

Cytokine-mediated blood brain barrier disruption as a conduit for cancer/chemotherapy-associated neurotoxicity and cognitive dysfunction

Hannah R. Wardill^{1,2}, Kimberley A. Mander^{1,3}, Ysabella Z.A. Van Seville¹, Rachel J. Gibson⁴, Richard M. Logan⁵, Joanne M. Bowen¹ and Stephen T. Sonis^{6,7}

¹School of Medicine, University of Adelaide, South Australia

²Centre for Nutrition and Metabolism, South Australian Health and Medical Research Institute, Adelaide, South Australia

³Adelaide Centre for Neuroscience Research and Discipline of Anatomy and Pathology, University of Adelaide, Adelaide, South Australia

⁴Division of Health Sciences, University of South Australia, Australia

⁵School of Dentistry, University of Adelaide, Adelaide, South Australia

⁶Brigham and Women's Hospital, Boston, MA

⁷Biomodels, LLC, Watertown, MA

Neurotoxicity is a common side effect of chemotherapy treatment, with unclear molecular mechanisms. Clinical studies suggest that the most frequent neurotoxic adverse events affect memory and learning, attention, concentration, processing speeds and executive function. Emerging preclinical research points toward direct cellular toxicity and induction of neuroinflammation as key drivers of neurotoxicity and subsequent cognitive impairment. Emerging data now show detectable levels of some chemotherapeutic agents within the CNS, indicating potential disruption of blood brain barrier integrity or transport mechanisms. Blood brain barrier disruption is a key aspect of many neurocognitive disorders, particularly those characterized by a proinflammatory state. Importantly, many proinflammatory mediators able to modulate the blood brain barrier are generated by tissues and organs that are targets for chemotherapy-associated toxicities. This review therefore aims to explore the hypothesis that peripherally derived inflammatory cytokines disrupt blood brain barrier permeability, thereby increasing direct access of chemotherapeutic agents into the CNS to facilitate neuroinflammation and central neurotoxicity.

Neurotoxicity and its associated cognitive manifestations are poorly characterized, dose-limiting side effects of chemotherapy treatment.¹ Clinically, the impact of chemotherapy on cognition has been most extensively studied in breast cancer patients,^{2–4} however it is becoming increasingly recognized that cognitive symptoms affect a large portion of patients with varying malignancies and treatments.^{5–8} Despite its prevalence, cognitive dysfunction, often referred to as *chemo-brain*, remains an under-reported and ill-defined

complication of anti-cancer treatment. Cognitive symptoms are vast, but are most commonly reported to affect memory and learning, attention, concentration, processing speeds and executive function.^{2,4,9,10} Importantly, unlike many acute chemotherapy-related toxicities, cognitive dysfunction presents both acutely and chronically, compromising quality of life for patients unable to return to prior levels of social and academic interaction.¹¹

Given its frequency, and its acute and chronic impact, the importance of better understanding chemotherapy-induced neurotoxicity has become a priority with an obvious goal of developing effective interventions. Like the overwhelming majority of regimen-related toxicities, changes in neurological function occur in a subset of cancer patients and curiously these changes may or may not be associated with structural and functional alterations in the brain.¹² Compounding our ability to attribute cognitive changes directly to treatment has been the finding that impairment has been reported among cancer patients who are treatment naïve.¹³

As yet, the molecular mechanism(s) involved in chemotherapy-induced neurotoxicity have not been clearly defined, however there is strong evidence implicating direct cytotoxicity and associated inflammatory mechanisms. Currently, the bulk of studies assessing the latter focus on neuro-inflammatory pathways, however, it is important to consider the impact of cytokines derived from the tumor, as well as

Key words: neurotoxicity, cognitive dysfunction, blood brain barrier, inflammation, chemotherapy-induced gut toxicity

Abbreviations: BBB–: blood brain barrier; BMECs: brain microvascular endothelial cells; CIGT: chemotherapy-induced gut toxicity; IL: interleukin; INF γ : interferon gamma; MMP: matrix metalloproteinase; MTX: methotrexate; Myd88: myeloid differentiation factor; TKI: tyrosine kinase inhibitors; TLR: toll-like receptor; TNF: tumour necrosis factor; VEGFR: vascular epidermal growth factor receptor; ZO: zonular occludens; 5-FU–: 5-fluorouracil

DOI: 10.1002/ijc.30252

History: Received 5 June 2016; Accepted 21 June 2016; Online 1 July 2016

Correspondence to: Hannah R. Wardill, School of Medicine, University of Adelaide, North Terrace, Adelaide 5005, South Australia, Australia, Tel.: +61-8-83133787, E-mail: hannah.wardill@adelaide.edu.au

those elicited by the effect of chemotherapy on normal or tumour tissue. Most likely, direct cytotoxicity and neuroinflammation occur in concert with cytokine-mediated disruption of the blood brain barrier (BBB) serving to enhance drug penetration to augment local levels and result in amplification of cognitive symptoms.

The finding of detectable levels of systemically administered chemotherapeutic agents within the central nervous system (CNS)¹⁴ supports this presumption and implies a level of BBB permeability that has not been previously appreciated. Increased levels of BBB permeability suggest that some chemotherapeutic agents are capable of disrupting its integrity, either directly or indirectly. Convincing evidence also exists linking BBB dysfunction with a proinflammatory state, with BBB dysfunction reported in patients with chronic inflammatory diseases as well as being a consequence of many forms of regimen-related peripheral toxicities. Among these, chemotherapy-induced mucosal injury, especially of the gastrointestinal tract, provides a compelling example of how focal chemotherapy-induced tissue damage can serve as a conduit for central neurotoxicity. Of interest, chemotherapy-induced gut toxicity (CIGT) has recently been shown to increase central markers of pain and neuroinflammation highlighting the ability of peripherally derived inflammation to profoundly affect CNS function.

Structural and Neuroimaging Studies That Define the Scope of Chemotherapy-associated Changes in the Brain

The neural basis for neurological deficiencies in cancer patients has been investigated with both structural and functional neuroimaging. Voxel-based morphometry (VBM) and diffusion-tensor imaging (DTI) are structural imaging techniques able to detect changes in both white and gray matter, whilst, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies enable assessment of functional deficiencies when structural changes are not evident. While these technologies have not been applied broadly, limited data suggest that chemotherapy is consistently associated with, changes in white matter (WM) structures. WM hyperintensities and hippocampal lesions have been identified using basic neuroimaging techniques in breast cancer patients treated with various chemotherapy regimens.^{15–17}

T1-VBM studies, an automated and quantitative method of neuroimaging which theoretically provides an unbiased, comprehensive and highly reliable assessment sensitive to local changes^{18,19} have demonstrated diffuse cortical and sub-cortical WM and bilateral neocortical gray matter (GM) volume reductions or deficiencies in the superior frontal gyrus, parahippocampal gyrus, cingulate gyrus and the precuneus gyrus.^{20–22} Based on the spectrum of neurocognitive symptoms seen in cancer patients and the well-documented function of the hippocampus, a growing body of research now shows impaired neurogenesis and hippocampal function likely contribute to neurotoxicity.^{23,24} In support of this,

hippocampal alterations have been identified in response to a spectrum of chemotherapeutic agents^{5,24–26} in a number of patient cohorts.

