

## PUBLISHED VERSION

Sultani, Masooma; Stringer, Andrea Marie; Bowen, Joanne Marie; Gibson, Rachel Jane. Anti-inflammatory cytokines: important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. *Chemotherapy Research and Practice*, 2012:490804

Copyright © 2012 Masooma Sultani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### PERMISSIONS

<http://www.hindawi.com/journals/chrp/guidelines/>

### Copyright

Open Access authors retain the copyrights of their papers, and all open access articles are distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided that the original work is properly cited.

The use of general descriptive names, trade names, trademarks, and so forth in this publication, even if not specifically identified, does not imply that these names are not protected by the relevant laws and regulations.

While the advice and information in this journal are believed to be true and accurate on the date of its going to press, neither the authors, the editors, nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Date URL accessed 6 May 2013

<http://digital.library.adelaide.edu.au/dspace/handle/2440/77237>

## Review Article

# Anti-Inflammatory Cytokines: Important Immunoregulatory Factors Contributing to Chemotherapy-Induced Gastrointestinal Mucositis

Masooma Sultani,<sup>1</sup> Andrea M. Stringer,<sup>2</sup> Joanne M. Bowen,<sup>1</sup> and Rachel J. Gibson<sup>1</sup>

<sup>1</sup> School of Medical Sciences, University of Adelaide, North Terrace, Adelaide, SA 5005, Australia

<sup>2</sup> School of Pharmacy and Medical Sciences, University of South Australia, North Terrace, Adelaide, SA 5001, Australia

Correspondence should be addressed to Rachel J. Gibson, rachel.gibson@adelaide.edu.au

Received 9 May 2012; Revised 25 July 2012; Accepted 25 July 2012

Academic Editor: Vassilios A. Georgoulas

Copyright © 2012 Masooma Sultani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

“Mucositis” is the clinical term used to describe ulceration and damage of the mucous membranes of the entire gastrointestinal tract (GIT) following cytotoxic cancer chemotherapy and radiation therapy common symptoms include abdominal pain, bloating, diarrhoea, vomiting, and constipation resulting in both a significant clinical and financial burden. Chemotherapeutic drugs cause upregulation of stress response genes including *NFκB*, that in turn upregulate the production of proinflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6), and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ). These proinflammatory cytokines are responsible for initiating inflammation in response to tissue injury. Anti-inflammatory cytokines and specific cytokine inhibitors are also released to limit the sustained or excessive inflammatory reactions. In the past decade, intensive research has determined the role of proinflammatory cytokines in development of mucositis. However, a large gap remains in the knowledge of the role of anti-inflammatory cytokines in the setting of chemotherapy-induced mucositis. This critical paper will highlight current literature available relating to what is known regarding the development of mucositis, including the molecular mechanisms involved in inducing inflammation particularly with respect to the role of proinflammatory cytokines, as well as provide a detailed discussion of why it is essential to consider extensive research in the role of anti-inflammatory cytokines in chemotherapy-induced mucositis so that effective targeted treatment strategies can be developed.

## 1. Introduction

Cancer patients receiving chemotherapy and/or radiation therapy often develop mucositis as a direct result of their treatment. The term “mucositis” specifically refers to the damage of mucous membranes throughout the entire gastrointestinal tract (GIT) following chemotherapy and radiotherapy [1–3]. It is a major oncological problem reported in approximately 40% of patients undergoing standard dose chemotherapy and in almost all patients receiving high-dose chemotherapy and stem cell transplantation [4–7]. The prevalence of mucositis also varies depending on the type of cancer and therefore the combination of cytotoxic drugs [8, 9]. For example, patients treated with 5-fluorouracil (5-FU), often experience more severe mucositis [9]. Patients with

mucositis exhibit severe clinical symptoms including pain due to ulceration of the GIT, nausea, vomiting, heartburn, diarrhoea, constipation, and therefore severe weight loss [1, 4, 7]. Furthermore, ulceration of the GIT is commonly associated with a high risk of systemic infection which poses a threat to patient health [10]. Mucositis can result in unplanned treatment interruptions including dosage reduction or premature cessation of cancer treatment [1, 3, 7]. Patients may require prolonged hospitalization and administration of antibiotic, antiviral therapy, or antifungal drugs depending on the severity of the condition [9, 10]. Currently, management of mucositis is largely supported with treatment limited to pain relief, maintenance of good oral hygiene, and the use of loperamide (a nonanalgesic opioid) to treat diarrhoea [11]. Hence, mucositis is a major

clinical and economic burden that severely impacts patients' quality of life and increases their risk of morbidity and mortality [12]. Within the previous decade, intense research has clarified the complex sequence of molecular events underlying the pathobiology of mucositis and the development of novel treatments and strategies in the management of mucositis.

