AGE) as the result of prolonged hyperglycemia may also contribute to nitrergic neuronal damage.³⁶ In the streptozotocin induced diabetic rat model nNOS dimerization was found to be abnormal. Active dimeric forms of nNOS were significantly reduced in female but not in male rats.³⁷ Restoration of nNOS dimerization reversed the delay in gastric emptying in diabetic rats.³⁸ Supplementation of the obligate co-factor for nNOS dimerization, BH4, has also been shown to improve gastric emptying in the diabetic rat.³⁹

Interstitial Cells of Cajal

Interstitial cells of Cajal (ICC) are pacemaker cells of the GI tract that generate and propagate slow waves, set the smooth muscle membrane potential and act as mechanotransducers.⁴⁰ Dysrhythmias of the gut caused by abnormal slow wave activity have been described^{41–43} and high-resolution electrical mapping has shown abnormal slow-wave activity in patients with gastroparesis.⁴⁴ Intrinsic enteric neurons communicate with ICC and ICC partly mediate nitrergic and cholinergic neurotransmission to the smooth muscle cells.^{45–47} These roles make loss of ICC of particular relevance to diabetic gastroparesis. The major cellular defect in diabetic gastroparesis is indeed loss of ICC, which has been consistently reported, in both humans and animal models.^{18, 20, 48–52}

In the diabetic NOD mouse model, loss of ICC networks in both the corpus and antrum was associated with the development of delayed gastric emptying. All mice with delayed gastric emptying had decreased Kit levels suggesting loss of ICC is a critical step in the development of delayed gastric emptying.^{20, 53}

There are several retrospective studies showing ICC pathology in patients with diabetes and gastroparesis.^{48, 49, 51} To systematically study the role of ICC in pathogenesis of gastroparesis, the Gastroparesis Clinical Research Consortium, funded by National Institutes of Health, performed a detailed investigation of the full-thickness biopsies from a cohort of patients with diabetic gastroparesis.^{18, 27} As seen in animal models, decreased numbers of ICC cell bodies, were observed in humans. At an ultra-structural level, ICC changes were seen in every patient except one.²⁷ These included abnormalities in intra-cytoplasmic vacuoles, mitochondrial matrix and rough endoplasmic reticulum. Apoptotic features consist of compact chromatin filling the entire nucleus or close to remnants of the nuclear envelope, decreased and abnormal mitochondria and increased lysosomes, which were observed in all of the patients with ultrastructural changes. Moreover, there was significantly less ICC cell-to-cell contact with smooth muscle cells, and nerve endings.²⁷

Multiple mechanisms have been proposed to be involved in the loss of ICC in diabetic gastroparesis. Decreased insulin and IGF-1 in diabetes results in ICC depletion secondary to smooth-muscle atrophy and a decrease in associated stem cell factor.⁵⁴ More recently with oxidative stress proposed as a primary pathology in diabetes, focus has turned to the loss of HO1 containing macrophages. Diabetes is a high oxidative stress state, and failure of up-regulation of macrophage HO1 increases ICC damage and loss of nNOS, a process that in animal models is reversible by the HO1 inducer hemin or the HO1 end product, carbon monoxide.^{20, 22}

Role of oxidative stress in the pathogenesis of diabetic gastroparesis

There is increasing evidence that oxidative stress orchestrates the paradigm of hyperglycemia induced cellular injury.⁵⁵ In mouse the presence of diabetes is associated with increased levels of oxidative damage as measured by malondialdehyde levels. Serum malondialdehyde levels were substantially higher in the mice that developed delayed gastric emptying compared with the diabetic mice with normal gastric emptying. In contrast, in mice treated with hemin, oxidative stress levels were substantially decreased.²⁰