Structural changes, indicative of direct neurotoxicity, are often seen in conjunction with neurocognitive functional deficiencies detected through DTI and digital symbol testing (DST); a measure of processing speed. Although studies are limited in size and number, results have indicated associations between the integrity of the corpus callosum and processing speeds of patients receiving adjuvant chemotherapy for breast cancer.²⁷ Further associations have been identified between processing speeds and frontal WM integrity.²⁸ The largest study to investigate this association was conducted by Deprez *et al.*²⁹ in premenopausal women with breast cancer. Patients receiving adjuvant chemotherapy exhibited worsening attention, psychomotor speed, verbal learning and memory, as well as decreased microstructural integrity in widespread regions of the corona radiata and the corpus callosum, compared to matched controls, reinforcing that WM changes may be the source of cognitive deficits seen in chemotherapy-treated patients.²⁹

Results of neuroimaging studies have been informative relative to describing observed structural and functional deficiency relationships associated with cancer- and chemotherapy-associated cognitive dysfunction. However, they are unable to mechanistically define the pathogenesis of chemotherapy-associated neurological toxicities. And at this early stage, their interpretation is limited by heterogeneity in experimental methodology, and confounded by neurological comorbidities commonly seen in cancer patients such as depression and anxiety, which can produce similar structural manifestations.¹ Furthermore, the inclusion of predominantly elderly patients, a lack pretreatment baseline controls and presence of structural deficits in treatment-naïve patients clouds the ability to make definitive conclusions regarding the mechanisms of chemotherapy-induced neurotoxicity.

Blood Brain Barrier Dysfunction: An Accelerant for Neurotoxicity?

The presence of chemotherapeutic agents in the CNS after systemic administration indicates their ability to cross the BBB, either physiologically or pathologically.³⁰ Early research has demonstrated detectable levels of intravenously administered cisplatin, bis-chloroethylnitrosourea (BCNU) and paclitaxel in the brains of rodents using PET.^{30,31} This phenomenon has also been seen in higher order primates, with detectable levels of 5-fluorouracil in the cerebrospinal fluid after intravenous administration.¹⁴ Although therapeutic drug levels effective for CNS malignancies were not seen, drug concentrations were sufficient to induce apoptosis and neuronal damage associated with neurological dysfunction.³² In addition to these findings, it is well established that a number of proinflammatory cytokines have detrimental effects on tight junctions and thus the integrity of the BBB. This is critically important when considering BBB breakdown

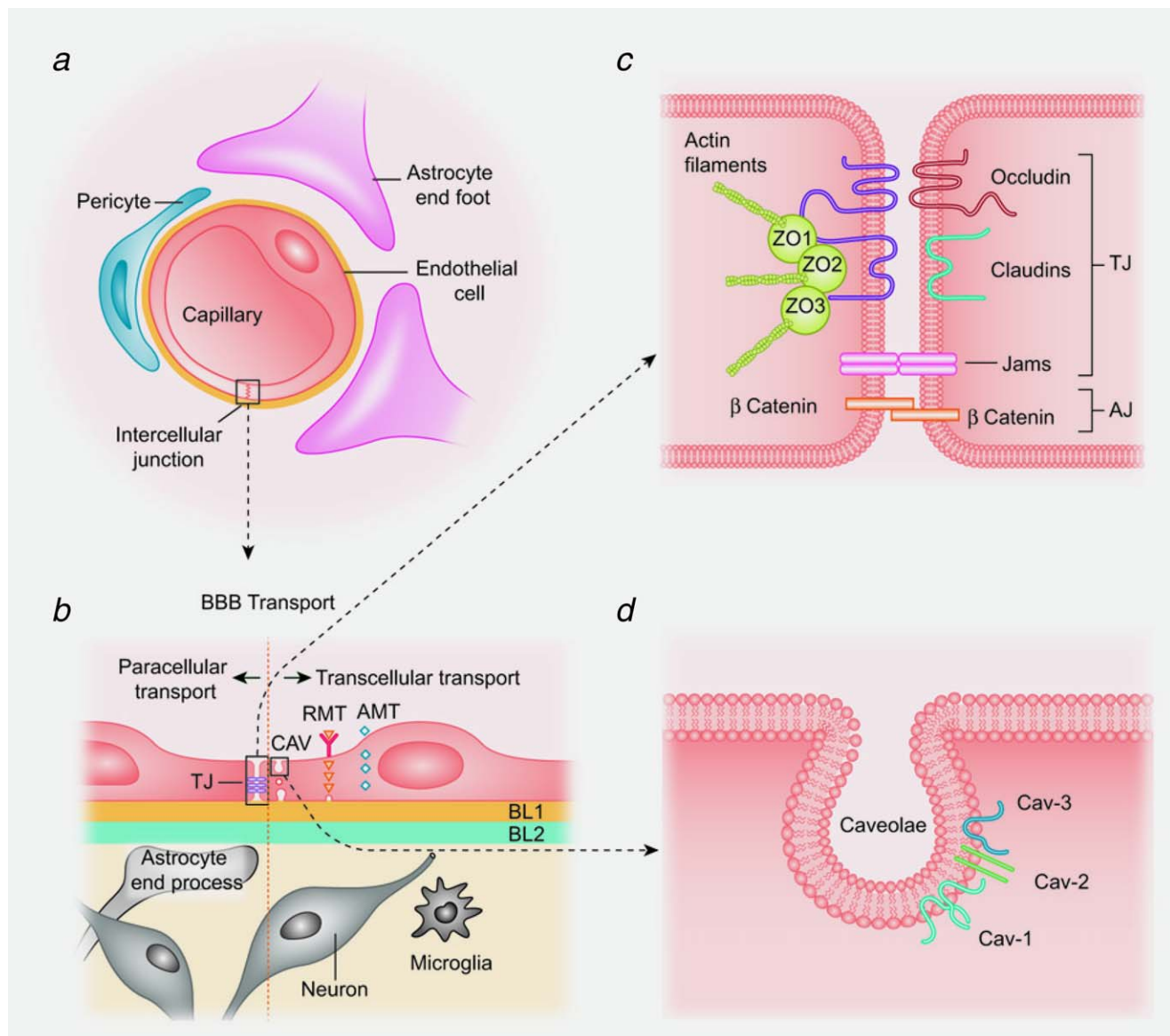


Figure 1. Blood brain barrier transport mechanisms. Cerebral endothelial cells of the blood brain barrier (Panel A) have specialised circumferential tight junctions and intracellular caveolae, which regulate blood brain barrier transit (Panel B). Tight junctions are highly plastic, multi-protein structures traversing the intercellular junction (Panel C). Each tight junction comprises the cytoplasmic protein family zonular occludens (–1, –2, –3) and the transmembraneous protein families claudin, JAMs and occludin. Caveolae, comprised of Cav-1, Cav-2 and Cav-3, control transcellular permeability within the blood brain barrier (Panel D). [Color figure can be viewed at wileyonlinelibrary.com]

in cancer patients, as there are a number of sources of proinflammatory cytokines, derived from the tumour and the effects of chemotherapy on normal tissue.

