Minireview

Chemotherapy-induced mucositis: the role of the gastrointestinal microbiome and toll-like receptors

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Abstract

Alimentary mucositis is a major clinical problem. Patients with mucositis are at significantly increased risk of infection and are often hospitalized for prolonged periods. More importantly, these patients often have to undergo reductions in their cytotoxic therapy, which may lead to reduced survival. Unfortunately, there are very limited therapeutic options for mucositis and no effective prevention. The human gut microbiome is receiving increased attention as a key player in the pathogenesis of alimentary mucositis with recent literature suggesting that changes in bacteria lead to mucositis. The bacteria which are found throughout the gut are tightly regulated by the toll-like receptor (TLR) family which currently has 13 known members. TLRs play a critical role in gut homeostasis and bacterial regulation. Furthermore, TLRs play a critical role in the regulation of nuclear factor kappa B, a key regulator of alimentary mucositis. However to date, no research has clearly identified a link between TLRs and alimentary mucositis. This critical literature review seeks to correct this.

Keywords: mucositis, gut microbiome, toll-like receptors, chemotherapy

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Introduction

Alimentary mucositis (AM) is a severe, dose-limiting, toxic side-effect of cytotoxic chemotherapy and radiotherapy, affecting the entire alimentary tract from mouth to anus.[1,2] AM occurs in more than 40% of standard dose chemotherapy patients and in 100% of high-dose chemotherapy patients,[3–5] causing a wide range of symptoms, including mouth ulceration, abdominal pain, nausea, vomiting, abdominal bloating and diarrhoea.[3,6] More importantly, patients with AM often have to undergo reductions in their treatment, which may lead to reduced survival.[1,7] Resource utilization for patients with AM is also significantly increased, with the need for nutritional adjuncts including fluid replacement, liquid diets and total parenteral nutrition.[8,9] Combined, this translates to an incremental cost increase of US$3500 per cycle of standard dose chemotherapy with AM (it is much higher for high-dose chemotherapy)[1,2] and hence a substantial burden on Medicare. Unfortunately, there is currently no effective prevention for AM.[8–13]

Nuclear factor kappa B and mucositis manifestation

Currently, the hypothesis for the development of AM suggests that there are five intertwined phases, namely; (1) initiation; (2) up-regulation and generation of messenger signals; (3) signal amplification; (4) ulceration and (5) healing.[11,14,15] This current hypothesis has been described in both animal[16–19] and human[20,21] studies. Nuclear factor kappa B (NFκB) has been demonstrated in numerous clinical and animal studies to be critically important in regulating mucositis.[21–24] Briefly, NFκB is a heterodimer of the p65/RelA and p50 or p52 subunits, which are found in the cytoplasm of cells.[25] When inactive, the classic form of NFκB is tightly bound to the IκB class of proteins, which act as an 'inhibitor' of NFκB function.[26] When stimulated, such as following cytotoxic therapy, the bound NFκB/IκB is phosphorylated, ubiquinated and NFκB is permitted to enter the cell nucleus.[21,25,26] Once in the nucleus, NFκB is able to up-regulate many genes associated with mucositis, including pro-inflammatory cytokines;[21–23,25] growth factors;[25] and pro- and antiapoptosis genes.[25]

Recent research has clearly shown that NFκB is up-regulated during the second phase of mucositis.[21–23] This up-regulation is associated with significant increases in the pro-inflammatory cytokines, tumor necrosis factor (TNF), interleukin (IL)-6 and IL-1β.[22,23] Furthermore, NFκB has been demonstrated to be involved in the signal amplification phase, where it acts to transcribe TNF and IL-6; these pro-inflammatory cytokines then positively feed back to NFκB which then amplifies the response.[22,23]

The Human Microbiome

With the establishment of the Human Microbiome Project, the human gastrointestinal microbiome is receiving significant attention.[27,28] The average human gut harbors approximately 100 trillion commensal microorganisms, constituting up to 1000 individual species[27,29] and carrying at least 100 times the genetic capacity of the human genome.[27–29] The human gut microbiome can influence host metabolism, physiology and gene expression.[28] Specific functions include, but are not limited to, the metabolism of serum proteins, cholesterol, hormones and vitamins, influencing natural resistance and preventing adherence of pathogenic enteric bacilli.[30–32]

