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VISIONS AND REFLECTIONS

Clostridium difficile-related postinfectious IBS: a case of enteroglial microbiological stalking and/or the solution of a conundrum?

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Abstract
Post-infectious irritable bowel syndrome is a well-defined pathological entity that develops in about one-third of subjects after an acute infection (bacterial, viral) or parasitic infestation. Only recently it has been documented that an high incidence of post-infectious irritable bowel syndrome occurs after *Clostridium difficile* infection. However, until now it is not known why in some patients recovered from this infection the gastrointestinal disturbances persist for months or years. Based on our in vitro studies on enteric glial cells exposed to the effects of *C. difficile* toxin B, we hypothesize that persistence of symptoms up to the development of irritable bowel syndrome might be due to a disturbance/impairment of the correct functions of the enteroglial intestinal network.

Keywords *Clostridium difficile* · Enteric glial cells · Post-infectious irritable bowel syndrome · Toxin B

Introduction

It has been estimated that irritable bowel syndrome (IBS) affects up to 18% of the population worldwide [1]. A defined risk factor for its development is infectious enteritis and it is known as postinfectious-IBS (PI-IBS) [2]. This IBS subtype has been reported following bacterial (*Campylobacter jejuni*, *Salmonella*, *Shigella* spp., *Escherichia coli*) and viral (norovirus) infections, and parasitic (*Giardia duodenalis*) infestations [3]. A recent review with meta-analysis carried out on more than 20,000 individuals with infectious enteritis reported than more than 10% of patients developed IBS later, at a rate four times higher than that found in nonexposed subjects [4].

These recent data did not take into account another enteric pathogen, *Clostridium difficile*. It is worth noting that asymptomatic colonization with toxigenic strains is frequent and up to more than 20% of patients with a suspected such infection may have alternative etiologies for persistent diarrhea [5, 6]. However, this pathogen, only in the United States, is responsible for half a million infections and it was associated with approximately 29,000 deaths in 2011 [7]. Some sporadic reports obtained from small cohort studies in patients with hospital-acquired infection [8, 9] and IBS outpatients [10] yielded conflicting results. Thereafter, a large study in a US military population revealed that more than 14% of subjects exposed to *C. difficile* later developed functional gastrointestinal disorders (IBS, gastroesophageal reflux disease, dyspepsia, and constipation) compared to 6% in nonexposed controls. Of interest, the largest rate ratios were seen for IBS among community- (rate ratio = 8.9) and health care-associated (rate ratio = 5.5) *C. difficile* infection [11]. A more recent study on a cohort of more than 300 patients infected with *C. difficile* reported that 25% of them developed PI-IBS at least 6 months after the infection, a percentage higher than that reported for other pathogens [12]. Interestingly, the analysis of the risk factors evidenced that only a symptoms' duration of more than 7 days was significantly associated with the development of PI-IBS.

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Pathophysiologic aspects

Several putative pathophysiologic mechanisms, such as altered immune activation, enterochromaffin cells hyperplasia, and impaired barrier function have been reported in PI-IBS [2]. However, information is lacking on a common core of the mechanisms responsible for an impairment of the neuromuscular gut apparatus, and hence of abnormal motility/perception [3, 13].

Concerning mechanisms related to specific infectious agents, these have been investigated in a very few cases. For instance, mucosal cellular changes (enterochromaffin cells hyperplasia) [14], abnormal intestinal permeability and cytokine increase [15] have been reported in PI-IBS related to *Campylobacter* species; abnormalities of immunoendocrine cells [16] have been described in PI-IBS related to *Shigella* infection; and mucosal cellular changes (enterochromaffin cells decrease and cholecystokinin-containing cell increase) [17] and visceral hypersensitivity [18] have been documented in *Giardia* infested patients.

Of interest, some of these abnormalities seem to be related to a dysfunction of the enteric glial cells (EGC) [19–21], a key cell population involved in several gastrointestinal conditions, especially those characterized by abnormal motility and inflammation [22–26]. The important role of EGC, besides mechanical/trophic support function [27], involves regulation of gut barrier functions [28], maintenance and modulation of enteric neurotransmission [29], enteric neurons homeostasis [30], and acting as mediators of interactions between the enteric nervous system and the immune system [25, 31]. These functions are particularly interesting in the setting of PI-IBS, in that there are emerging data that immune-mediated barrier defects are found in IBS patients [32, 33]. It is worth noting, however, that EGC, if targets of pathogenic enteric microorganisms, could be the pivotal crossroad from which the most relevant dysfunctions originate and persist.