Mediators of inflammation disrupt blood brain barrier integrity

The BBB (Fig. 1) is highly plastic and can undergo significant modification in response to a raft of physiological and pathologies cues. Subsequently, impairment of the BBB has been implicated in a number of CNS pathologies, particularly those characterized by a proinflammatory state.^{33,34} For example, traumatic brain injury (TBI) is often accompanied

by a large inflammatory response resulting in grossly abnormal BBB permeability, the influx of inflammatory cells and subsequent oedema.^{35,36} A similar mechanism is now also hypothesized to play a role in the pathogenesis of stroke in which a weakening of the BBB, associated with a transient breakdown of tight junction proteins, is thought to contribute to the haemorrhagic transformation manifested by a heightened inflammatory state and worsened prognosis.³⁷

In light of these clinical associations between inflammation and BBB breakdown, there is now a wealth of *in vitro* and *in vivo* data demonstrating the ability of proinflammatory cytokines to disrupt the BBB (Table 1). From these

Table 1. The action of established mediators of gut toxicity on blood–brain barrier regulation and dysfunction

Mediator	Effect on tight junctions and paracellular permeability	Effect on caveolae-mediated transcytosis	Established role in gut toxicity
Proinflammatory cytokines	IL-1 β , IL-1 and TNF exposure caused increased paracellular permeability and reduced ZO-1 expression in an <i>in vitro</i> model of the blood brain barrier (THBMEC). Consequently, many inflammatory CNS pathologies are characterised by increased blood brain barrier permeability. ⁴⁷	IL-1 β induces translocation of IL-1R1 and recruitment of signaling molecules to caveolin-enriched lipid rafts, preceding caveolin-dependent endocytosis. ⁴⁸ Caveolae are involved in TNF endocytosis in BBB endothelia and eventual transport across the BBB from the luminal to abluminal side. ⁴⁹ TNF also induces translocation of its receptors, TNFR1 and TNFR2, to endothelial caveolae. ⁵⁰	Well-documented mediators of toxicity; significantly elevated levels of IL-1 β , IL-6 and TNF following cytotoxic insult. Initiate an inflammatory response, and amplify NF κ B signaling. ^{51–54}
MMPs	BMECs exposed to oxidative stress expressed significantly elevated MMP-9 activity paralleled by downregulation and redistribution of occludin. Elevated levels of MMP-2/-9 have also been reported in several CNS pathologies such as cerebral ischemia, leading to increased barrier permeability and cerebral oedema. Inhibition of both MMP-2/-9 has been shown to attenuate oedema formation.	Membrane-type 1 (MT1) MMP is present at endothelial caveolae with caveolin-1 constituting a novel pathway for MT1-MMP internalization in human endothelial cells. ⁵⁵	Altered tissue and serum levels of various MMPs and their inhibitors (TIMPs) have been reported following irinotecan. Hypothesized to contribute to development of gut toxicity through inflammatory pathways, altered extracellular matrix composition, adhesion molecules and tight junctions. ^{56–59}
ROS	Increased presence of ROS correlates with cytoskeleton rearrangements, redistribution and disappearance of TJ proteins claudin-5 and occludin. ⁶⁰	Peroxyinitrite (OONO) exposure is related to the impaired expression of Cav-1 in endothelial membrane associated with vascular disturbances of diabetes. ⁶¹ Specifically, regulation of eNOS within caveolae is an important physiological mechanism for control of vascular reactivity, and is thought to have a role in the suppression of inflammatory signaling pathway. ⁶² During IBD the loss of Cav-1, and thus caveolae, results in reduced tissue pathology likely due to decreased inflammatory and angiogenic signaling; this protection is also lost with endothelial cell-specific restoration of Cav-1. ⁶²	Upregulated ROS is a key step in the initiation of gut toxicity, occurring almost instantaneously after cytotoxic treatment. It is currently part of the universally accepted model of gut toxicity. ⁶³
Substance P	Substance P (SP) shown to induce changes in ZO-1 and claudin-5 in HBMECs and correlate with increased permeability. ^{69–69} SP is released early as part of a neurogenic inflammatory response. In so doing, it facilitates an increase in the permeability of the blood–brain barrier. At the cellular level, SP has been shown to directly result in neuronal cell death. ⁶⁷	SP receptor the NK-1R is localized within endothelial caveolae though their role requires further elucidation. ⁶⁸	Preclinical studies have shown elevated levels of SP, and NK-1R, in the small bowel following cytotoxic insult; 5-fluorouracil caused a 3-fold increase in the mRNA expression of NK-1R and significant elevations 5HT3 and NK-1R immunopositive cells. Regulated through NF κ B, SP is thought to activate MMPs and initiate an inflammatory response. ⁶⁹

Table 1. The action of established mediators of gut toxicity on blood–brain barrier regulation and dysfunction (Continued)

Mediator	Effect on tight junctions and paracellular permeability	Effect on caveolae-mediated transcytosis	Established role in gut toxicity
TLR4	Ischaemic stroke is associated with heightened blood brain barrier permeability, and elevated TLR4 expression. Pharmacological inhibition or genetic deletion of TLR4-attenuated barrier and tight junction disruption. ⁷⁰	Cav-1 phosphorylation is required for the interaction with TLR4 and activation of TLR-related signaling during sepsis-induced lung inflammation. ⁷¹ Inhibition of cav-1 phosphorylation and resultant inactivation of TLR4 signaling in pulmonary vascular endothelial cells has been suggested as a novel strategy for preventing sepsis-induced lung inflammation and injury.	The innate immune system is critically important in the development of gut toxicity following cancer treatment, with elevated levels of TLR4 observed preclinically following methotrexate and irinotecan. ⁷²
VEGF	VEGF-A disrupts claudin-5 and occludin expression in CNS endothelial cultures and induces BBB breakdown and immune cell infiltration <i>in vivo</i> . ⁷³ Downregulation of claudin-5 by VEGF-A constitutes a significant mechanism in BBB breakdown. ⁷⁴ These results suggest that overexpression of VEGF may exacerbate the inflammatory response in autoimmune diseases of the CNS by inducing focal BBB breakdown and migration of inflammatory cells into the lesions. ⁷⁵	Evidence of colocalization of VEGFR-2 and Nox2 in caveolae/lipid rafts is involved in the negative modulation of glucose uptake. ⁷⁶	VEGFR inhibitors (TKIs) are known to exacerbate chemotherapy-induced diarrhoea, implicating VEGF activity in cytotoxic therapy-induced gut toxicity. ^{70,77}

observations, it is clear that some cytokines exclusively affect paracellular barriers (e.g., IL-1 β), through breakdown and translocation of tight junction proteins, whilst others target transcellular processes mediated by caveolae (e.g., TNF α). A number of vasogenic agents (histamine, substance P) and proteases associated with inflammation, have also been identified to promote BBB remodeling. Of interest is the impact of matrix metalloproteinases (MMP) on tight junction integrity (Table 1) given the high levels of circulating MMPs observed after chemotherapy.³⁸

It is well documented that increased MMP activity correlates with elevated permeability of both endothelial and epithelial barriers (Table 1), strongly implying MMP-mediated tight junction disruption.^{39–42} Particularly robust evidence supports a role for MMP-mediated tight junction disruption in the BBB as endothelial cells, astrocytes and pericytes are all potent sources of these signaling proteins.³⁹ Brain-derived microvascular endothelial cells (BMECs) exposed to oxidative stress expressed significantly elevated MMP-9 activity paralleled by downregulation and redistribution of occludin.⁴³ Numerous preclinical studies also support a role for MMP-mediated tight junction disruption.^{40,41,44} For example, MMP-2/-9 levels have been shown to be significantly elevated following cerebral ischemia leading to tight junction protein degradation, increased BBB permeability and oedema.^{40,45} Furthermore, inhibition of MMP-2/-9 has been shown to reduce vascular permeability and attenuate tight junction disruption.⁴⁰ This is further supported by evidence showing that MMP-9 knockout mice have greater ZO-1 expression coupled with decreased BBB permeability and reduced oedema following stroke.⁴⁶ The impact of MMPs on caveolae-mediated transcytosis is now also being recognized for its potential role in CNS pathologies characterized by BBB disruption (Table 1).

