



Complications of Treatment

Toll-like receptor 4 signaling: A common biological mechanism of regimen-related toxicities

An emerging hypothesis for neuropathy and gastrointestinal toxicity

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ARTICLE INFO

Article history:

Received 24 October 2014

Received in revised form 24 November 2014

Accepted 27 November 2014

Keywords:

Chemotherapy

Neurotoxicity

Neuropathy

Gut toxicity

Toll-like receptor 4

Glial activation

ABSTRACT

Regimen-related toxicities remain a priority concern within the field of supportive care in cancer. Despite this, many forms of toxicity are under reported and consequently poorly characterised. Although there have been significant improvements in our understanding of regimen-related toxicities, symptom management continues to occur independently raising concerns such as drug interactions and the tendency to emphasise management of a single symptom at the expense of others. This review focuses on two important toxicities induced by chemotherapy; neuropathy/pain and gastrointestinal toxicity, introducing the Toll-like receptor (TLR) 4 pathway as a common component of their pathobiology. Given the global observation of toxicity clusters, identification of a common initiating factor provides an excellent opportunity to simultaneously target multiple side effects of anticancer treatment. Furthermore, identification of common biological underpinnings could perhaps reduce polypharmacy and have pharmacoeconomic benefits.

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Introduction

Regimen-related toxicities are universally underappreciated and often seen as the trade-off for remission [1]. Studies suggest this is due to oncology follow-up clinics focusing on disease recurrence whilst rarely addressing symptom management and referral pathways [1]. While research efforts into supportive care in cancer have seen significant improvement, regimen-related toxicities are viewed as biologically independent, but simultaneous events, perpetuating the silo mentality that typically exists within the supportive care domain. Individual, symptom-oriented therapeutic strategies also raise some important concerns, such as polypharmacy and drug side effects, and the tendency to emphasise

management of a single symptom at the expense of others. Furthermore, this approach ignores global observations that regimen-related toxicities occur in symptom clusters [2] which point to commonalities in their underlying biology, or at the least, overlapping mechanisms. In fact, in a retrospective review of 1000 cancer patients admitted for palliative care, each patient was reported to have greater than 10 symptoms [3,4]. Based on these observations, we suggest a paradigm shift, moving towards the idea that toxicities should be approached more holistically [5], combining efforts of neurologists, gastroenterologists, oncologists and other leading experts to identify common mechanisms between these pathologies. This critical review will focus on two important regimen-related toxicities, neurotoxicity and gastrointestinal (GI) toxicity, introducing the Toll-like receptor (TLR) 4 pathway as a common component of their pathobiology.

Neurotoxicity is a poorly characterised, dose limiting side effect of chemotherapy treatment [6] with symptoms typically falling under three broad categories, cognitive dysfunction, fatigue and neuropathy. Most commonly associated with platinum compounds (cisplatin and oxaliplatin), spindle poisons/antitubulins

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(vincristine and paclitaxel) and the newer targeted agents such as the proteasome inhibitors (bortezomib, ixabepilone, thalidomide) [7,8], heightened pain perception (hyperalgesia) and allodynia remain under reported and ill-defined side effects of chemotherapy. Given the profound personal impact of these neurological symptoms, chemotherapy-induced neurotoxicity is now considered a priority concern within the oncology arena, bringing together oncologists and neurologists to shed light on the mechanisms that underlie this pathology. Recent neuroimaging techniques suggest performance changes in neurological function occur in a subset of cancer patients, and that these changes may be associated with structural and functional alterations in the brain [9]. However, the molecular mechanism(s) involved in chemotherapy-induced neurotoxicity, specifically heightened pain perception (chemoneuropathy), remain unclear and poorly studied. Recent speculation has led to several candidate mechanisms for neurotoxicity including oxidative stress, inflammation and DNA damage [10,11]. It has also been proposed that some cytotoxic agents may damage neurons through binding to axonal microtubules to subsequently alter axonal transport [12]. This is however contradicted by a wealth of evidence showing no morphological changes in centrally-located neurons following various cytotoxic insults [13,14]. The lack of pathological changes observed in these neurons suggests that direct cytotoxicity is not sufficient to fully account for the range and severity of neurological symptoms experienced by patients, and more complex mechanisms are likely to be involved.