Hyperglycemia increases AGE that cause oxidative damage, leading to mitochondrial dysfunction.⁵⁶ Excess intracellular glucose also activates alternative glycolytic pathways. End products such as sorbitol and fructose increase cellular osmolality and deplete NADPH, which leads to oxidative stress in endothelial and neuronal cells.⁵⁶ Further, accumulation of AGE in diabetes can cause cell injury not only directly by changing protein function, but also by binding to the receptor for AGE, causing an inflammatory signaling cascade leading to oxidative⁵⁷ and nitrosative stress.⁵⁸ The receptor for AGE is expressed in enteric neurons³⁶ but it is not known if it is also expressed in ICC. In addition to hyperglycemia, dyslipidemia and increased free fatty acids in diabetes can cause oxidative stress. Oxidized and glycated LDLs activate NADPH oxidase, which produce superoxide radicals and depletes NADPH levels.⁵⁹ Decreased Nrf2 (nuclear factor erythroid 2-related factor), a transcriptional factor that protects the cells from oxidative stress has been shown to be associated with dysfunction in nitrgic relaxation and decreased NOS dimerization in apolipoprotein E knockout mice, an animal model of oxidative stress.⁶⁰

The oxidative and nitrosative stress pursuant to AGE receptor activation is one of the mechanisms underlying decreased nNOS expression in myenteric neurons.³⁶ Prevention of the formation of AGE in the streptozotocin induced diabetic rat, results in preservation of nNOS expression.³¹ Reactive oxygen species further increase apoptosis in enteric neurons. This process can be prevented *in vitro* by lipoic acid.⁶¹ In a mouse model with heme-free guanylate cyclase, hypertrophy of the smooth muscle layers of the fundus and pylorus was associated with delayed gastric emptying.⁶²

In recent work Nezami et al. showed that mitochondrial oxidative stress is one of the underlying mechanisms involved in enteric nerve apoptosis secondary to accumulation of the free fatty acid palmitate. Reactive oxygen species induced lipid peroxidation of palmitate free fatty acids in the mitochondria and led to apoptosis of enteric neurons. A microRNA, Mir 375 expressed in myenteric ganglia was identified as responsible for induction of apoptosis in enteric neurons and inhibition of this microRNA prevented the palmitate-induced enteric neuronal cell death, both *in vitro* and *in vivo*.⁶³

These data, taken together, suggest that oxidative stress may underlie several of the cellular abnormalities seen in diabetic gastroparesis.

Heme oxygenase, macrophages and oxidative stress

Heme oxygenase (HO) is increasingly recognized as an essential enzyme for protection against oxidative stress through catalysis of heme to its end products, in particular carbon monoxide and biliverdin. Compelling evidence suggests a role for HO as a strong anti-inflammatory agent in health and disease.⁶⁴ Heme oxygenase protects tissues from oxidative stress not only directly but also through macrophage activation and polarization.⁶⁵

There are 2 major isoforms of heme oxygenase. Heme oxygenase 1 (HO1) is the inducible isoform of heme oxygenase and is up-regulated in response to stressors such as oxidative stress. Heme oxygenase 2 (HO2), is the constitutive isoform and its role in the regulation of oxidative stress is not as well established.⁶⁶

Both isoforms of heme oxygenase are implicated in prevention of development of gastroparesis. In streptozotocin-induced diabetic rats, the population of nNOS-containing neurons that also contain HO2 within the myenteric plexus are resistant to changes induced by oxidative stress.⁶⁷ As outlined above, in the NOD mouse model of type 1 diabetes, loss of expression of HO1 in macrophages leads to development of delayed gastric emptying. Furthermore, in this model, treatment with hemin polarized macrophages towards the M2 phenotype, increased HO1 levels and activity, decreased the high levels of oxidative stress and reversed delayed emptying. This was accompanied by increased expression of Kit and nNOS²¹ and has also been reported for other models of diabetes.⁶⁸ The downstream product of HO1, carbon monoxide appears to be sufficient to mediate the protective effects of HO1 as carbon monoxide inhalation reversed delayed gastric emptying in diabetic mice. Carbon monoxide treatment reduced oxidative stress and restored Kit expression even in the presence of a heme oxygenase activity inhibitor²² suggesting the effects of carbon monoxide are down stream of HO1. In the stomach muscle wall macrophages are the main source of HO1.²¹

Macrophages in the rodent and human GI tract

Macrophages are critical for innate immunity and have diverse roles not only limited to inflammation and host defense but are also involved in tissue remodeling, hemostasis, and tumorigenesis.⁶⁹ Gut macrophages outnumber all other tissue macrophages.⁷⁰ In the GI tract, macrophages show significant heterogeneity and have distinct phenotypes and functions.