Chemotherapy-induced Cytokine Production, Blood Brain Barrier Dysfunction and Clinical Implications for Patients

Virtually every biological substance known to have the capacity to alter the integrity of the BBB has been shown to be generated by tissues and organs that are targets for chemotherapy-associated toxicities. Among these, chemotherapy-induced mucosal injury, especially of the gastrointestinal tract, provides a compelling example of how focally induced chemotherapy tissue damage can serve as a conduit for central neurotoxicity. While suggested by Seigers and Fardell,⁷⁸ the potential impact peripheral inflammatory mediators as a driver of central toxicity has hardly been explored.

Chemotherapy-induced gut toxicity: A potential facilitator of CNS pathology

We have previously highlighted strong epidemiological data linking the development of neurotoxicity and gut toxicity following chemotherapy, and suggest that these off targets toxicities may in fact have common molecular roots.^{79,80} The development of CIGT is a dynamic process, characterized by

overlapping and simultaneous biological events. Logan *et al.*⁵¹ demonstrated that the administration of irinotecan, 5-fluorouracil and methotrexate induced significant elevations in TNF α , IL-1 β and IL-6 in tumour-bearing rats. Importantly, these proinflammatory cytokines not only damage surrounding tissue through pro-apoptotic signals (*e.g.*, caspases 3 activation), but they are also highly efficient activators of NF κ B thus amplifying the mucotoxic cascade. Furthermore, TNF α and IL-1 β induce MMP-1, MMP-2 and MMP-3 activation, which is thought to contribute to development of gut toxicity through inflammatory pathways, altered extracellular matrix composition, adhesion molecules and tight junction disruption. Toll-like receptor (TLR)4-dependent mechanisms have also been linked to the development of gut toxicity, with increases in its expression seen following chemotherapy⁸¹ as well as improvements in symptomatic parameters seen following genetic manipulation of its downstream signaling molecules (*e.g.*, MyD88, MD-2).^{82,83} This aspect of CIGT has significant implications for BBB maintenance, as it is becoming increasingly clear that TLR4-dependent signaling pathways are critical for tight junction integrity.⁸⁴

TLR4-mediated barrier modulation has been shown in both endothelial and epithelial models. For example, Gao *et al.* (2015)⁸⁵ recently showed that traumatic brain injury was not only associated with traditional proinflammatory markers, but also elevated TLR4 signaling and uncontrolled BBB transit. Importantly, administration of a vascular endothelial growth inhibitor (VEGI) up-regulated the tight junction proteins (claudin-5, ZO-1, occludin) and attenuated TLR4 activation, NF- κ B signaling and the production of proinflammatory cytokines, as well as improving markers of brain injury. Alcohol-induced steatohepatitis is also well documented to present with acute intestinal barrier disruption, resulting from impaired tight junction protein expression.⁸⁶ In this study, administration of a TLR4 monoclonal antibody attenuated both functional and molecular markers of barrier function, emphasizing the importance of TLR4-mediated tight junction disruption in an inflammatory setting.

TLR4-dependent tight junction disruption has also been shown to occur in response to irinotecan treatment in a TLR4 knockout (−/−) mouse model of gut toxicity.⁸⁰ Following irinotecan, increased permeability of both the intestinal barrier and BBB were detected, both seen at 24 h post-treatment. Although TLR4 knockout animals only showed improvements in intestinal barrier disruption, this study is the first to demonstrate central neurotoxic changes in a model of chemotherapy-induced gut toxicity and reinforces the bidirectional communication that exists between the gastrointestinal system and CNS. It is likely this communication that underpins the prevalent comorbidities affecting these organ systems.

Intestinal inflammation drives CNS changes

A number of intestinal pathologies are associated with an increased risk of behavioral comorbidities as indicated by increased rates of depression, mood disorders and cognitive

dysfunction in patients with inflammatory bowel disease (IBD).⁸⁷ For example, elevated circulating proinflammatory cytokines, increased in intestinal permeability and the number of circulating monocytes are commonly reported in acute phases of trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice.⁸⁸ Importantly, these are accompanied by localized breaches in the BBB⁸⁹ leading to increased neuroinflammation^{90,91} and associated cognitive disturbance. These findings are consistent with those of Zonis *et al.*⁹² who, using a different murine IBD model (dextran sodium sulfate), found increased microglial and astrocytic reactivity in the hippocampus of treated mice. These results also compliment data indicating altered neuronal function and increased anxiety-like behavior in models of parasitic gut inflammation.^{93,94} Other reports support the concept that patients with chronic inflammatory states initiated by autoimmune diseases, cancer or infections are at higher risk for central neurological pathology and that there is a high likelihood that such changes are mediated by proinflammatory cytokines directly impacting the brain.⁹⁵

Neuroinflammation and Cognitive Dysfunction

Increased systemic proinflammatory cytokine production has been previously suggested as a candidate mechanism for cognitive dysfunction in cancer patients.²⁶ It is therefore possible that proinflammatory cytokines may be involved in several aspects of neurotoxicity by; (i) increasing BBB transit, and (ii) permitting neuroinflammation and associated tissue manifestations.

Substantial data, from a spectrum of clinical settings, highlight links between peripheral inflammation and cognitive symptoms. For example, peripheral activation of the immune system by a subseptic dose of lipopolysaccharide (LPS) has been shown to increase cytokine expression within the brain^{96–98} at levels associated with learning and memory disruption in both models of disease and health.^{99–101} In healthy volunteers, LPS leads to increased levels of IL-1, TNF α and IL-6 resulting in impaired working memory and cognitive dysfunction.¹⁰² Similarly, increased peripheral inflammation has also been associated with gradual cognitive decline and the development of dementia in the elderly population.¹⁰³

Interestingly, the use of IFN α and IL-2 (proinflammatory cytokines) as anti-cancer agents is highly linked to the development of depression and other cognitive impairments,^{104,105} however, there is only limited clinical data from cancer patients in which correlations between circulating cytokines and cognitive function have been evaluated. Meyers *et al.*¹⁰⁵ reported an association between elevated levels of circulating IL-6 and worsened executive function in patients with acute myeloid leukemia. In addition, elevated IL-6 and TNF α seen in chemotherapy-treated breast cancer survivors correlated with persistent hippocampal structural changes and reduced verbal memory performance^{107,108} well beyond the period during which patients received drug infusions.