It has been suggested that systemic proinflammatory and immune factors released following chemotherapy [15,16] cause localised glial activation to further exacerbate neuronal responses and potentiate pain [17]. Glia have long been overlooked for their role in pain signaling, viewed only as structural supports of neurons of the CNS. It was not until the early 1990's when the actions of glia in varying pain states were appreciated and it is now a well-documented component of neuropathic pain [17,18]. The most recent advent in the area of glia-mediated nociception is the role of the Toll-like receptor (TLR) family, specifically TLR4. TLRs are a family of transmembrane protein receptors that recognise a diverse range of signals on exogenous and endogenous substances considered to be danger signals, and hence warrant activation of the innate immune system for the survival of the host [19]. TLR4 has been most extensively characterised as it recognises lipopolysaccharide (LPS) from gram-negative bacteria. TLR4 agonists activate similar downstream intracellular signaling pathways to those previously documented for interleukin (IL)-1, binding to its co-receptor, activating nuclear factor kappaB (NFκB) and resulting in a powerful proinflammatory cascade [20].

In addition to severe neurotoxicity, chemotherapy is also recognised for causing severe gastrointestinal side effects. Gut toxicity is often a dose-limiting manifestation of chemotherapy treatment that affects a large proportion of patients, dependent on the dose of chemotherapy administered [21]. Clinically, chemotherapy-induced gut toxicity (CIGT) is associated with severe gastrointestinal symptoms such as diarrhoea, infection and rectal bleeding [1]. Characterised by severe ulceration, inflammation and pain, CIGT has recently been implicated with glial activation [22], elevated proinflammatory cytokines (IL-1β, IL-6, TNF) [15] and, importantly, excessive TLR4 activation [23]. Like the CNS, the enteric nervous system is comprised of neurons and glia [24]. The traditional role of glia has also been challenged in the enteric nervous system with research suggesting that enteric glia are capable of regulating gastrointestinal homeostasis, and critically, transmission of sensory information from the gut to the CNS [25–27]. It is therefore tangible to suggest that peripheral toxicity, such as CIGT, may drive glial activation and thus exacerbate neuronal damage and pain perception.

Indirect neuromodulation through glial activation

The emerging role of glia in neuropathic pain

Glia is the collective term used to describe both astrocytes and microglia, the key supportive cells of the CNS. Traditionally, glia were viewed as structural supports for neurons, providing typically homeostatic roles including immune surveillance, clearance of debris, regulation of the ionic and chemical composition of the extracellular matrix and maintenance of blood brain barrier (BBB) integrity; glia are therefore considered pivotal to not only CNS homeostasis but also the survival of the host [18]. It was not until the early 1990's where these static, neurosupportive roles of glia were challenged and their roles under varying pain states acknowledged [28]. This paradigm shift in our understanding of glia followed early evidence showing an associative link between astrocyte activation and neuropathic pain [28]. The earliest evidence came from Garrison et al. (1991) where significantly elevated glial fibrillary acidic protein (GFAP) staining in the lumbar spinal cord was noted following sciatic nerve constriction. Garrison and colleagues furthered this work in 1994, showing activated glia in neuropathic animals [29]. Importantly, when an N-methyl-D-aspartate (NMDA) receptor inhibitor – MK-801 – was applied, both glial activation and neuropathic pain were improved. Several studies also report comparable changes in glial activity in various preclinical models of neuropathic pain [28,30,31] and subsequently glia are considered a vital step in its pathobiology.

It is now well established that glia have two distinct states; a quiescent basal state and an activated state. Microglia have a classic quiescent phenotype under normal pain responses, responsible for surveying the extracellular space in search of potential danger, but producing no neuroexcitatory substances [32]. In contrast, astrocytes are active players in synaptic signaling even under basal conditions. They maintain house-keeping functions, providing energy sources and neurotransmitter precursors to neurons, cleaning debris and resorbing excess neurotransmitters. Upon activation, these glia shift from their basal state, to an activated state characterised by a reactive, proinflammatory response profile [17]. A variety of glial activation signals have been identified, some of which are very well characterised including neuronally released fractalkine and traditional neuronal nociceptive modulators and transmitters, such as reactive oxygen species (ROS), nitric oxide, prostaglandins, excitatory amino acids, substance P and proinflammatory cytokines [33]. Upon activation, glia release substances (ROS, nitric oxide, prostaglandins, proinflammatory cytokines) that increase neuronal excitability, leading to pain enhancement. These neuroexcitatory mediators directly enhance neuronal excitability [33,34], increase pain associated neurotransmitter release from sensory afferents [35], upregulate the number and conductance of calcium permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors [36] and downregulate expression of glial glutamate transporters; all of which potentiate pain [37].