GI macrophages comprise 2 major populations; mucosal macrophages and muscularis propria macrophages. Mucosal macrophages are located in the lamina propria, Peyer's patches and mesenteric lymph nodes, and are associated with the epithelial innate immune defense against exogenous pathogens.⁷¹ Muscularis propria macrophages populate the muscle wall and are particularly common in the myenteric plexus region but also found throughout the circular and longitudinal muscle layers.^{72–74} This class of macrophages develops independently of foreign antigens.⁷⁵ Muscularis propria layer macrophages have a different phenotype from macrophages in the subserosa and submucosa. Muscularis propria macrophages are characterized by a central nucleus and between 2 to 5 long slender cell processes. Muscularis propria macrophages express MHC class-II antigen constitutively. While MHCII positive macrophages are not present in newborn and germ-free adult mice, the intestinal microbiota up-regulates their expression.⁷⁵ Muscularis propria macrophages are mostly acid phosphatase negative, and have large numbers of primary lysosomes while secondary lysosomes are rare.⁷³

Although macrophages are present in rodent gastric muscular layers, as compared to other parts of the gastrointestinal tract, there is no detailed histological information on the distribution of macrophages in the stomach and their relation to ICC and other key cell types associated with control of gastric emptying. In humans, a close proximity between macrophages and myenteric ICC is reported in the colon.⁷⁶ Studies of rodent GI tract macrophages have shown that macrophages are in close spatial contact with ICC and neurons at the level of myenteric plexus in the mouse small intestine. In contrast, at the level

of the deep muscular plexus they are surrounded by ICC and fibroblast-like cell processes but not nerves.^{72, 77} Powley et al. recently showed the structural relationship between muscularis propria macrophages and intrinsic/extrinsic neurons in rat GI tract.⁷⁸ Macrophages were noted to outline and engulf myenteric plexus ganglia with extensive appositions with myenteric plexus neurons. They proposed a role for muscularis propria macrophages in performing housekeeping function in the GI tract with regards to normal aging, apoptosis and maintenance of synaptic homeostasis, a well-defined role for macrophages in the central nervous system.⁷⁸ Muscularis propria macrophages are also involved in regulation of gut motility. Extensive studies have shown a crucial role for muscularis propria macrophages in the pathogenesis of postoperative ileus⁷⁹ and here we review their role in diabetic gastroparesis.

Macrophages polarization and plasticity

Macrophages show remarkable plasticity and may be activated to either classically activated macrophages (M1) or alternatively activated macrophages (M2) in response to adaptive and innate stimuli. They are also able to switch from one activation state to another, which suggests that the different phenotypes represent a continuum of the activated phenotype rather than stable distinct ones.^{80, 81}

The change in the phenotype in response to the environmental cues is dictated by the expression of transcription factors and surface markers. Activation of differing transcription factors determines the outcome of macrophage polarization.⁸² Activation of toll-like receptor (TLR) and subsequent nuclear factor NF- κ B heterodimerization and STAT1 activation, results in M1 macrophages.^{83, 84} IL-4 dependent STAT6 activation, as well as IL-10 dependent NF- κ B homodimerization, and STAT3 activation promotes M2 polarization.^{85–89} A number of epigenetic mechanisms including microRNAs have recently emerged as important regulators of macrophage polarization.⁹⁰