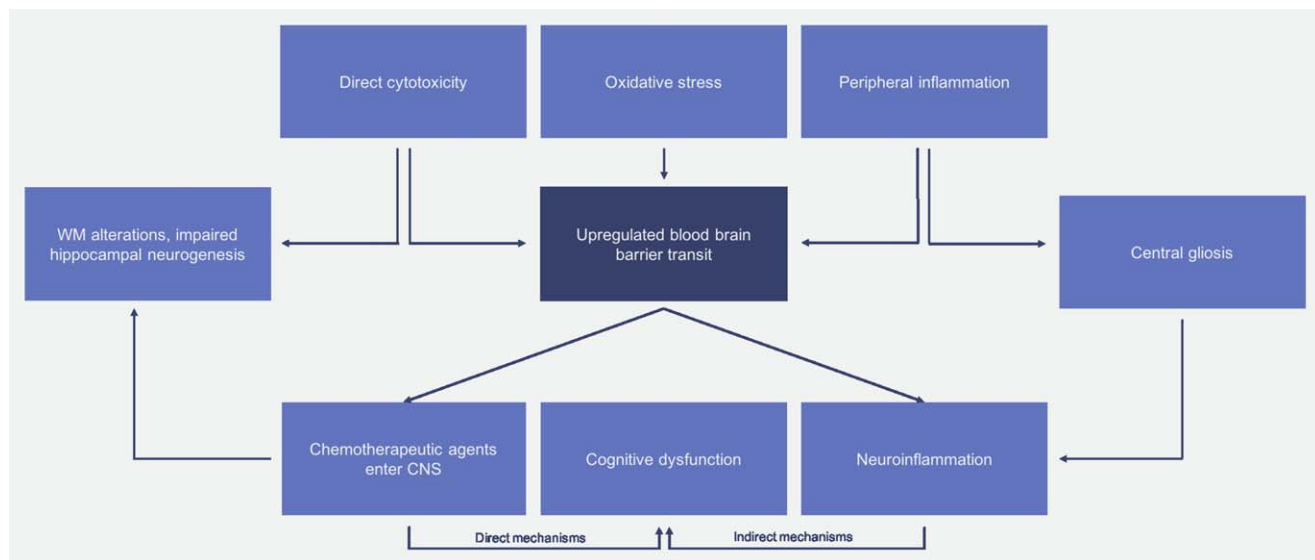


Figure 2. Schematic highlighting multifactorial pathobiology of cognitive dysfunction. Many cognitive disorders are characterised by blood brain barrier disruption and neuroinflammation. The impact of peripheral inflammation on central neurovascular integrity and the subsequent development of neuroinflammation is now considered a key driver in many neurological disease characterised by systemic inflammation. Blood brain barrier disruption may therefore compliment what is currently understood about neurotoxicity, by enhancing exposure of the CNS to chemotherapeutic agents (direct neurotoxicity) and permitting neuroinflammation (indirect). [Color figure can be viewed at wileyonlinelibrary.com]

When looking at other CNS pathologies characterized by neuroinflammation and cognitive impairment, Alzheimer's disease (AD) provides additional insight. Patients with AD often have elevated levels of TNF α in the cerebrospinal fluid and parenchyma,¹⁰⁹ as well as expressing TNF α -related polymorphisms.¹¹⁰ The pathological level of cytokines resulting in this chronic inflammatory state mimics that noted in cancer patients and perpetuates neuronal loss and cognitive decline.¹¹¹ Importantly, treatment with anti-TNF α agents in patients with AD has been shown to favorably impact the development of cognitive dysfunction.^{112,113} Of particular importance to our proposed hypothesis is that AD is often accompanied by increased BBB transit,¹¹⁴ leading to heightened inflammatory influx and worsened clinical outcomes.

Tumour-dependent cytokine production

Further confounding our understanding of how peripheral inflammation, BBB disruption and neuroinflammation contribute to cognitive dysfunction is the fact that results from clinical studies are neither uniform nor concrete. Cognitive dysfunction has been reported in patients with breast cancer or colorectal cancer after diagnosis, but before the administration of any anti-cancer treatment.¹¹⁵ Similarly, in a recent study comparing patients with localized colorectal cancer ($n = 289$) who received or did not receive chemotherapy, patients with metastatic or recurrent colorectal cancer (CRC) ($n = 73$) and healthy controls, Vardy *et al.*¹³ reported significant differences in cognitive dysfunction prior to, and following, treatment between patients with CRC and healthy controls. Surprisingly, there was no difference in the degree

of cognitive dysfunction between patients who received chemotherapy and those who did not. In addition, the extent of disease (local vs. metastatic) did not effect neurological function clouding understanding of tumour-driven effects on cognition. While levels of proinflammatory cytokine levels were elevated in the CRC cohorts vs. controls, there was no statistically significant relationship between them and cognitive dysfunction. Patel *et al.*'s¹¹⁵ study of 174 newly diagnosed patients with breast cancer also reported baseline levels of cognitive dysfunction and elevations in proinflammatory cytokine levels (TNF). However, elevations in TNF were no higher in cancer patients compared to a noncancer, demographically similar control group.

The findings of these, and similar studies, demonstrate the complexities of both the clinical and biological elements associated with cancer treatment-related cognitive dysfunction. First, the intrinsic and extrinsic biological activities associated with tumours have likely been underestimated. Tumours may actively produce inflammatory mediators as a consequence of local oxidative stress or stimulate inflammation in response to their presence.¹¹⁶ These findings might account for baseline cognitive dysfunction. Nonetheless, the lack of significant differences in cytokine levels between recently diagnosed cancer patients and noncancer controls clouds definitive conclusions.

The Significance of Symptom Chronicity

Despite patient and study heterogeneity and methodological limitations, neurotoxicity is defined by the chronicity of symptoms which often persist long after treatment

cessation.²⁹ It has been reported that patients treated with high dose chemotherapy and those that receive autologous haemopoietic stem cell transplantation have significant impairments in cognitive function up to 1 year after cessation of their treatment.¹¹⁷ The longevity of cognitive symptoms has also been assessed in survivors of childhood acute lymphoblastic leukaemia 6–18 years after remission.¹¹⁸ Deficits in figural memory as well as reduced hippocampal volume were also noted. Similarly, in breast cancer patients, chemotherapy treatment resulted in reduced gray matter volume in the right parahippocampal gyrus compared to untreated cancer patients, which correlated with reduced memory performance at 1 but not 3 years post-treatment.²¹ These studies are somewhat complimented by two prospective studies that show hippocampal volume reductions at 1 month following chemotherapy treatment, which was lost at further time points (1 year).¹¹⁹ More persistent changes were observed in a recent investigation of 19 breast cancer patients,¹²⁰ who showed right hippocampal gray matter volume reductions at both 1 month and 1 year after treatment completion.

Although variations exist in the time-course of these symptoms, chronicity is almost always reported and is a defining characteristic of neurotoxicity. Given the relatively short half-life of many chemotherapeutic agents, the chronicity of symptoms is biologically significant, and suggests that the impact of direct cytotoxicity would presumably be minimal; with chronic, reactive inflammatory processes, a more likely candidate. However, it is important to consider that neurotoxicity is unlikely to be attributable to a single mechanism, rather, various mechanisms may converge additively or synergistically to result in the heterogeneous symptoms seen in patients (Fig. 2). This is well described by Dietrich *et al.*⁵ who highlights key mechanistic drivers such as oxidative stress, direct cellular toxicity and inflammation that contribute to altered cellular kinetics in the hippocampus as well as neurovascular/BBB disruption. If a prolonged period of inflammation is present, either systemically or centrally, altered BBB transit could parallel the chronic, long-term

changes seen in patients and may provide a better biological understanding.

Conclusion

Regimen-related toxicities are poorly characterized and often have significant effects on patient quality of life. We have previously highlighted the importance of symptom clusters, emphasizing the possibility of common underlying mechanisms, which could perhaps be simultaneously targeted.¹²¹ Cognitive impairment is particularly devastating to patients, and currently has no universally accepted mechanism. This review has proposed that, contrary to traditional beliefs, chemotherapeutic agents can in fact gain access to the CNS. Importantly, we suggest that this is likely due to upregulated and uncontrolled BBB transit. The BBB, like many interfaces within the body, is subject to intense modification highlighting the plasticity of tight junctions and transcytotic mediators. Based on symptom clustering and potential linkages between the gut and CNS, we suggest that peripherally derived inflammatory mediators are responsible for inducing BBB dysfunction, thus permitting central neurotoxicity. Importantly, neurotoxic changes may occur through the direct actions of the chemotherapeutic drug itself, or present as the behavioral manifestation of neuroinflammation. BBB disruption may therefore be the missing link in our understanding of how gut/CNS communication is involved in the development of two critically important regimen-related toxicities. Furthermore, it presents as an exciting opportunity to target peripheral inflammation and achieve wider reaching clinical outcomes.