Interlinks of enteric glial cells with enteric neurons and neurotransmitters

Enteric glial cells play an active role within the neural circuits that control gut motility [34]. To exert this role, EGC strongly interact with the enteric neurons and neurotransmitters [27, 29]. Most EGC within enteric ganglia are contacted by vesiculated nerve processes with presynaptic specializations [35], and respond to transmitters released by these terminations by expressing a series of receptors for neurotransmitters and neuromodulators [36–39]. Thus, EGC can modulate enteric neural circuits in several ways, such as by supplying neurotransmitters precursors to neurons [40, 41], terminating the actions of neurotransmitters from synapses [42, 43], and through the generation of neuroactive compounds [44]. In addition, EGC are needed to assure survival of the enteric neurons, as demonstrated by the experimental ablation of the intestinal glial network [45]. Thus, it is conceivable that every damage to the EGC network may in turn reflect on the function of the enteric neurons; on the other hand, since the EGC and the enteric neurons networks are now considered also as a functional entity with reciprocal regulation between its cellular components, especially regarding the intestinal barrier [46], damages to the enteric neurons may influence EGC functions.

PI-IBS after *C. difficile* infection: a case of a microbiological stalker, or the way to resolve the conundrum?

The main virulence factors of *C. difficile* are represented by two exotoxins, toxin A (TcdA) and toxin B (TcdB), similar in structure and mechanisms of action [47] (although TcdB is about 1000-fold more potent than TcdA [48]). Both toxins are able to cause adverse effects in enteric cell populations, such as enterocytes, colonocytes, and enteric neurons [47, 49].

A few studies on the effects of *C. difficile* toxins on enteric neurons showed that toxin A stimulates extrinsic sensory nerves and causes uncontrolled gut inflammation, likely mediated by substance P from primary afferent neurons [50]; this inflammation was prevented by extrinsic surgical denervation [51, 52]. On the other hand, neuronal activation of VIP-positive pathways after exposure to toxin B has been hypothesized as an attempt of a protective adaptive response toward a pathogenic agent aggression of the intestinal mucosa [53]. However, to date there is a lack of information on the possible persistence of functional abnormalities in neurons surviving the effects of *C. difficile* toxins.

Recently, we reported that the adverse effects of toxin B extend to EGC [54]. In fact, TcB in vitro caused in a dose- and time-dependent manner cytopathic and cytotoxic effects on EGC, correlated with Rac1 glucosylation, such as cytoskeleton disorganization, cell-cycle arrest, apoptosis, increased susceptibility to apoptosis induced by proinflammatory cytokines [54]. More importantly, in EGC surviving the cytotoxic effect of TcdB persistently impaired cell functions were observed, such as Rac1 glucosylation and cell-cycle arrest, even though there was evidence of a self-rescuing mechanism as suggested by an increased production of glial-derived neurotrophic factor [54]. Moreover, surviving EGC were more susceptible to TNFα and IFNγ

action, which induces apoptosis to doses of cytokines not affecting control EGC.

By extrapolating these in vitro data to in vivo infection, we could hypothesize three scenarios based on early effects of TcdB on EGC and late consequences of these early effects (Fig. 1): (a) EGC interact with low concentrations of TcdB (Fig. 1a). The early effects are represented by low and transient Rac1 glucosylation and temporary cell-cycle arrest, with none or modest effects on symptoms as late effects; (b) EGC interact with increased concentrations of TcdB which induce moderate apoptosis allowing the survival of a certain percentage of these cells, but with reduced functionality (Fig. 1b). The early effects are represented by persistent Rac1 glucosylation, with an alteration of the molecular pathways downstream to Rac1 such as cell-cycle arrest and cytoskeletal disorganization. The late effects could cause a progressive increase of gastrointestinal symptoms (especially those related to motility/perception) due to a persistent abnormal function and to a slow loss of EGC, partly balanced by a reactive gliogenesis, a mechanism able to counteract pathogenic offences to the gut [55]; (c) EGC interact with high concentrations of TcdB (Fig. 1c). The early effects are represented by a strong apoptosis of EGC, whereas the late effects, with the loss of a consistent number of EGC, might cause disruption of the network of this cell population, resulting in delay of mucosal healing [56] and, if the loss of EGC is massive and overcomes the reactive gliogenesis, most of their function is lost, with devastating effects on other cell populations (e.g., enteric neurons) and persistence of clinical consequences (abnormal motility and visceral perception).