## 2. Pathobiology of Mucositis

The pathobiology of mucositis is complex and involves the interplay of multiple intricate pathways including molecular and cellular events that occur in all layers of the gastrointestinal mucosa [13]. Historically, it was assumed that mucositis development was simply an epithelial phenomenon and occurred due to the direct toxic effect of cytotoxic chemotherapeutic agents on the basal cells of the gastrointestinal tract epithelium [9, 14, 15]. However, recent investigations have clearly identified that mucositis development is complicated with the involvement of molecular pathways between all mucosal compartments [8, 9, 15–17]. Initially, mucositis development was proposed in a four-stage model by Sonis in 1998 [18]. However subsequent studies have further extending this model into a five stage model comprising of the (1) *initiation*, (2) *upregulation and message generation*, (3) *signalling and amplification*, (4) *ulceration and inflammation*, and (5) *healing phase* [9, 14, 19, 20].

Briefly, the initiation phase occurs immediately following exposure to cytotoxic therapy resulting in direct damage to cellular DNA leading to immediate cell injury or death in the basal epithelia and submucosal cells [9, 13]. Furthermore, extensive tissue injury can result in the generation of reactive oxygen species (ROS). ROS are known to cause damage to cells and tissues, stimulate macrophages, and trigger a cascade of inflammatory pathways including the SP1-related retinoblastoma control protein, p53, and the nuclear factor kappa-B (NF- $\kappa$ B) inflammatory pathway which is described in detail later in this paper [14, 21, 22].

The upregulation and message generation phase involves the activation of a number of signalling pathways and transcription factors, most importantly NF $\kappa$ B [13, 23]. NF $\kappa$ B in turn mediates gene expression and synthesis of various inflammatory molecules including proinflammatory cytokines such as tumour-necrosis factor (TNF), interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), adhesion molecules, and cyclooxygenase-2 (COX-2) from adjacent connective tissue [21, 22, 24]. The initiation of this inflammatory cascade has been confirmed in the clinical setting where studies have demonstrated the presence of these inflammatory factors in peripheral blood samples of chemotherapy patients [24, 25].

Signal amplification is the third phase of mucositis development where the inflammation signal is further amplified as a consequence of proinflammatory cytokines such as TNF acting in a positive feedback loop to reinforce NF $\kappa$ B activation [13, 18, 21]. The net effect of this amplified inflammatory positive feedback loop is enhanced production of proinflammatory cytokines (TNF, IL-6, and IL-1 $\beta$ ) and further tissue damage as a result of increased apoptosis.

Mucositis is clinically evident during the fourth ulcerative phase where the gastrointestinal epithelium integrity is destroyed. The GIT epithelium is characterised by the formation of pseudomembranes and ulcers [13, 15, 18, 21]. Bacterial colonisation at the site of ulceration further induces inflammation and activates the infiltration of macrophages and other inflammatory cells to the site of tissue injury [26].

The final healing phase occurs within approximately two weeks following cessation of treatment [21] and is a spontaneous self-resolving process where the intestinal epithelium is renewed. Although it remains the least understood stage of mucositis, it is thought that COX-2 may play an important “rebuilding” role in the healing phase by initiating angiogenesis which is predominantly seen in this phase [21, 27].

## 3. Inflammatory Signalling Pathways in Mucositis

*3.1. The Role of Cytokines in Tissue Inflammation.* Cytokines are pleiotropic endogenous inflammatory and immunomodulating mediators that exhibit both negative and positive regulatory effects on various target cells [28–30]. These cell-derived polypeptides closely orchestrate both acute and chronic inflammatory processes by acting locally or systemically on the site of tissue infection via autocrine and paracrine pathways [30]. Briefly, inflammation at the site of infected tissue arises from the activation of various resident inflammatory cells such as fibroblasts, endothelial cells, tissue macrophages, and mast cells as well as the recruitment of monocytes, lymphocytes, and neutrophils [30]. This aggregation of inflammatory cells at the site of inflammation is initiated by a number of soluble mediators such as cytokines, inflammatory lipid metabolites such as platelet activating factor (PAF), and derivatives of arachidonic acid such as prostaglandins [30]. Such inflammatory effects can give rise to swelling due to fluid accumulation, increased blood flow and vascular permeability resulting in redness, and pain [30]. As inflammation closely correlates with the production of cytokines, inflammatory events that occur during mucositis development have also been thought to be associated with the generation of cytokine signalling cascade [29].