It is well known that the gut is an immunological organ in its own right.[33,34] In healthy individuals there is a high level of immunological activity, as a direct result of ongoing stimulation by the normal microbiome.[34] Due to the symbiotic relationship between the intestine and microbes, the gut mucosa has immunologically adapted to the extensive capacity of potentially antigenic microbes.[35] The aerobic and anaerobic bacteria found in the gut are tightly regulated by toll-like receptor (TLR) signaling, a family that currently has 13 known members, 10 of which are known to be present in humans.[36,37] TLR signaling is stimulated by the host flora and plays a protective role within the gut.[38,39] The immune system constantly monitors the gut microbiome by allowing bacteria through the epithelial barrier into Peyer's patches, where immune cells initiate an innate immune response.[35] This occurs as the result of TLR binding to the bacterial products and activating immune cells,[35] ultimately resulting in the body being aware of gut bacteria but not mounting a full immune response against them.[36] The microbiome regulates the host cytokine levels and has been recently suggested to regulate specific immune responses by controlling the profile of locally released cytokines.[27,35] A systemic response is prevented through the inhibition of NFκB expression.[35] Combined, this provides compelling evidence of a tight relationship between expression of toll-like receptors and the development of alimentary mucositis.

Gut microbiome and alimentary mucositis

There are two main groups of microflora found in the gut; commensal and pathogenic. Both groups have their own unique molecular patterns known as commensal-associated molecular patterns and pathogen-associated molecular pattern, respectively.[40] Furthermore, a third kind of molecular patterns, damage-associated molecular patterns (DAMPs), are released from necrotic cells and the extracellular matrix.[41] The gut microbiome is of particular interest in AM, as our research has implicated microbiome changes as integral in the development of AM.[1,7,31,33] These findings have clearly shown that the gut microbiome is adversely affected by chemotherapy with changes seen in the microbiome composition in the stomach, and small and large intestines.[30–32] In particular, a clear shift from commensal bacteria, specifically *Bifidobacterium* spp., between 24 and 72 h corresponding with mucositis represented as diarrhoea, toward *Salmonella* spp. and *Escherichia coli* has been demonstrated. The decrease in commensal bacteria, represented by *Bifidobacterium* spp., inversely followed the pattern of diarrhoea induced by chemotherapy.[30–32] These studies (using quantitative real-time polymerase chain reaction) were among the first worldwide to show that changes in the gut microbiome are clearly associated with the underlying pathophysiology of subsequent AM.[30–32]

These findings are consistent with studies undertaken in rats treated with irinotecan and 5-fluorouracil (5FU), where results demonstrated an altered microbiome allowing different genera of bacteria to proliferate.[16,30–32] Irinotecan has been shown to be associated with an increase in bacteria that produce β-glucuronidase, which may result in SN-38G (the non-toxic metabolite of irinotecan) being converted back to SN-38 (the toxic metabolite of irinotecan) at an increased rate, causing significant intestinal damage.[16,30–32] The changes seen in both β-glucuronidase and bacteria producing β-glucuronidase coincided with the incidence of diarrhoea.[30–32] These findings are supported in clinical studies where a recent study demonstrated a decrease in anaerobic bacteria and an increase in potentially pathogenic bacteria in fecal samples.[42] Investigations in patients undergoing chemotherapy for acute myeloid leukemia showed a decrease in the number of anaerobic bacteria, subsequently leading to an increase in numbers of aerobic and potentially pathogenic bacteria.[42] The idea that alterations in the intestinal microbiome are, at least in part, responsible for toxicity is further supported by a pediatric placebo-controlled study whereby 42 patients were randomized to receive either a probiotic containing *Bifidobacterium breve*, or control. Patients who received the probiotics demonstrated an enhanced anaerobic population, whereas control patients had increased levels of Enterobacteriacae.[43] These results have led to the suggestion that probiotics are an effective way of managing toxicity after chemotherapy by improving the gut microbiome.

TLRs and intestinal homeostasis

TLRs are known to play a key role in maintaining gut epithelial homeostasis.[38] Of specific interest to the gut microbiome are family members TLR2, TLR4, TLR5 and TLR9, all of which have been shown to play a role in controlling homeostasis of the intestinal epithelium.