Although this mechanism of pain potentiation is well described in the setting of peripheral nerve damage, limited data exists regarding its role in chemotherapy-induced pain. Of the limited data, both microgliosis and astrocytosis are reported following administration of vincristine, paclitaxel, bortezomib and oxaliplatin [38–42]. Robinson et al. (2014) characterised patterns of glial activation in response to chemotherapy and typical spinal nerve ligation [42]. Consistent with previous peripheral nerve injury models, microglia activation was evident following spinal nerve ligation, but not chemotherapy administration. In contrast, astrocytes were activated following both oxaliplatin and bortezomib treatment in a manner that paralleled chemotherapy-evoked behavioural changes. Despite this disparity, the behavioural

phenotype and activation of astrocytes were prevented by co-administration of minocycline hydrochloride – a microglial inhibitor – in both models, suggesting a common mechanism between both neuropathies. Similarly, Ji et al. (2013) reported significant astrocytic hypertrophy and activation, demonstrated by increased glial GFAP expression in the dorsal horn of vincristine-treated rats with mechanical allodynia [38]. This was coupled by increased astrocytic expression of IL-1 β and phosphorylation of the NMDA receptor in spinal dorsal horn neurons. Importantly, treatment with pentoxifylline, an anti-inflammatory agent and an IL-1R antagonist, attenuated phosphorylation of NMDA receptors and mechanical allodynia. Most recently, oxaliplatin treatment was also associated with microglia activation, however this was only transient [40]. Microglia displayed a highly ramified phenotype, similar to that of vehicle-treated animals. The number of GFAP-expressing cells in the dorsal horn superficial laminae was significantly increased in oxaliplatin treated animals at 1, 2 and 3 weeks following treatment, correlating with the pain-profile. Importantly, although application of minocycline attenuated pain and glial activation, the efficacy of fluorocitrate – an astrocyte inhibitor – was significantly greater. Together, these studies highlight that glial activation, specifically astrocyte activation, is an important component of chemoneuropathy and associated pain. Despite these promising findings, the initiating factor for glial activation following chemotherapy remains unclear. One potential candidate is the release of endogenous danger signals. Several neurological conditions such as peripheral nerve damage have been shown to elicit the release of these endogenous danger signals [43] which communicate cellular/tissue damage and/or stress independent of the release of classic neurotransmitters or neuromodulators [17]. On release of these danger signals, the innate immune pattern recognition receptor, TLR4, causes activation of TLR4-expressing cells including both microglia and astrocytes [17]. Given the extensive peripheral tissue damage observed following cytotoxic treatment, TLR4-mediated glial activation therefore presents as a novel pathway in the pathobiology of chemoneuropathy.

TLR4-mediated glial activation

TLRs are a family of approximately ten single transmembrane receptors that recognise a diverse range of moieties or 'patterns' on exogenous and endogenous substances considered to be danger signals, and hence warrant activation of the innate immune system [18]. Of the many TLR subtypes, TLR4 has been most extensively characterised with established roles in the host immune response. When activated, typically by lipopolysaccharide (LPS), TLR4 recruits adaptor molecules and kinases, initiating a downstream signaling cascade that culminates in the secretion of proinflammatory cytokines and chemokines [44–46]. This signaling cascade can be MyD88-dependent or -independent, with the MyD88-dependent pathway most commonly associated with translocation of NF κ B and proinflammatory cytokine secretion. MyD88-dependent signaling typically requires the adaptor proteins TIRAP (TIR domain containing adaptor protein) and MyD88 to initiate the rapid production of proinflammatory cytokines, chemokines and their receptors TNF, IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, IL-10, IL-12p40, IL-23, macrophage inflammatory protein (MIP)-1 α , and MIP-1 β [47]. These factors facilitate the inflammatory response by increasing vascular permeability, directing dendritic cells and initiating macrophage migration from the periphery [48]. In contrast, the independent signaling pathway is reliant on Toll-like receptor adaptor molecule (TICAM)-1, -2, the TIR-domain-containing adaptor inducing interferon- β (TRIF) or TRIF-related adaptor molecule (TRAM) resulting in the production of interferon- β and chemokines.