*3.2. Nuclear Factor  $\kappa$ B (NF $\kappa$ B): A “Gate-Keeper” Secondary Messenger.* Evidence from previous literature clearly describes the role of nuclear factor  $\kappa$ B (NF $\kappa$ B) transcription factor in the development of mucositis [8, 9, 31, 32]. NF $\kappa$ B is a ubiquitous transcription factor that collectively comprises of the following five members: NF- $\kappa$ B1 (p50/p105), NF- $\kappa$ B2 (p52/p100), p65 (Rel A), Rel 3, and cRel [9, 33]. It typically resides as an inactive heterodimer of p65/RelA and p50 or p52 subunits bound to the members of I $\kappa$ B (Inhibitor kappa B) proteins in the cell cytoplasm [32, 34]. Upon activation by an extensive range of agents such as bacteria and bacterial cell wall products, viruses, cytokines, free radicals scavengers from oxidative stress, ionizing radiation,

and even the use of antineoplastic agents (cisplatin, doxorubicin, taxol, paclitaxel, and etoposide), NF $\kappa$ B acts to induce gene expression of many cytokines involved predominantly in mucosal inflammation, and angiogenesis, chemokines, immunoreceptors, cell adhesion molecules, proapoptotic and antiapoptotic as well as stress response genes [9, 31–33]. On a molecular level, NF $\kappa$ B stimulation signals cause the phosphorylation of NF $\kappa$ B inhibitory protein I $\kappa$ B, located in cell cytoplasm [35]. This results in the dissociation of bound NF $\kappa$ B from I $\kappa$ B which is subsequently translocated to the cell nucleus where it upregulates the expression of approximately 200 genes [8]. I $\kappa$ B is further degraded by proteases [14].

In the past decade, much attention has been given to the diverse roles NF $\kappa$ B plays in generating tissue response by targeting a wide range of genes [8, 34]. Its role in potentiating inflammatory and immune responses by inducing various proinflammatory cytokines production such as TNF, IL-6 and IL-1 $\beta$  involved in the development of mucositis has been widely recognised with the use of animal models and in the clinical setting [5, 34]. NF $\kappa$ B is considered a key “driver” of chemotherapy-induced mucositis as its activation correlates with the production of TNF, IL-6 and IL-1 $\beta$  the hallmarks of mucositis inflammation [8, 36]. In particular, cytotoxic drug administration results in the upregulation of NF $\kappa$ B and subsequently proinflammatory cytokine (TNF, IL-6, and IL-1 $\beta$ ) levels [8, 37, 38]. In further support of this, Logan et al. (2008) reported a significant rise in serum NF $\kappa$ B, TNF, IL-6, and IL-1 $\beta$  levels following administration of three different chemotherapeutic drugs known to cause mucositis [37]. Irinotecan, a commonly used cytotoxic agent, has also been shown to significantly elevate NF $\kappa$ B production in the oral mucosa, jejunum, and colon [8, 34]. This elevation ultimately culminates in villus blunting, epithelial atrophy, and increased inflammatory cell infiltration in all tissues [8]. Further research clearly indicates that NF $\kappa$ B activation is stimulated by chemotherapeutic agents thus leading to the production of proinflammatory cytokines, TNF, IL-6, and IL-1 $\beta$ , resulting in mucosal damage [8, 37, 38].

In support of the animal studies, clinical evidence is also available. Research from our group has further demonstrated a significant rise in tissue NF $\kappa$ B levels in the oral buccal mucosa of cancer patients undergoing chemotherapy [34]. Yeoh et al. (2005) published data correlating histopathological changes with increased NF $\kappa$ B and COX-2 expression in the colonic mucosa of patients treated with ionizing radiation [5]. In addition, ionizing radiation-induced NF $\kappa$ B activation has been reported in other studies where radiation is known to cause the generation of oxygen free radicals, damage to cellular components, and breakage of the DNA double strands [39–41]. These inflammatory markers are essential in the pathogenesis of mucositis development and therefore in assessing the severity of tissue damage.