Classically activated M1 macrophages are pro-inflammatory. IFN γ alone or along with lipopolysaccharide (LPS) or granulocyte-macrophage colony-stimulating factor (GM-CSF) can polarize mouse macrophages to the M1 phenotype.^{69, 91, 92} M1 macrophages are phenotypically IL-12^{high}, IL-23^{high}, IL-10^{low} and have enhanced MHCII expression levels. In mice, they secrete a wide range of pro-inflammatory cytokines such as tumor necrosis factor TNF α , interleukin IL- β , and NO, which directly contribute to their antimicrobial function. They also secrete a wide range of chemokines that recruit T cells for further defense in Thelper1 (Th1) immunity. M1 macrophages play a major bactericidal role by endocytosis, elimination of iron and nutrients, acidification of the phagosome, synthesis of reactive oxygen and release of NO.^{69, 84, 91, 92} When the M1 pro-inflammatory response is uncontrolled it causes significant collateral tissue damage.^{93, 94}

Alternatively activated M2 macrophages are anti-inflammatory and participate in Thelper2 (Th2) responses essential for countering parasitic infections, tissue remodeling, wound healing, and fibrosis.^{76, 97} M2 macrophages have an IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and express scavenger receptors such as galactose-type and the mannose receptor (CD206).⁹⁶ M2 macrophages exert anti-inflammatory effects through induction of HO1 in

response to several substances including IL-10, hemin and 15-deoxy- 12,14 prostaglandin J2.^{20, 95} M2 macrophages secrete distinct cytokines and chemokines. A number of cytokines have been identified to be involved in M2 polarization, among which IL-4 and IL-13 are the most potent inducers studied.^{96–98} IL-4 has been also shown to be an inducer of M2 macrophage proliferation in a variety of tissues without recruitment from the circulating blood.⁹⁹

Along with their opposing roles, mouse M2 and M1 macrophages differ significantly in utilizing L-arginine as a substrate through arginase and inducible nitric oxide synthase (iNOS) activity. In mice, M1 macrophages mainly express iNOS and generate reactive NO species with pro-inflammatory effects and M2 macrophages, in contrast to M1, have high levels of arginase 1 which competes with iNOS for arginine and generates L-ornithine as part of the urea cycle.^{89, 100, 101} However human macrophages do not appear to express iNOS and M2 macrophages lack arginase.^{102, 103}

Another interesting difference between M1 and M2 macrophages, shared in both human and mouse, is in iron handling. While iron sequestration through ferritin is a bacteriostatic strategy in M1 macrophages, M2 macrophages express heme scavenger receptors (CD163) and utilize this substrate with strong anti-inflammatory effects.^{104, 105}

M1 and M2 macrophages share many of same surface markers and therefore better measures such as quantification of the expression of surface markers and/or transcription factors are required to fully differentiate the 2 (or more) subtypes of macrophages.

Macrophages in gastroparesis

A key role for macrophages in pathophysiology of diabetic gastroparesis is emerging (Figure 1). In models of both type 1 and type 2 diabetes, the presence of diabetes was associated with an increase in the number of macrophages in the muscularis propria.²⁰

Macrophage plasticity appears to play an important role in pathogenesis of gastroparesis. Polarized activation of gastric macrophages during diabetes in mice determines the development or protection against the development of gastroparesis. M2 macrophages accounted for the majority of the macrophages in diabetic mice, although they represent less than 10% of resident macrophages in the non-diabetic mice. The polarization to M2 macrophages in diabetes is consistent with the anti-inflammatory role of the M2 population in this high free radical state. M2 macrophage HO1 appears to serve as an effective protective mechanism against the oxidative damage of diabetes. This was shown in diabetic mice with delayed gastric emptying who were treated with hemin to activate HO1. Repopulation of the gastric muscle layers with M2 macrophages caused a decrease in oxidative stress and reversal of delayed gastric emptying.

