EDITORIAL

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What are the predictive factors in the risk and severity of chemotherapy-induced gastrointestinal toxicity?

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Damage to the mucous membranes of the GI tract by chemotherapy is known as mucositis or chemotherapy-induced gastrointestinal toxicity (CIGT). Injury can occur anywhere along the GI tract, or may be localized to a particular region, depending on the chemotherapy regimen. CIGT has huge clinical and economic implications affecting >50% of cancer patients, and can necessitate treatment reductions and/or treatment breaks. Patients that experience severe CIGT have twice the infection risk leading to a fourfold higher chance of death and threefold longer hospital stays, compromising survival and creating a burden on patients' quality of life. Economically, US data from 2012 estimated a combined cost of US\$15,500 for each hospitalization due to severe CIGT, adding millions to health-care expenditure [1]. Patients with CIGT typically experience severe consequences including, but not limited to, oral mucositis, increased infection rates and diarrhea [2]. Currently, there is no prophylactic intervention available for CIGT and its severity varies widely across patients receiving

identical therapy. Thus there is an urgent need to be able to accurately predict which patients may develop CIGT and its severity. In light of our recent preclinical and clinical research [3,4], we propose that mediators in the Toll-like receptor 4 (TLR4) signaling pathway may provide specific predictive markers of CIGT risk and severity.

Key mediators of CIGT: TLR4 & proinflammatory cytokines

Homeostasis of the GI tract is carefully balanced by interactions between the resident microflora, epithelial barrier function and the mucosal immune system [5]. Data from our laboratories have demonstrated that CIGT disrupts the delicate balance of these three factors, leading to a substantial inflammatory response, changes in epithelial turnover and changes in microflora characteristics [6]. TLR signaling is a key component of the host-immune response, translating signals from the gut to activate innate immune responses, as well as providing links to the adaptive immune system. Our laboratory has provided compelling

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data indicating that TLRs, in particular TLR4, modulate the pathology of CIGT [3,4]. TLR4 detects both pathogens and endogenous danger signals [7], of which, the best characterized agonist is lipopolysaccharide [8], that is present throughout the GI tract. When lipopolysaccharide binds to TLR4 in subepithelial locations, a profound inflammatory signal via the TLR pathway occurs; the end result being upregulation of proinflammatory cytokines (such as TNF, IL-1 β and IL-6) [9].

We believe that TLR4 activation occurs during multiple events during CIGT. Specifically we believe that there is both direct and 'off-target' activation of TLR4 by chemotherapeutic agents. *In silico* docking studies have shown that chemotherapies including irinotecan and SN-38, (the major toxic metabolite of irinotecan in humans), are able to bind directly to the accessory protein of TLR4, MD2, with high specific energy [HUTCHINSON MR, PERS. COMM.]. We also believe that indirect activation of TLR4 will occur as a result of increased exposure to bacterial influx due to compromised gut barrier integrity. In support of this, our research has shown significant epithelial damage during CIGT occurrence in response

to increased apoptosis and subsequent rebound hyperplasia resulting in changed epithelial barrier integrity [10]. Importantly, the time course of epithelial damage correlates with that of CIGT development observed clinically [11], highlighting the possible connection between the two events. Finally we believe that TLR4 activation will also occur from increased inflammatory mediators in a positive feedback fashion. We know that over the time course of CIGT, there is increased production of proinflammatory cytokines, in particular TNF, IL-1 β and IL-6, and NF- κ B, a key transcriptional regulator of these proinflammatory cytokines in the gastrointestinal mucosa [12]. These are excellent markers of, and play an important role in, the pathogenesis of the chemotherapy-induced inflammatory response and CIGT.

TLR4 pathway genetic variability impacts on CIGT & may be an excellent predictive marker

We know that the risk of cancer patients' developing CIGT depends on several variables, some of which are related to drug treatment. For example, CIGT incidence and severity is altered depending on whether the patient receives chemotherapy alone, or receives chemotherapy in combination with radiotherapy and/or the newer targeted therapies [13,14]. Other variables are related to each patient individually. For example, each individual's oral hygiene, cancer diagnosis and importantly genetic factors play key roles. Much of the previous research in this area has focused on toxicity risk associated with variability in drug-metabolizing enzyme genes that determine chemotherapeutic concentrations [15–17]. However, we believe that with the emerging role of patient innate immunity in CIGT, the variability of genes encoding mediators of the TLR4/IL-1 β pathway are critically important markers of risk. In support of our position are studies documenting associations between proinflammatory cytokine genotypes and CIGT and other types of chemotherapy toxicity. For example, a promoter variant of the *IL-1B* gene, which is known to increase IL-1 β expression was more highly expressed in patients with thrombocytopenia after treatment with chemotherapy [18]. In the same group of patients, a promoter variant of *TNFA*, which alters TNF expression was associated with CIGT [18]. Similar associations between *TNFA* and *TNFB* variants, again which alter TNF- α expression, and subsequent toxicity severity have been reported in

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patients receiving high-dose chemotherapy prior to stem-cell transplantation [19].

To prove our hypothesis that the risk of developing CIGT depends on TLR pathway genetic variability our laboratory conducted a small retrospective patient study [4]. Using TLR pathway genetic variability we successfully predicted severe CIGT risk in patients treated with 5-fluorouracil-based therapies [4]. Specifically, 34 patients (ten with severe toxicity) were genotyped for 21 loci in TLR pathway mediators and a general linear model (multivariate logistic regression) was generated incorporating genetic and demographic covariates. The final model combining genetic variability in *TLR* (*TLR2*, dimerizes with *TLR4* and uses the same downstream pathway) and *TNF* together with cancer type (gastric and colorectal) could predict up to 87% of severe CIGT risk. Importantly, this is the first clinical study to directly link TLR genetic variability with severe CIGT and hence demonstrate the likelihood that preclinical observations linking TLR with CIGT are similar in the clinical setting.

Blockade of TLR4 signaling to improve clinical management of CIGT

In order to confirm TLR4 signaling is the key predictor of risk and severity for CIGT we need to establish that blocking the pathway will lead to reduced incidence of toxicity. In a small study using TLR4 knockout mice, our laboratory demonstrated that TLR4 plays a key role in the development of CIGT. Following irinotecan, a chemotherapy agent known to cause significant

CIGT, TLR4 knockout mice had reduced clinical symptoms compared with wild-type counterparts. Specifically these mice had significant reductions in diarrhea ($p < 0.0001$) and weight loss ($p < 0.021$) and had no increase in mucosal tissue injury scores compared with wild-type mice who had significantly increased mucosa tissue injury ($p < 0.002$) [3]. These observations align with a TLR2 knockout study that demonstrated protection against doxorubicin-induced CIGT [20]. The next step to prove this theory is to block TLR2/4 signaling with an appropriate pharmacological agent that is safe to use in cancer patients.

Conclusion

We have provided strong preclinical and the first clinical evidence to suggest that TLR4 is perfectly positioned to be an accurate predictive marker of CIGT risk and severity. This strengthens the push to personalize medicine for all cancer patients.

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