#### **4. Proinflammatory Cytokines and Inflammation**

As described earlier, the activation of NF $\kappa$ B results in the production of proinflammatory mediators such as TNF, IL-6, and IL-1 $\beta$  [8, 9, 18, 21, 28]. This subclass of cytokines

is referred to as “*proinflammatory cytokines*” due to their ability to promote inflammation in response to tissue injury and infection [42]. Another subclass of cytokines is the “*anti-inflammatory cytokines*” which are involved in suppressing the activity of proinflammatory cytokines hence downregulating the inflammatory response [43]. Overexpression of anti-inflammatory cytokines is known to lead to depression of the immune system thus rendering the host at risk of systemic infection [43, 44]. Previous studies have directly implicated the presence of TNF, IL-6, and IL-1 $\beta$  proinflammatory cytokines in the pathogenesis of a number of inflammatory diseases, such as inflammatory bowel disease (IBD) [45], rheumatoid arthritis [46], sepsis [47], and most importantly, in mucositis [8, 13, 21, 34]. The role of proinflammatory cytokines has been discussed in detail in other critical reviews and is outside the scope of this paper. For further details please refer to Logan Review [8]. However the role of anti-inflammatory cytokines is discussed in detail below.

#### **5. Anti-Inflammatory Cytokines and Inflammation**

Under normal physiological conditions, the human immune system comprises of multiple redundant pathways and immunoregulatory control elements that act in concert to coordinate the immune response initiated upon an external signal [43]. Of these multifaceted components of the immune system, the anti-inflammatory cytokine component has been subject to in-depth research for years. Studying the regulation of inflammation by these cytokine inhibitors is complicated as a number of related external factors must be considered for appropriate understanding and analysis of net effect of these cytokines. It has been postulated that factors such as timing of cytokine release, local environment in which it acts, presence of competing or synergistic elements, nature of target cells, availability and density of specific cytokine receptors, and tissue-specific response to each cytokine determine their net effect [43]. In addition, the discovery of various proinflammatory cytokines has further added to the complexity of the intricate pathways that occur during an immune response. Collective findings from a wide range of cytokine investigations indicate that the net effect of the inflammatory response is determined by a delicate balance between pro- and anti-inflammatory cytokines [43] as shown in Figure 1. Perturbations in this equilibrium can drive the host defence immune response either towards chronic inflammation or towards healing [43]. To date, various anti-inflammatory cytokines have been acknowledged in literature and these include IL-1ra, IL-4, IL-6, IL-10, IL-11, IL-13, TGF- $\beta$ , and various soluble cytokine receptors [43]. This literature paper will address four of these anti-inflammatory cytokines in detail: IL-4, IL-10, IL-11, and IL-1ra.

**5.1. Interleukin-4 (IL-4).** IL-4 is a 20-kDa polypeptide secreted by mature Th-2 type helper T cells, mast cells, and basophils [48, 49]. IL-4 has marked inhibitory effects on