Acknowledgements

Ms Hannah Wardill is the recipient of the Florey Medical Research Foundation Doctor Chun Chung Wong and Madam So Sau Lam Memorial Postgraduate Cancer Research Top Up Scholarship 2015. Ms Hannah Wardill, Ms Ysabella Van Sebille and Ms Kimberly Mander are recipients of an Australian Postgraduate Award.

References

- Holmes D. Trying to unravel the mysteries of chemobrain. *Lancet Neurol* 2013;12:533–4.
- Ahles TA, Saykin AJ, McDonald BC, et al. Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. *J Clin Oncol* 2010;28:4434–40.
- Jenkins V, Beveridge H, Low R, et al. Atypical hearing loss in women with breast cancer receiving adjuvant treatment. *Breast* 2006;15:448–51.
- Jenkins V, Shilling V, Deutsch G, et al. A 3-year prospective study of the effects of adjuvant treatments on cognition in women with early stage breast cancer. *Br J Cancer* 2006;94:828–34.
- Dietrich J, Prust M, Kaiser J. Chemotherapy, cognitive impairment and hippocampal toxicity. *Neuroscience* 2015;309:224–32.
- Horky LL, Gerbaudo VH, Zaitsev A, et al. Systemic chemotherapy decreases brain glucose metabolism. *Ann Clin Transl Neurol* 2014;1:788–98.
- Janelins MC, Kohli S, Mohile SG, et al. An update on cancer- and chemotherapy-related cognitive dysfunction: current status. *Semin Oncol* 2011;38:431–8.
- Wu LM, Diefenbach MA, Gordon WA, et al. Cognitive problems in patients on androgen deprivation therapy: a qualitative pilot study. *Urol Oncol* 2013;31:1533–8.
- Sodeck GH, Domanovits H, Sterz F, et al. Can brain natriuretic peptide predict outcome after cardiac arrest? An observational study. *Resuscitation* 2007;74:439–45.
- Tannock IF, Ahles TA, Ganz PA, et al. Cognitive impairment associated with chemotherapy for cancer: report of a workshop. *J Clin Oncol* 2004;22:2233–9.
- Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat Rev Cancer* 2007;7:192–201.
- Komaki R, Meyers CA, Shin DM, et al. Evaluation of cognitive function in patients with limited small cell lung cancer prior to and shortly following prophylactic cranial irradiation. *Int J Radiat Oncol Biol Phys* 1995;33:179–82.
- Vardy JL, Dhillon HM, Pond GR, et al. Cognitive function in patients with colorectal cancer who do and do not receive chemotherapy: a prospective, longitudinal, controlled study. *J Clin Oncol* 2015;33:4085–92.
- Bourke RS, West CR, Chheda G, et al. Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following

- intravenous injection in a primate. *Cancer Res* 1973;33:1735–46.
15. Brown MS, Simon JH, Stemmer SM, et al. MR and proton spectroscopy of white matter disease induced by high-dose chemotherapy with bone marrow transplant in advanced breast carcinoma. *AJNR Am J Neuroradiol* 1995;16:2013–20.
 16. Brown MS, Stemmer SM, Simon JH, et al. White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. *AJNR Am J Neuroradiol* 1998;19:217–21.
 17. Ferguson RJ, McDonald BC, Saykin AJ, et al. Brain structure and function differences in monozygotic twins: possible effects of breast cancer chemotherapy. *J Clin Oncol* 2007;25:3866–70.
 18. Simo M, Rifa-Ros X, Rodriguez-Fornells A, et al. Chemobrain: a systematic review of structural and functional neuroimaging studies. *Neurosci Biobehav Rev* 2013;37:1311–21.
 19. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage* 2000;11:805–21.
 20. Ahdieh M, Vandenbos T, Youakim A. Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-gamma. *Am J Physiol Cell Physiol* 2001;281:C209–38.
 21. Inagaki M, Yoshikawa E, Matsuoka Y, et al. Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. *Cancer* 2007;109:146–56.
 22. McDonald BC, Conroy SK, Ahles TA, et al. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. *Breast Cancer Res Treat* 2010;123:819–28.
 23. Dietrich J, Monje M, Wefel J, et al. Clinical patterns and biological correlates of cognitive dysfunction associated with cancer therapy. *Oncologist* 2008;13:1285–95.
 24. Nokia MS, Anderson ML, Shors TJ. Chemotherapy disrupts learning, neurogenesis and theta activity in the adult brain. *Eur J Neurosci* 2012;36:3521–30.
 25. Han R, Yang YM, Dietrich J, et al. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *J Biol* 2008;7:12.
 26. Seigers R, Loos M, Van Tellingen O, et al. Neurobiological changes by cytotoxic agents in mice. *Behav Brain Res* 2015;299:19–26.
 27. Abraham J, Haut MW, Moran MT, et al. Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. *Clin Breast Cancer* 2008;8:88–91.
 28. Deprez S, Amant F, Yigit R, et al. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. *Hum Brain Mapp* 2011;32:480–93.
 29. Deprez S, Amant F, Smeets A, et al. Longitudinal assessment of chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning. *J Clin Oncol* 2012;30:274–81.
 30. Ginos JZ, Cooper AJ, Dhawan V, et al. [13N]cisplatin PET to assess pharmacokinetics of intra-arterial versus intravenous chemotherapy for malignant brain tumors. *J Nucl Med* 1987;28:1844–52.
 31. Gangloff A, Hsueh WA, Kesner AL, et al. Estimation of paclitaxel biodistribution and uptake in human-derived xenografts in vivo with (18)F-fluoropaclitaxel. *J Nucl Med* 2005;46:1866–71.
 32. Dietrich J, Han R, Yang Y, et al. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. *J Biol* 2006;5:22.
 33. Rochford KD, Collins LE, Murphy RP, et al. Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. *PLoS One* 2014;9:e101815.
 34. Wardill HR, Bowen JM, Gibson RJ. Chemotherapy-induced gut toxicity: are alterations to intestinal tight junctions pivotal? *Cancer Chemother Pharmacol* 2012;70:627–35.
 35. Corps KN, Roth TL, McGavern DB. Inflammation and neuroprotection in traumatic brain injury. *JAMA Neurol* 2015;72:355–62.
 36. Chodobski A, Zink BJ, Szymdynger-Chodobska J. Blood-brain barrier pathophysiology in traumatic brain injury. *Transl Stroke Res* 2011;2:492–516.
 37. Jickling GC, Liu D, Stamova B, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab* 2014;34:185–99.
 38. Al-Dasooqi N, Gibson RJ, Bowen JM, et al. Matrix metalloproteinases are possible mediators for the development of alimentary tract mucositis in the dark agouti rat. *Exp Biol Med* 2010;235:1244–56.
 39. Cummins PM. Occludin: one protein, many forms. *Mol Cell Biol* 2012;32:242–50.
 40. Bauer A, Burgers H, Rabie T, et al. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. *J Cereb Blood Flow Metab* 2010;30:837–48.
 41. Lischper M, Beuck S, Thanabalasundaram G, et al. Metalloproteinase mediated occludin cleavage in the cerebral microcapillary endothelium under pathological conditions. *Brain Res* 2010;1326:114–27.
 42. Vermeer P, Denker J, Estin M, et al. MMP9 modulates tight junction integrity and cell viability in human airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L751–62.
 43. Liu W, Hendren J, Qin X, et al. Normobaric hyperoxia attenuates early blood-brain barrier disruption by inhibiting MMP-9-mediated occludin degradation in focal cerebral ischemia. *J Neurochem* 2009;108:811–20.
 44. Chen W, et al. Matrix metalloproteinases inhibition provides neuroprotection against hypoxia-ischemia in the developing brain. *J Neurochem* 2009;111:726–36.
 45. Higashida T, et al. The role of hypoxia-inducible factor-1alpha, aquaporin-4 and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury. *J Neurosurg* 2011;114:92–101.
 46. Asahi M, Wang X, Mori T, et al. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. *J Neurosci* 2001;21:7724–32.
 47. de Vries HE, Kuiper J, de Boer AG, et al. The blood-brain barrier in neuroinflammatory diseases. *Pharmacol Rev* 1997;49:143–55.
 48. Blanco AM, Perez-Arago A, Fernandez-Lizarbe S, et al. Ethanol mimics ligand-mediated activation and endocytosis of IL-1RI/TLR4 receptors via lipid rafts caveolae in astroglial cells. *J Neurochem* 2008;106:625–39.
 49. Pan W, Stone KP, Hsueh H, et al. Cytokine signaling modulates blood-brain barrier function. *Curr Pharm Des* 2011;17:3729–40.
 50. Pan W, Tu H, Yu C, et al. Differential role of TNF receptors in cellular trafficking of intact TNF. *Cell Physiol Biochem* 2007;20:559–68.
 51. Logan RM, Gibson RJ, Bowen JM, et al. Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis. *Cancer Chemother Pharmacol* 2008;62:33–41.
 52. Logan RM, Stringer AM, Bowen JM, et al. The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. *Cancer Treat Rev* 2007;33:448–60.
 53. Sonis ST. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol* 1998;34:39–43.
 54. Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. *Ann Rev Med* 2000;51:289–98.
 55. Galvez BG, Matias-Roman S, Yanez-Mo M, et al. Caveolae are a novel pathway for membrane-type 1 matrix metalloproteinase traffic in human endothelial cells. *Mol Biol Cell* 2004;15:678–87.
 56. Wardill HR, Bowen JM, Al-Dasooqi N, et al. Irinotecan disrupts tight junction proteins within the gut: implications for chemotherapy-induced gut toxicity. *Cancer Biol Ther* 2014;15:236–44.
 57. Al-Dasooqi N, Sonis ST, Bowen JM, et al. Emerging evidence on the pathobiology of mucositis. *Supp Care Cancer* 2013;21:3233–41.
 58. Al-Dasooqi N, Sonis ST, Bowen JM, et al. Emerging evidence on the pathobiology of mucositis. *Supp Care Cancer* 2013;21:2075–83.
 59. Stringer AM, Al-Dasooqi N, Bowen JM, et al. Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. *Supp Care Cancer* 2013;21:1843–52.
 60. Schreibelt G, Kooij G, Reijerkerk A, et al. Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. *FASEB J* 2007;21:3666–76.
 61. Henriksen EJ. A radical concept on caveolae and endothelial dysfunction in coronary microvascular disease in diabetes. *Diabetes* 2014;63:1200–2.
 62. Chidlow JH, Jr, Sessa WC. Caveolae, caveolins, and cavinins: complex control of cellular signalling and inflammation. *Cardiovasc Res* 2010;86:219–25.
 63. Stringer AM. Interaction between host cells and microbes in chemotherapy-induced mucositis. *Nutrients* 2013;5:1488–99.
 64. Sharma R, Young C, Neu J. Molecular modulation of intestinal epithelial barrier: contribution of microbiota. *J Biomed Biotechnol* 2010;2010:305879.
 65. Sharma HS, Vannemreddy P, Patnaik R, et al. Histamine receptors influence blood-spinal cord barrier permeability, edema formation, and spinal cord blood flow following trauma to the rat