In addition to the well-documented roles of TLR4 signaling in the host immune response, recent evidence has also linked this

immune receptor to a number of neurodegenerative disorders such as Alzheimer's and Parkinson's disease [44]. TLR4 expression in the CNS was, until recently, limited to microglia, astrocytes and oligodendrocytes. Recent evidence has now shown that TLR4 is expressed on CNS structures exposed to the blood stream such as the choroid plexus, circumventricular organs and leptomeninges. This newly emerging distribution of TLR4 expression may therefore explain the innate immune response observed in the brain, which originates from areas devoid of a blood-brain barrier [49]. Furthermore, recent evidence has shown altered neuronal TLR4 expression in response to ischaemia/reperfusion [50]. This is further supported by knockout studies, where the extent of energy deprivation-induced cell death and associated neurological deficit were significantly reduced in TLR4 deficient mice compared to wild-type [51].

TLR4 in the central nervous system

There is accumulating evidence that TLR4 contributes to neuronal death, BBB damage and inflammatory responses in the brain [52,53]. Consequently, TLR4 has been implicated with several CNS pathologies, particularly those characterised by neuroinflammation and subsequent degeneration. It has been postulated that TLR4-mediated NF κ B signaling plays a critical role in the development of neuroinflammation, leading to the secretion of proinflammatory cytokines, chemokines and enzymes such as cyclooxygenase (COX)-2 and matrix metalloproteinases (MMPs) [54,55]. Furthermore, it is suggested that these neuroinflammatory mediators are able to activate microglia leading to neuronal excitation or neuronal loss [56,57]. In fact, this phenomenon was recently demonstrated in the setting of Alzheimer's disease; a neurodegenerative disease characterised by microgliosis. Importantly, activated microglia have been identified surrounding senile plaque in the brains of Alzheimer's disease patients and have been shown to express increased levels of TLR4 [58]. Additionally, treatment of microglia with senile plaque material was shown to induce sharp peaks in the mRNA expression of many TLR subtypes, including TLR4, when compared with age-matched plaque-free tissue [59]. It is therefore suspected that TLR4-mediated glial activation results in the production of nitric oxide, oxygen derived free radicals, proteases, adhesion molecules and proinflammatory cytokines which, when produced in excess, have detrimental effects on neuronal homeostasis and contribute to the development of neurodegenerative conditions such as Alzheimer's disease [60].

In addition to Alzheimer's disease, TLR4 signaling has gained momentum regarding the pathobiology of Parkinson's disease; a chronic, neurodegenerative condition characterised by loss of dopaminergic neurons in the substantia nigra pars compacta and the striatum of the basal ganglia [61]. Although the mechanisms responsible for Parkinson's disease remain unclear, emerging evidence suggests a neuroinflammatory component to the condition [62]. The presence of cytoplasmic alpha-synuclein (AS), or Lewy bodies, is the hallmark trait of Parkinson's disease and the subject of significant molecular research. Stefanova and colleagues (2007) were the first to show elevated levels of TLR4 in AS cytoplasmic inclusions [63]. These findings have since been extended with research now showing that TLR4 is essential for the AS-dependent activation of microglia, leading to the production of proinflammatory cytokines and ROS [64]. Importantly, this mechanism is unique to microglia, with astrocytic uptake of AS shown to be TLR4-independent. In contrast, the role for TLR4 in Parkinson's disease is confounded by evidence showing that genetic TLR4 deletion results in reduced phagocytic activity of microglia, leading to heightened AS accumulation and exacerbated neurodegeneration [65,66]. These results suggest that despite initiation of an inflammatory response, TLR4-mediated glial activation may be important

in the clearance of AS, thus exerting a protective effect in Parkinson's disease.