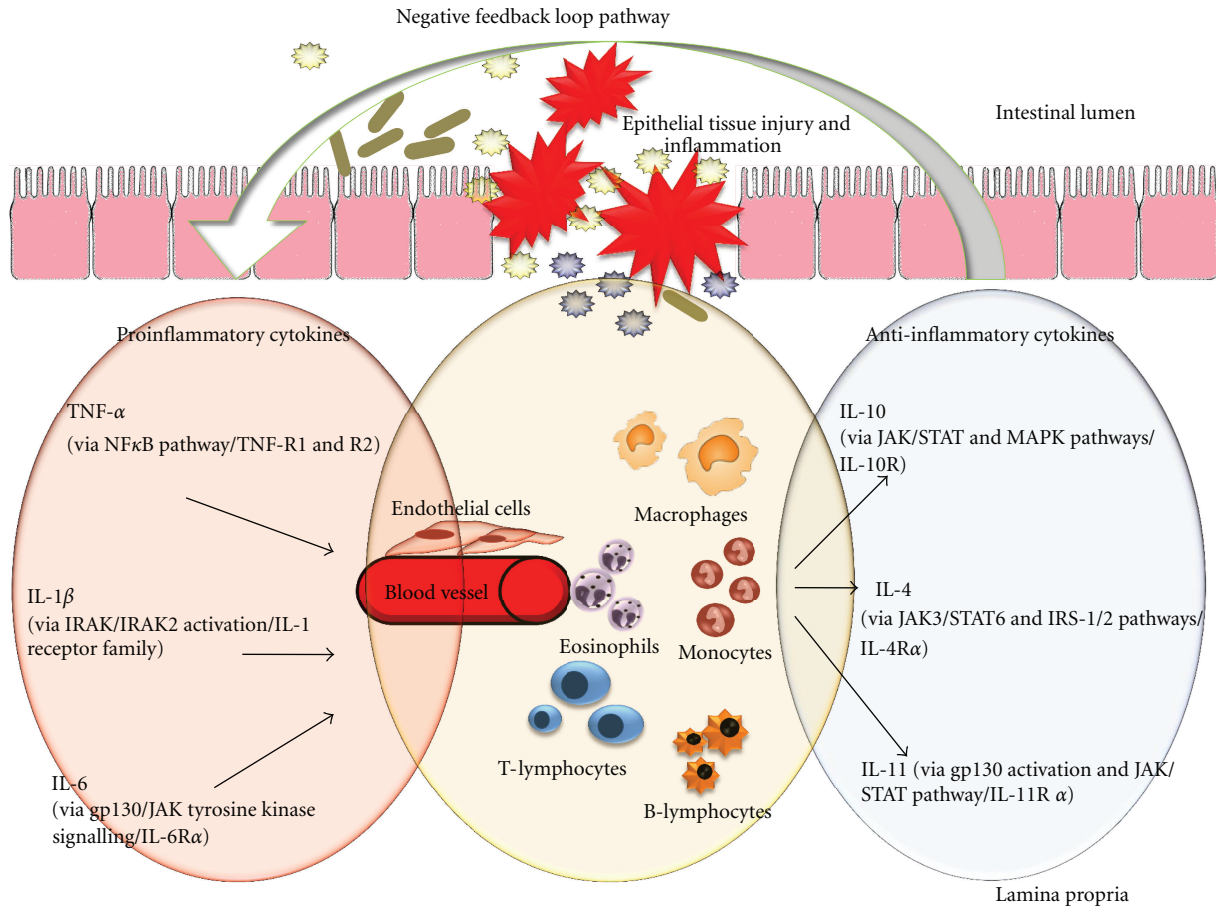


FIGURE 1: Possible negative feedback mechanism between pro- and anti-inflammatory cytokines IL-10, IL-4, and IL-11 following intestinal damage and inflammation.

the expression and release of proinflammatory cytokines [43]. Molecular and structural analysis of the IL-4 receptor has identified the 140-kDa IL-4 $\alpha$  chain where high-affinity binding with IL-4 occurs and further dimerization of this complex with the  $\gamma$ -chain mediates intracellular cell signalling [50, 51]. These cell surface IL-4 receptor complexes have been discovered in an extensive range of tissues including hematopoietic, endothelial, epithelial, muscle, fibroblast, hepatocytes, and brain tissues thus accounting for their broad range of activity [50].

IL-4 suppresses IL-1 $\beta$  synthesis, a major proinflammatory cytokine involved in inducing inflammation [52]. Furthermore, IL-4 enhances the expression of IL-1 receptor antagonist (IL-1ra), an antagonist that blocks the binding of proinflammatory cytokines, IL-1 $\alpha$  and IL-1 $\beta$ , to their specific receptors [52, 53]. By mediating its action through the widely expressed IL-4 receptor  $\alpha$  (IL-4 $\alpha$ ), IL-4 plays an important role in tissue adhesion and inflammation as well as potentiates immunological effects against gram-negative bacterial infections [54, 55]. This highlights the diverse complex biological effects of IL-4 in mediating immunity through a unique array of cellular responses. Although evidence suggests that IL-4 has potential anti-inflammatory effects, its role during mucositis remains undefined. To date, there is

no evidence to suggest whether IL-4 is produced at tissue levels in mucositis and if it is upregulated during different stages of mucositis. Thus it is obligatory for forthcoming studies to address these issues in order to develop better understanding of the anti-inflammatory properties of IL-4 during inflammation in mucositis.

**5.2. Interleukin-10 (IL-10).** IL-10 is the central anti-inflammatory cytokine well researched in the pathogenesis of Inflammatory Bowel Disease (IBD). Active IL-10 is secreted by CD4<sup>+</sup> Th2 cell, Treg, monocyte, and macrophage cells of the immune system [56, 57]. Figure 2 shows the IL-10 receptor activation that induces a wide range of inflammatory controlling genes during tissue injury.