Interestingly, vagal stimulation was found to be protective against development of postoperative ileus by inactivation of macrophages.¹⁰⁶ In animal models of postoperative ileus the protective effect of vagal nerve stimulation was suggested to be secondary to the activation of resident macrophages through the STAT3/Jak2 signaling pathway.¹⁰⁷ Although in these series of investigations the distinct subtype of macrophages were not elucidated, in

light of the known role of STAT3 signaling in activation of M2 macrophages⁸⁹, it would be interesting to determine if vagal nerve stimulation preserves gut motility through activation of M2 macrophages.

In NOD mice, development of diabetes was associated with appearance of M2 macrophages with high levels of arginase 1 expression, consistent with expression of the M2a macrophage subtype. Arginase 1 in M2a macrophages will compete with iNOS for Larginine and thus protects the tissue from production of NO free radicals. Delay in gastric emptying was evident only after M2 macrophages phenotypically switched to M1. The activation of M1 pro-inflammatory macrophages and subsequent inflammatory cascade response is likely responsible for damage to ICC and potentially other cell types as well as decreased nNOS expression; pathologies that are extensively studied in diabetic gastroparesis. On the other hand, conversion of M2a to M2c macrophages protects diabetic mice from the development of delayed gastric emptying.²¹ M2c macrophages secrete high levels of IL-10, which may protect and preserve the ICC network and thereby prevent the development of diabetic gastroparesis. Indeed in a recent study, treatment of diabetic mice with delayed gastric emptying with IL-10 reversed the delay in transit and improved pathophysiology.¹⁰⁸

Abnormal macrophage infiltration in muscularis propria in diabetic gastroparesis has been also noted in human full thickness biopsy samples. Using antibodies against CD68, a general macrophage marker, CD68+ cells were identified in the myenteric plexus.¹⁸ Interestingly, it appears that in human, like mouse, there is a significant correlation between the number of ICC and CD206+ cells (M2 macrophages) in the presence of diabetes with or without gastroparesis.¹⁰⁹ The scarcity of reliable human macrophage markers is a significant limitation in performing detailed studies to evaluate the possible phenotypic switch of macrophages in human gastroparesis and needs to be addressed in future studies.

Differences between human and mouse macrophages

Studies of macrophages show significant interspecies differences. Most of the current data on macrophage polarization is from mouse and cannot be generalized to humans. Mouse and human macrophages have different surface markers. Many of the reliable mouse macrophage markers, including the pan mouse macrophage marker, F4/80, are not useful in human tissues. The protein recognized by F4/80 antibodies is EMR1, a member of the epidermal growth factor EGF-transmembrane family that is not selectively expressed on the human macrophage.¹¹⁰ Therefore antibodies to F4/80 that are widely used to label mouse macrophages cannot be used to label human macrophages. Also there is a significant need for good markers to differentiate between the two macrophage subtypes in humans. Arginase 1 and iNOS activity are commonly used to differentiate M2 and M1 macrophages in mice¹⁰⁰ however, human macrophages are devoid of arginase 1.¹⁰²

Other than than arginase 1, resistin-like molecule alpha (RELM α) and Ym1/2 are two distinct mouse M2 macrophage markers^{97, 111} that are also not found in human macrophages. In recent years, extensive work has been done on identifying human M2 macrophage markers and the signature genes and proteins that determine human and mouse M2 macrophages. Transglutaminase 2 (TGM2) was found to be a conserved M2

macrophage marker in both human and mouse.¹¹² TGM2 is expressed in response to IL4 and activates TGF- β and therefore prompts anti-inflammatory and profibrotic cytokine cascade.¹¹³ A combination of CD206 (mannose receptor) and TGM2 has been suggested to identify human M2 macrophages.¹¹²