TLR2

TLR2 is plays a pivotal role in the protection and regulation of the gut epithelial barrier. TLR2 recognizes lipoproteins, peptidoglycan, lipoteichoic acid and zymosan and leads to the activation of NFκB through the Toll/IL-1R domain with the adaptor protein MyD88 (Figure 1).[44] TLR2 activation results in antiapoptotic effects, resulting in regulation of tight junctions between intestinal epithelial cells.[44] This occurs through the activation of the PI3K/Akt pathway through the phosphorylation of Akt and activation of downstream p70S6K and ribosomal protein S6.[44]

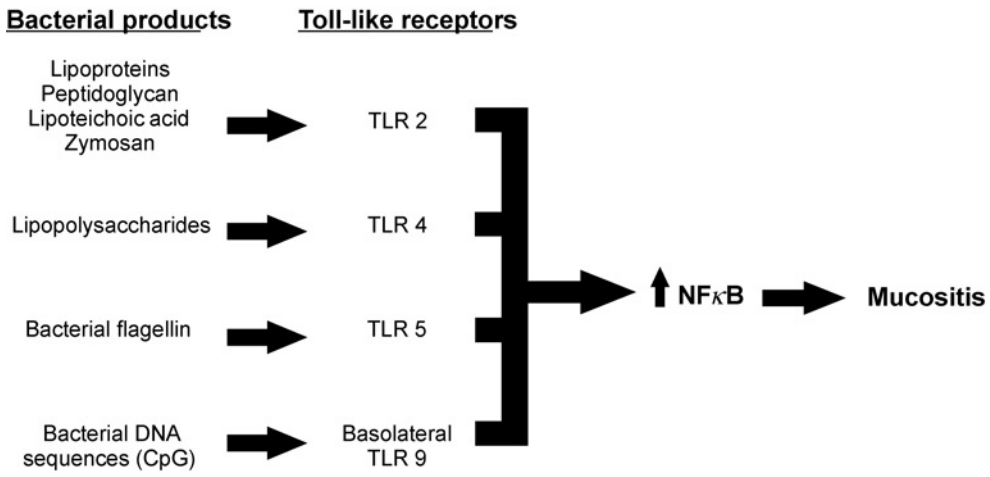

Figure 1 Bacterial products activate nuclear factor kappa B (NFκB) through toll-like receptors (TLRs). Bacterial products affect NFκB expression through toll-like receptors, lipoproteins, peptidoglycan, lipoteichoic acid and zymosan through TLR2, lipopolysaccharides through TLR4, bacterial flagellin through TLR5 and bacterial DNA through basolateral TLR9. NFκB expression has also been implicated in the development of mucositis, indicating a possible link between the bacterial changes and NFκB expression

Therefore, it is suggested that TLR2 is involved in mucosal homeostasis through balancing intestinal inflammation and tight junction regulation.[44]

TLR2 has also been implicated in doxorubicin (10 mg/kg of doxorubicin in 0.2 mL of 0.9% NaCl administered intraperitoneally)-induced mucositis in C57BL/6 mice.[45] TLR2−/− mice showed significantly decreased pathological damage ($P < 0.001$) based on histological scores compared with controls, when compared with wild-type (wt) mice in the ileum 72 h after chemotherapy.[45] TLR2−/− mice also had a significant decrease in the number of apoptotic cells ($P < 0.001$) in the ileum compared with wt mice following chemotherapy.[45]

DAMPs have also been shown to activate TLR2. Heat shock proteins (Hsps) in particular, are a type of DAMP which are released from necrotic cells and bacteria, activating TLR2.[46] Previous research has shown that Hsps are altered following cytotoxic insult.[24,47] In a hamster radiation model of oral mucositis, Sonis *et al.*[49] demonstrated that Hsp70 and Hsp90 increased early after radiotherapy.[24] Hsp70 has been suggested to have a cytoprotective effect, via initiation of DNA damage and subsequent cell death,[48] whereas Hsp90 is known to be associated with increased apoptosis. In contrast, Bowen *et al.*[47] demonstrated that Hsp27 was down-regulated in a rat model of intestinal mucositis following chemotherapy. Hsp27 is known to be involved in the stress response. Despite the conflicting up- and down-regulation of Hsps, they may play a significant role in mucositis. An increase in Hsp70 in a cell line from NIH3T3 mice is associated with an increase in resistance cytotoxic insult, hypothesized to be due to an increase in reactive oxygen species (ROS) tolerance.[48] Furthermore, in the same cell line an increase in Hsp70 has been associated with a resistance to mitotic cell death.[48] These results suggest that TLR2 may be associated with alimentary mucositis through Hsp responses of cytoprotection and/or stress.

TLR4

TLR4 and myeloid differentiation protein 2 create a receptor complex which is mediated by CD14.[50] TLR4 signaling is induced by endotoxins such as lipopolysaccharides (Figure 1).[50] Although TLR4 is located on the cell surface, its exact location on the surface varies from the apical to basolateral.[50,51] In ulcerative colitis, TLR4 is located basolaterally; however, in Crohns disease TLR4 is located apically.[51] Activation of TLR4 leads to activation of both MyD88-dependent and MyD88-independent pathways.[50,52] The MyD88-dependent pathway results in rapid activation of interferon regulatory factor 3 resulting in beta-interferon release and delayed NFκB activation.[50,52] In contrast, the MyD88-independent pathway results in rapid NFκB activation,[50,52] resulting in an increased complexity in TLR4-induced inflammation. In a recent study by Ferreira *et al.*[53] TLR4 expression has been shown to increase significantly ($P = 0.061$) at three days after administration of 5FU (intraperitoneally 200 mg/kg) in a Swiss mouse model of mucositis.