TLR4 and neuropathic pain

TLR4 has received significant attention for its roles in several neuroinflammatory disorders characterised by neurodegeneration. In addition to these emerging roles, TLR4 has also gained momentum for its role(s) in modulating neuropathic pain. Within the CNS, TLR4 is predominantly expressed by microglia, but expression may be upregulated on astrocytes under neuroinflammatory settings [67]. TLR4 appears to be directly relevant to the pathobiology of neuropathic pain, as it recognises and responds to endogenous danger signals, and thus has the ability to modulate pain signaling. TLR4 knockout and knockdown studies have demonstrated this emerging role for TLR4, with knockout/knockdowns suppressing the development and/or maintenance of nerve injury-induced allodynia [68–71]. Additionally, administration of a selective TLR4 antagonist has been shown to suppress well-established neuropathic pain induced by chronic constriction injury [18]. In the setting of chemoneuropathy, recent research has shown that paclitaxel treatment is associated with elevated TLR4 expression and glial activation in the dorsal root ganglion. Additionally, application of both TLR4 and MyD88 antagonists significantly reduced peripheral neuropathy and associated pain [39]. Taken together, these studies suggest that ongoing TLR4 activation and peripheral endogenous danger signaling is at the core of neuropathic pain, and may therefore contribute to the development of chemoneuropathy and its associated clinical features.

Peripheral tissue damage activates central TLR4

Although the development of neuropathic pain through TLR4 activation is most extensively characterised in the setting of

peripheral nerve injury, the production of these endogenous danger signals and other TLR4 ligands is not unique to this form of tissue damage. In fact, it is well established that chemotherapy treatment causes significant gut toxicity, which is characterised by excessive production of endogenous danger signals (pathological- and danger-associated molecular patterns; PAMPs/DAMPs) [43]. In addition to this, recent research has shown enterocyte-expressed TLR4 is intimately involved in the initiation of gut toxicity following chemotherapy treatment, activating NF κ B and mounting an immune response [72]. Given that TLR4 is activated by endogenous danger signals, centrally-located TLR4 and glia are well positioned to enhance pain resulting from inflammation in the periphery such as gut toxicity following chemotherapy. We therefore hypothesise that the molecular signals derived from gastrointestinal toxicity drive glial activation and subsequent neuropathy in a TLR4-dependent manner (Fig. 1). This pathway appears to be initiated by damage that originates in the periphery, and thus the pathobiology of chemoneuropathy may point to the existence of a gut-CNS axis.

The existence of a gut-CNS axis is not a new phenomenon [73]. Based on paralleled comorbidities of gastrointestinal and neurological origin, there has been an appreciation gained for the existence of a gut-CNS axis and the roles it may play in governing neurological function [74,75]. While candidate mechanisms of the gut-CNS axis include neural, endocrinal and immune pathways, the gut microbiota has emerged as a predominant player, although the mechanisms underpinning the gut-CNS axis remains unclear [76]. Although a wealth of data exists supporting a role for the gut microbiota in modulating neurological function, there is evidence to suggest that immune cells produced within the gut may also exhibit neuromodulatory effects [77]. Disruption of the homeostatic state between the microbiota and the innate mucosal