Potential therapeutic avenues

Our increased understanding of the role of macrophages in the pathophysiology of diabetic gastroparesis may open up new therapeutic options targeting the macrophages. One potential strategy is to re-polarize macrophages to the M2 cyto-protective phenotype. An example would be the use of IL-10 or other 'protective' cytokines such as IL-4. Similarly, polarization of macrophages as a therapeutic strategy has been under scrutiny in a variety of cancers where macrophages are important for progression and invasion to distant locations. A number of different clinical trials; IFN γ in patients with soft tissue sarcomas or bisphosphonates in metastatic bone disease, are currently investigating the possibility of use of these agents in polarizing the macrophages and will inform on their use in other disease states including gastroparesis. Another option is to directly induce HO1, as induction of HO1 appears to be critical in the prevention of development of delayed gastric emptying and reversal of delayed gastric emptying in mice. A third strategy is to identify the injurious substances released by M1 macrophages and block their release. In mice an obvious candidate would be iNOS and NO, but human M1 macrophages do not appear to express iNOS and we therefore need to identify the equivalent injurious substance from human gastric M1 macrophages.¹⁰³ Yet another option would be to bypass the macrophage and deliver the end product of HO1 activity, biliverdin and or carbon monoxide. Very low amounts of inhaled carbon monoxide (100 ppm) can reverse delayed gastric emptying in mice.²² However there are considerable real and perceived hurdles in safely delivering inhaled carbon monoxide and therefore other delivery systems for carbon monoxide are needed. Currently there are clinical trials using the gaseous form of carbon monoxide and the oral or parenteral CO-releasing molecules (CO-RMs)¹¹⁴ which will inform on their potential use in diabetic gastroparesis.

Future research and conclusions

Our current knowledge of the protective effects of M2 macrophages in diabetic gastroparesis comes predominantly from mouse studies. These studies highlight the importance of macrophage polarization in the pathogenesis of diabetic gastroparesis. However identification of macrophage subtypes in human gastrointestinal smooth muscle lags behind the detailed characterization that has been done in mice and there are no well-established markers to differentiate human macrophage subsets. Further work is required to identify the subtypes of macrophages that are present in the normal human stomach and show how their expression changes along with the development of disease. As outlined above we need to understand the molecules released by M1 and M2 macrophages and how these agents result in damage or protection from damage of key cell types such as ICC and enteric nerves.

In conclusion, an increasing body of evidence from both outside and within the gastrointestinal tract highlights the importance of the balance between pro-inflammatory and

anti-inflammatory macrophages in development of a variety of diseases including diabetic gastroparesis. Changes in the macrophage phenotype in the stomach may underlie and link the diverse cellular changes seen in diabetic gastroparesis. As our knowledge base continues to grow, macrophages may hold promise as new therapeutic options to target the underlying disease rather than manage symptoms in diabetic gastroparesis.

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Key Messages

- Activation and polarization of macrophages may underlie the diverse cellular changes seen in diabetic gastroparesis including loss of interstitial cells of Cajal (ICC) and nNOS in enteric neurons.
- We review current knowledge on the role of macrophages in pathogenesis of diabetic gastroparesis.
- A review of the literature suggests that oxidative stress associated with diabetes activates macrophages. Activation of CD206 +, anti-inflammatory M2 macrophages expressing heme oxygenase 1 (HO1) is protective while activation of pro-inflammatory M1 macrophages which lack HO1 is injurious and leads to the development of delay in gastric emptying.

Figure 1. A proposed model for the development of diabetic gastroparesis

Muscularis propria macrophages are located in close proximity to ICC and enteric neurons. Diabetes causes macrophage activation and polarization. Activated macrophages based on genetic predisposition and environmental cues can switch to M2 anti-inflammatory or M1 pro-inflammatory phenotype. M2 macrophages are CD206+ and have increased levels of HO1 expression. HO1 from CD206+ M2 macrophages protects ICC and nNOS expression in enteric neurons from oxidative damage and prevent development of delayed gastric emptying (left panel). Activation of M1 macrophages and loss of HO1 expression (right panel) is associated with loss of ICC and nNOS expression as well as possibly underlying some of the other commonly seen changes in diabetic gastroparesis including loss of smooth muscle cells and increased collagen fibrils in the connective stroma.