Conclusions

We hypothesize that *C. difficile*, by means of its toxins, may behave as a microbiological stalker towards the intestinal EGC network and, according to the grade of exposure of these toxins to the gut, disorganize and/or disrupt this network. Since EGC function is of paramount importance for a correct gut homeostasis, the clinical consequences may vary according to the intensity and the duration of the exposure to these toxins. We feel that exposure to amounts of TcdB able to derange EGC function in the time course might explain the persistence of symptoms in patients after *C. difficile* infection, and to indicate a possible way of developing PI-IBS.

However, it must be stressed that our hypothesis is based on in vitro findings, in conditions very simplified compared to in vivo. In fact, the EGC in vitro lack both the complex cellular environment with which interact (neurons, myocytes, immunocytes, etc.) and the soluble factors produced

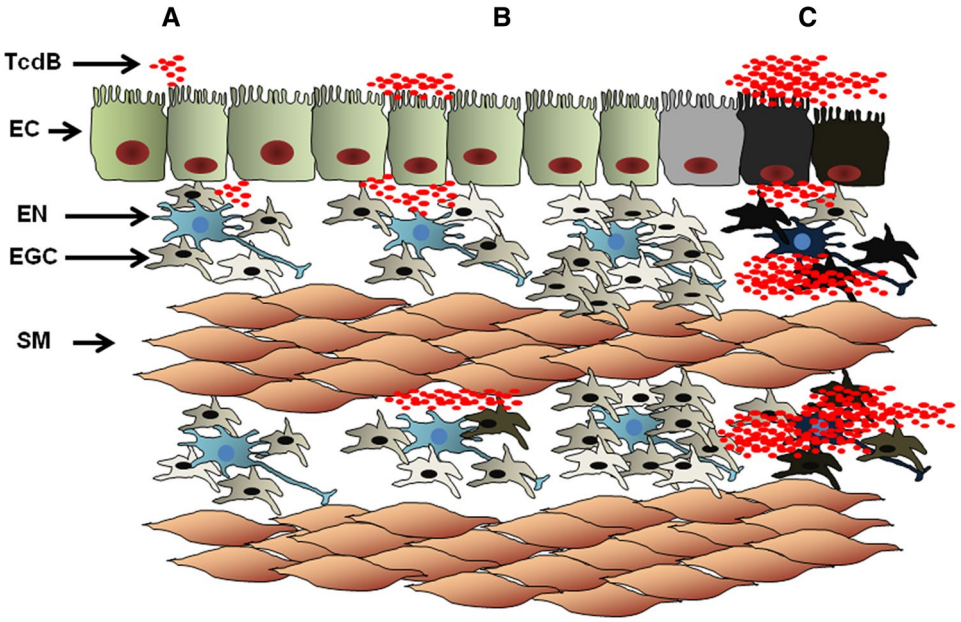

Fig. 1 Hypothesized in vivo effects of TcdB on the enteric glial cell network. **a** Exposure to low doses of toxin, with transient effects on a few cells (evidenced in lighter colour); **b** exposure to increased doses of toxin, which impair EGC function and cause low grades of apoptosis (left, evidenced, respectively, in lighter and darker color), and subsequent reactive gliogenesis with persistent impairment of part of the network (right, lighter color); **c** exposure to high doses of toxin, with massive apoptosis (dark color) overcoming gliogenesis, loss of the ECG network and of its functions relative to neurons and mucosal healing. EC, epithelial cells; EGC, enteric glial cells; EN, enteric neurons; SM, smooth muscle; TcdB, *Clostridium difficile* toxin B

by the interacting cells, such as cytokines and biochemically active metabolites characterizing the inflammatory response evoked by *C. difficile* infection, that in turn evolves in the time course. Thus, further studies are obviously needed to support our observations and hypothesis, and to show whether this approach may be useful to address targeted therapeutic interventions to protect the EGC network.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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