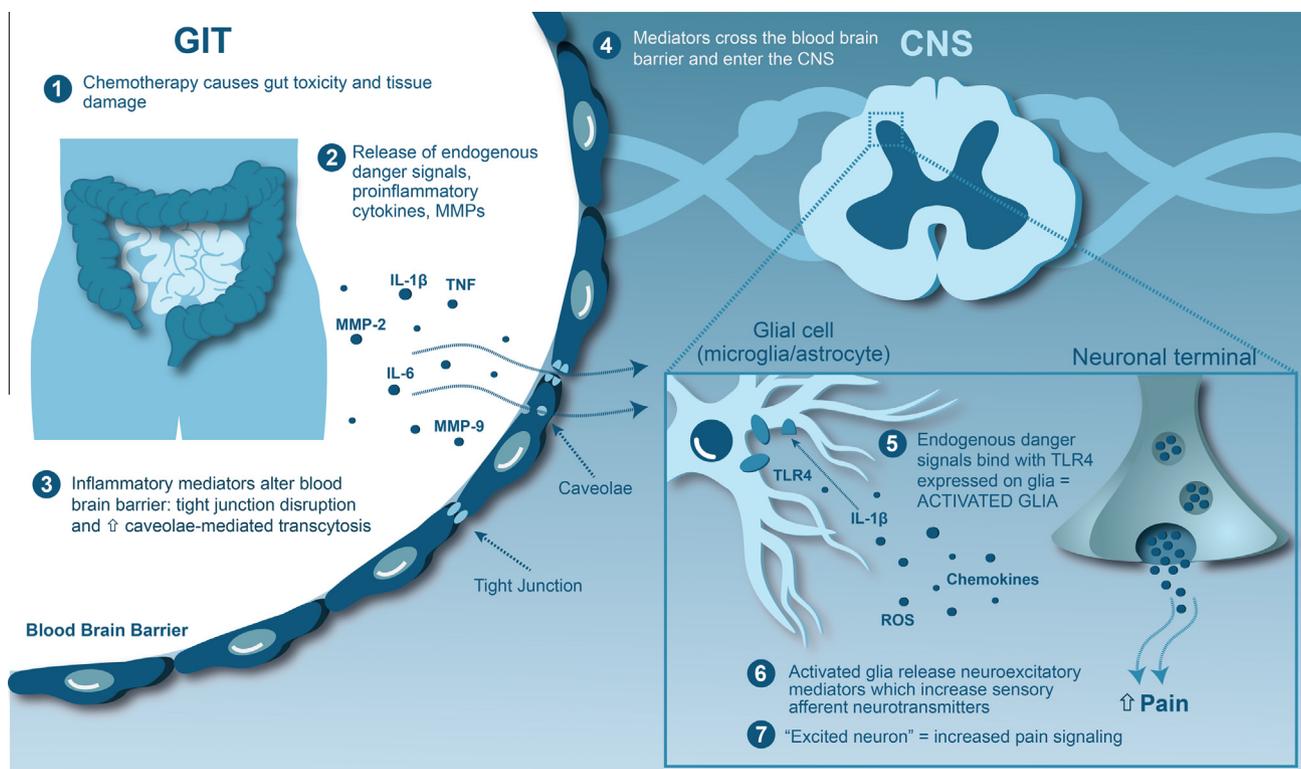


Fig. 1. Peripheral inflammation modulates central pain signaling through TLR4. Here we proposed that inflammatory mediators, released from the gastrointestinal tract upon chemotherapy-induced damage, are able to cross the blood brain barrier and enter the CNS. These mediators are potent ligands for TLR4, located on glial cells through the CNS, causing extensive glial activation. Upon activation, glia release substances that increase neuronal excitability, leading to pain enhancement. These neuroexcitatory mediators directly enhance neuronal excitability, increase pain associated neurotransmitter release from sensory afferents, upregulate the number and conductance of calcium permeable AMPA and NMDA receptor and downregulate expression of glial glutamate transporters; all of which potentiate pain.

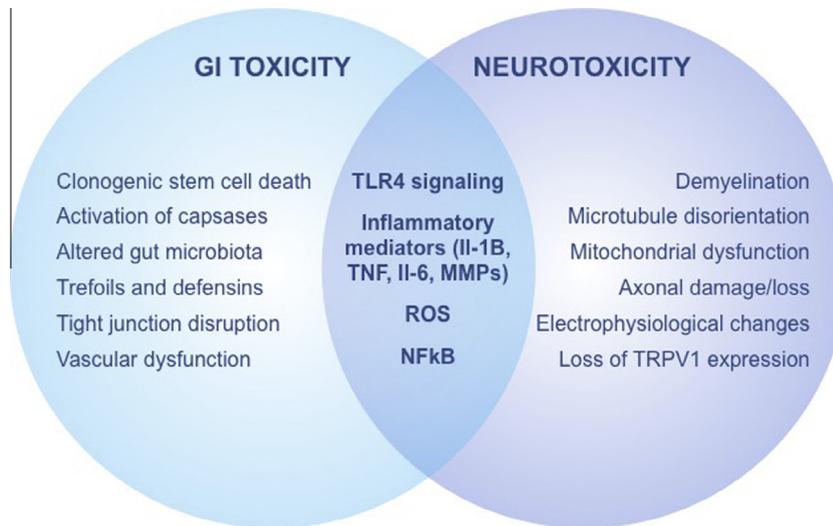


Fig. 2. Independent and common mechanisms of gastrointestinal and neurotoxicity.