- spinal cord. *Acta Neurochir Suppl* 2006;96:316–21.
66. Rodriguez PL, Jiang S, Fu Y, et al. The proinflammatory peptide substance P promotes blood-brain barrier breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions. *Int J Cancer* 2014;134:1034–44.
 67. Vink R, van den Heuvel C. Substance P antagonists as a therapeutic approach to improving outcome following traumatic brain injury. *Neurotherapeutics* 2010;7:74–80.
 68. D'Alessio A, Al-Lamki RS, Bradley JR, et al. Caveolae participate in tumor necrosis factor receptor 1 signaling and internalization in a human endothelial cell line. *Am J Pathol* 2005;166:1273–82.
 69. Mustian KM, Devine K, Ryan JL, et al. Treatment of nausea and vomiting during chemotherapy. *US Oncol Hematol* 2011;7:91–7.
 70. Wardill HR, Gibson RJ, Logan RM, et al. TLR4/ PKC-mediated tight junction modulation: a clinical marker of chemotherapy-induced gut toxicity? *Int J Cancer* 2013;135:2483–92.
 71. Jiao H, Zhang Y, Yan Z, et al. Caveolin-1 Tyr14 phosphorylation induces interaction with TLR4 in endothelial cells and mediates MyD88-dependent signaling and sepsis-induced lung inflammation. *J Immunol* 2013;191:6191–9.
 72. Villena J, Kitazawa H. Modulation of intestinal TLR4-inflammatory signaling pathways by probiotic microorganisms: lessons learned from *Lactobacillus jensenii* T12937. *Front Immunol* 2014;4:512.
 73. Argaw AT, Asp L, Zhang J, et al. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J Clin Invest* 2012;122:2454–68.
 74. Argaw AT, Gurfein BT, Zhang Y, et al. VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. *Proc Natl Acad Sci U S A* 2009;106:1977–82.
 75. Proescholdt MA, Jacobson S, Tresser N, et al. Vascular endothelial growth factor is expressed in multiple sclerosis plaques and can induce inflammatory lesions in experimental allergic encephalomyelitis rats. *J Neuropathol Exp Neurol* 2002;61:914–25.
 76. Caliceti C, Zamboni L, Rizzo B, et al. Role of plasma membrane caveolae/lipid rafts in VEGF-induced redox signaling in human leukemia cells. *BioMed Res Int* 2014;2014:857504.
 77. Boussios S, Pentheroudakis G, Katsanos K, et al. Systemic treatment-induced gastrointestinal toxicity: incidence, clinical presentation and management. *Ann Gastroenterol* 2012;25:106–18.
 78. Seigers R, Fardell JE. Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. *Neurosci Biobehav Rev* 2011;35:729–41.
 79. Wardill HR, Van Seville YZ, Mander KA, et al. Toll-like receptor 4 signaling: a common biological mechanism of regimen-related toxicities: an emerging hypothesis for neuropathy and gastrointestinal toxicity. *Cancer Treat Rev* 2015;41:122–8.
 80. Wardill HR, Gibson RJ, Van Seville YZ, et al. Irinotecan-induced gastrointestinal dysfunction and pain are mediated by common TLR4-dependent mechanisms. *Mol Cancer Ther* 2016;15:1376–86.
 81. Bowen JM, Collier J, Hutchinson M, et al. Expression of TLRs in the rat intestine following chemotherapy for cancer. *Brain Behav Immun* 2012;26:S27–S.
 82. Wong DV, Lima-Junior RC, Carvalho CB, et al. The adaptor protein Myd88 is a key signaling molecule in the pathogenesis of irinotecan-induced intestinal mucositis. *PLoS One* 2015;10:e0139985.
 83. Frank M, Hennenberg EM, Eyking A, et al. TLR signaling modulates side effects of anticancer therapy in the small intestine. *J Immunol* 2015;194:1983–95.
 84. Wardill HR, Gibson RJ, Logan RM, et al. TLR4/ PKC-mediated tight junction modulation: a clinical marker of chemotherapy-induced gut toxicity? *Int J Cancer* 2014;135:2483–92.
 85. Guo S, Nighot M, Al-Sadi R, Alhmod T, Nighot P, Ma TY. Lipopolysaccharide Regulation of Intestinal Tight Junction Permeability Is Mediated by TLR4 Signal Transduction Pathway Activation of FAK and MyD88. *J Immunol* 2015;195:4999–5010.
 86. Li X, Wang C, Nie J, et al. Toll-like receptor 4 increases intestinal permeability through up-regulation of membrane PKC activity in alcoholic steatohepatitis. *Alcohol* 2013;47:459–65.
 87. Nyuyki KD, Pittman QJ. Toward a better understanding of the central consequences of intestinal inflammation. *Ann N Y Acad Sci* 2015;1351:149–54.
 88. Brown KA, Back SJ, Ruchelli ED, et al. Lamina propria and circulating interleukin-6 in newly diagnosed pediatric inflammatory bowel disease patients. *Am J Gastroenterol* 2002;97:2603–8.
 89. Natah SS, Mouhate A, Pittman QJ, et al. Disruption of the blood-brain barrier during TNBS colitis. *Neurogastroenterol Motility* 2005;17:433–46.
 90. Wang K, Yuan CP, Wang W, et al. Expression of interleukin 6 in brain and colon of rats with TNBS-induced colitis. *World J Gastroenterol* 2010;16:2252–9.
 91. Riazi K, Galic MA, Kuzmiski JB, et al. Microglial activation and TNF α production mediate altered CNS excitability following peripheral inflammation. *Proc Natl Acad Sci U S A* 2008;105:17151–6.
 92. Zonis S, Pechnick RN, Ljubimov VA, et al. Chronic intestinal inflammation alters hippocampal neurogenesis. *J Neuroinflammation* 2015;12:65.
 93. Bercik P, Verdu EF, Foster JA, et al. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology* 2010;139:2102–12 e1.
 94. Goehler LE, Gaykema RP, Opitz N, et al. Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with *Campylobacter jejuni*. *Brain Behav Immun* 2005;19:334–44.
 95. Dantzer R, O'Connor JC, Freund GG, et al. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;9:46–56.
 96. Gatti S, Bartfai T. Induction of tumor necrosis factor- α mRNA in the brain after peripheral endotoxin treatment: comparison with interleukin-1 family and interleukin-6. *Brain Res* 1993;624:291–4.
 97. Laye S, Parnet P, Goujon E, et al. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Brain Res Mol Brain Res* 1994;27:157–62.
 98. Quan N, Stern EL, Whiteside MB, et al. Induction of pro-inflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat. *J Neuroimmunol* 1999;93:72–80.
 99. Holden JM, Overmier JB, Cowan ET, et al. Effects of lipopolysaccharide on consolidation of partial learning in the Y-maze. *Integr Physiol Behav Sci* 2004;39:334–40.
 100. Gahtan E, Overmier JB. Performance more than working memory disrupted by acute systemic inflammation in rats in appetitive tasks. *Physiol Behav* 2001;73:201–10.
 101. Sparkman NL, Buchanan JB, Heyen JR, et al. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. *J Neurosci* 2006;26:10709–16.
 102. Krabbe KS, Reichenberg A, Yirmiya R, et al. Low-dose endotoxemia and human neuropsychological functions. *Brain Behav Immun* 2005;19:453–60.
 103. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* 2004;39:687–99.
 104. Capuron L, Ravaut A, Dantzer R. Timing and specificity of the cognitive changes induced by interleukin-2 and interferon- α treatments in cancer patients. *Psychosom Med* 2001;63:376–86.
 105. Trask PC, Esper P, Riba M, et al. Psychiatric side effects of interferon therapy: prevalence, proposed mechanisms, and future directions. *J Clin Oncol* 2000;18:2316–26.
 106. Meyers CA, Albitar M, Estey E. Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. *Cancer* 2005;104:788–93.
 107. Bower JE, Ganz PA, Aziz N, et al. Inflammatory responses to psychological stress in fatigued breast cancer survivors: relationship to glucocorticoids. *Brain Behav Immun* 2007;21:251–8.
 108. Collado-Hidalgo A, Bower JE, Ganz PA, et al. Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clin Cancer Res* 2006;12:2759–66.
 109. Cheng X, Yang L, He P, et al. Differential activation of tumor necrosis factor receptors distinguishes between brains from Alzheimer's disease and non-demented patients. *J Alzheimers Dis* 2010;19:621–30.
 110. Di Bona D, Candore G, Franceschi C, et al. Systematic review by meta-analyses on the possible role of TNF- α polymorphisms in association with Alzheimer's disease. *Brain Res Rev* 2009;61:60–8.
 111. Minter MR, Taylor JM, Crack PJ. The contribution of neuro-inflammation to amyloid toxicity in Alzheimer's disease. *J Neurochem* 2015;136:457–74.
 112. in't Veld BA, Launer LJ, Hoes AW, et al. NSAIDs and incident Alzheimer's disease. The Rotterdam Study. *Neurobiol Aging* 1998;19:607–11.
 113. Alkam T, Nitta A, Mizoguchi H, et al. Restraining tumor necrosis factor- α by thalidomide prevents the amyloid β -induced impairment of recognition memory in mice. *Behav Brain Res* 2008;189:100–6.