Up-regulation of TLR4 causes changes to epithelial cell proliferation,[54] NFκB expression[51] and pro-inflammatory cytokine expression.[55] All three of these are known to be altered during mucositis leading to the suggestion that TLR4 may be a key driver in the pathogenesis of mucositis.

TLR5

TLR5 is located on the basal surface of epithelial cells, and is specifically activated by bacterial flagellin, (present on *Salmonella typhimurium*). Bacteria flagella are known to elicit a two to three times greater response on the basolateral epithelial surface and this activation causes subsequent NFκB activation and adaptive immune response (Figure 1).[56,57] NFκB activation is a key driver in mucositis development; therefore, flagella activation of NFκB

through TLR5 suggests a link between the microbiome change, which occurs during mucositis and NFκB activation through TLR5. A recent study investigating the role of TLR5 agonists in radiotherapy demonstrated that these agonists decreased apoptosis levels and improved survival of animals following a lethal dose of total body irradiation.[39] Briefly, a polypeptide drug CBLB502 (derived from *Salmonella* flagellin and known to bind to TLR5) was injected into mice, and was found to reduce the toxicity associated with radiotherapy. It was hypothesized that activation of NFκB by the TLR5 agonist led to multiple genes being expressed, including apoptosis inhibitors, ROS scavengers and cytokines.[39] This success of this study strongly suggests that TLR5 is important in protecting the gut mucosa from damage.[41]

TLR9

TLR9 is thought to play a key role in the development of an appropriate response to bacteria within the gut, as it recognizes specific bacterial DNA sequences.[58] TLR9 is an intracellular receptor in dendritic cells and macrophages, and a surface receptor in intestinal epithelial cells and tonsil cells, occurring both apically and basolaterally in epithelial cells.[59] It interacts with CpG (Figure 1).[58,59] However, CpG affects signaling differently depending on whether it is apical or basolateral.[37] Apically IKB kinase mediated phosphorylation and polyubiquitination of inhibitors kappa B alpha (IκBα) fails to undergo proteasomal degradation resulting in NFκB inhibition by IκBα.[37] Basolaterally, IκBα degrades resulting in no inhibition of NFκB.[37] Different reactions to apical and basolateral CpG result in an appropriate response to breaches of the epithelium, providing a control mechanism for the innate immune system.[37]

TLR9 has also been implicated in doxorubicin (10 mg/kg of doxorubicin in 0.2m L of 0.9% NaCl administered intraperitoneally)-induced mucositis in C57BL/6 mice, in a study also investigating TLR2 in the same scenario.[45] TLR9−/− mice were found to have significantly ($P < 0.001$) decreased histological scores as a measurement of damage compared with controls when compared with wt mice in the ileum at 72 h after doxorubicin administration. TLR9−/− mice also showed a significant ($P < 0.001$) decrease in apoptotic cells in the ileum compared with wt mice following administration of doxorubicin.[45] Wt mice were then co-administered ODN2088 (an agonist of TLR9) with doxorubicin, compared with wt mice treated with just doxorubicin. At 72 h both the histological and apoptotic scores were significantly ($P < 0.05$) decreased in co-administered mice compared with just doxorubicin-treated mice,[45] strongly suggesting inhibition of TLR9 may be a possible therapeutic target for preventing mucositis.

Conclusion

Research has clearly demonstrated that NFκB is up-regulated in alimentary mucositis. Further, the dynamics of the gut microbiome alter from commensal to pathogenic bacteria following chemotherapy administration. However, there have been no studies which have investigated how changes in the microbiome activate the pathways which up-regulate pro-inflammatory cytokines. TLRs have been shown to mediate inflammatory response and maintain epithelial barrier homeostasis,[44,54,60–62] and are highly likely to be involved in the activation of a number of pathways following cancer therapy. Furthermore, TLR2, 4, 5 and 9 have been demonstrated to increase NFκB activation as a result of bacterial products.[44,50,56,57,59] Further research in this area is now warranted to elucidate the complete effects of TLRs and the microbiome in mucositis, to develop new targets for intervention to attenuate mucositis.

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