immune system of the host has been shown to result in activation of TLRs and consequent alteration of cytokine profiles, leading to impaired neurological function. It is suggested that these immune cells disrupt the BBB and upon crossing, are subsequently reactivated within the CNS. This phenomenon was recently demonstrated in mice receiving peripheral surgery, displaying BBB disruption and elevated TNF signaling which facilitated macrophage migration into the hippocampus and subsequent neurological decline [78]. Additionally, administration of proinflammatory cytokines in rodents has been reported to induce depressive like symptoms, disrupted circadian rhythm and reduced appetite [79,80]. Although a gut-CNS axis has not been applied to the setting of chemotherapy-induced pain, these results support the hypothesis that CIGT is able to modulate CNS homeostasis, and may contribute to the development of chemoneuropathy. Furthermore, TLR4 may be uniquely positioned to modulate inflammatory responses in both the gut and CNS thus contributing to both toxicities and it therefore presents as an attractive therapeutic target.

Clinical translation

There is strong evidence suggesting that peripheral toxicity drives glial activation through TLR4 signaling; this review has highlighted evidence using the examples of CIGT and pain. Where the complexity lies is the sequence of these toxicities. Identifying whether these toxicities occur in unison or sequentially will shed light on the role of TLR4 as a common underlying mechanism. It is likely that TLR4-mediated pain is agent specific, and may be a case where one size, on a theoretical basis, does not fit all. For example, the clinical observation of neuropathy is rare amongst patients being treated with agents typically associated with high rates of gut toxicity (irinotecan, 5-fluorouracil, methotrexate), indicating potential independent mechanisms (Fig. 2). However, the hypothesis of dual-toxicities of common biology is compelling with regards to the clinical use proteasome inhibitors (bortezomib, thalidomide, ixabepilone, ixazomib) [81–83] and taxanes (paclitaxel, docetaxel) [84–86], which commonly induce toxicities of both gastrointestinal and neurological origin, raising the question of equivalent risk.

Conclusions and future directions [87]

Regimen-related toxicities remain a priority concern within the field of supportive care in cancer. Despite this, many forms of toxicity are under reported and consequently poorly characterised. This holds particularly true for chemotherapy-induced gastrointestinal toxicity and neurotoxicity, specifically the symptom of pain. This review has highlighted TLR4 signaling as a common underlying pathway of both toxicities. Given the global observation of toxicity clusters, identification of a common initiating factor would provide an excellent opportunity to simultaneously target multiple side effects of anticancer treatments. Despite strong epidemiological evidence highlighting toxicity clusters, it remains unclear why some patients are more susceptible to severe toxicity. Evidence has shown TLR4 gene mutations (Thr399Ile) contribute to the severity of acute Graph versus Host Disease, influencing the risk in patients undergoing allogeneic transplantation [88]. The TLR4 Asp299Gly polymorphism has also been identified as a risk factor for Crohn's disease potentially contributing to disease phenotype [87]. This emerging hypothesis for TLR4-mediated toxicities could therefore have the potential to drive biomarker development and risk evaluation techniques, presenting an attractive avenue for future research. Furthermore, identification of common biological underpinnings could perhaps reduce polypharmacy, lessen drug side effects, and have pharmacoeconomic benefits.

The ubiquitous involvement of the innate immune system in regimen-related toxicity makes TLR4 an overlooked candidate in the pathophysiology of other dose-limiting side effects of chemotherapy. For example, recent speculation suggests that proinflammatory cytokines are able to disrupt the hypothalamic–pituitary–adrenal (HPA) axis, to alter circadian rhythm and thus induce fatigue – an established side effect of chemotherapy [89]. In fact, decreased circulating levels of serum cortisol have been reported immediately following treatment with the platinum compounds cisplatin and carboplatin [89], indicating impaired HPA axis function [90,91]. Given the role of TLR4 in neuroinflammation, it is conceivable that activation of enterocyte-expressed TLR4 initiates the induction of a 'cytokine storm' which is able to modulate the CNS and thus impact on the function of the HPA axis. Activation of TLR4 may therefore be the missing link in the initiation of this

cascade where circulating proinflammatory cytokines can access the CNS and exert profound effects on behaviour and cognitive function. The induction of this ‘cytokine storm’ appears to be predominantly initiated by the activation of TLR4 in the gut – the largest immunological organ – and must therefore be adequately acknowledged if we are to adopt a holistic approach to toxicity.

Conflict of interest statement

There are no conflicts of interest to declare on behalf of any authors.

Acknowledgement

Thank you Mr. Tavik Morgenstern for kindly providing the illustration for this manuscript.

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