114. Sharma HS, Castellani RJ, Smith MA, et al. The blood-brain barrier in Alzheimer's disease: novel therapeutic targets and nanodrug delivery. *Int Rev Neurobiol* 2012;102:47–90.
115. Patel SK, Wong AL, Wong FL, et al. Inflammatory biomarkers, comorbidity, and neurocognition in women with newly diagnosed breast cancer. *J Natl Cancer Inst* 2015;107. doi: 10.1093/jnci/djv131.
116. Roque AT, Gambeloni RZ, Felitti S, et al. Inflammation-induced oxidative stress in breast cancer patients. *Med Oncol* 2015;32:263
117. Hjermstad M, Holte H, Evensen S, et al. Do patients who are treated with stem cell transplantation have a health-related quality of life comparable to the general population after 1 year? *Bone Marrow Transplant* 1999; 24:911–8.
118. Genschaft M, Huebner T, Plessow F, et al. Impact of chemotherapy for childhood leukemia on brain morphology and function. *PloS One* 2013;8:e78599
119. McDonald BC, Conroy SK, Ahles TA, et al. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. *Breast Cancer Res Treat* 2010;123: 819–28.
120. Lepage C, Smith AM, Moreau J, et al. A prospective study of grey matter and cognitive function alterations in chemotherapy-treated breast cancer patients. *SpringerPlus* 2014;3:444
121. Wardill HR, Van Seville YZ, Mander KA, et al. Toll-like receptor 4 signaling: a common biological mechanism of regimen-related toxicities: an emerging hypothesis for neuropathy and gastrointestinal toxicity. *Cancer Treat Rev* 2015;41:122–8.