IL-10 controls inflammatory processes by suppressing the expression of proinflammatory cytokines, chemokines, adhesion molecules, as well as antigen-presenting and costimulatory molecules in monocytes/macrophages, neutrophils, and T cells [57, 58]. Early *in vitro* studies demonstrated IL-10 suppresses monocytes/macrophage-derived proinflammatory cytokines such as TNF- $\alpha$ , IL-1, IL-6, IL-8, and IL-12 [59, 60]. Additional studies support the notion that IL-10 attenuates TNF-receptor expression and further

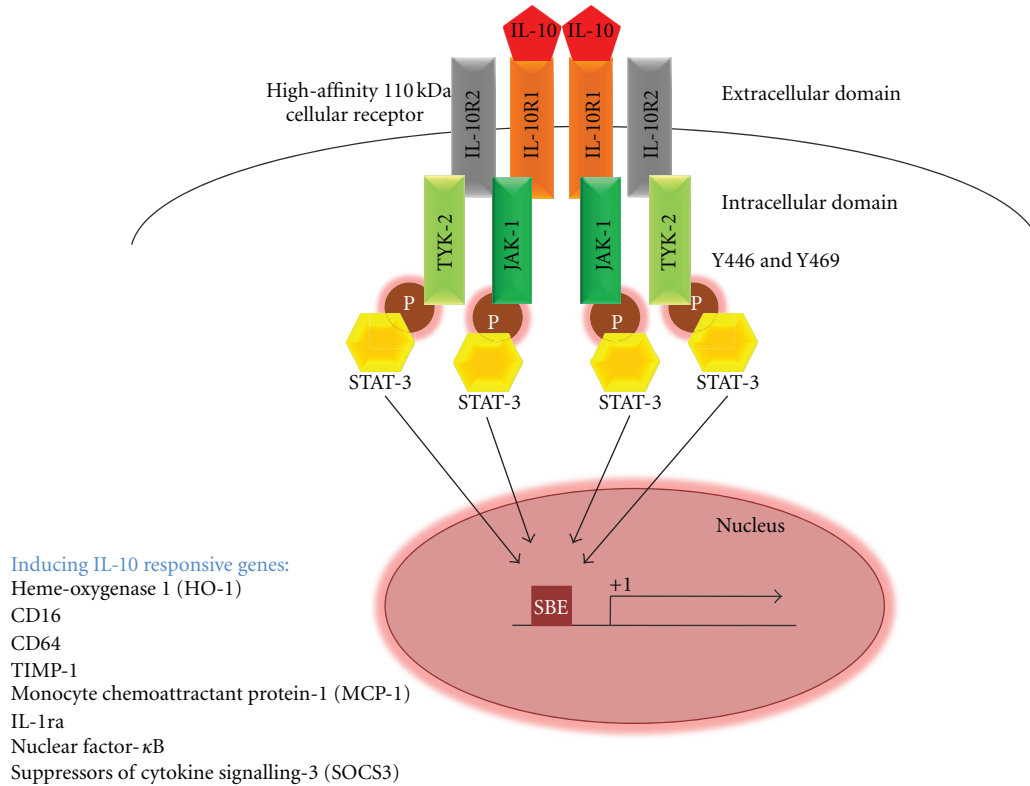


FIGURE 2: Diagram illustrating the JAK-STAT signal transduction pathway activated by anti-inflammatory cytokine, Interleukin-10. IL-10 binds its high-affinity extracellular IL-10 receptors in a homodimeric form which leads to intracellular receptor-associated Janus tyrosine kinases, JAK-1 and TYK-2. These activated kinases serve a temporary docking-sites for the cytosolic STAT-3 via their phosphorylated SH-2 domain. Activated STAT-3 translocates to the nucleus and binds to STAT Binding Element (SBE) to promote a wide range of IL-10 responsive genes.

promotes its shedding into systemic circulation [61, 62]. Together these findings indicated IL-10 is an important immunoregulatory factor that significantly contributes to decreasing the intensity of inflammatory response by down-regulating proinflammatory cytokine production at the site of tissue damage.

In an attempt to report the effect of IL-10 on NF $\kappa$ B, *in vitro* analysis by Clarke and Colleagues (1998) showed that IL-10 is capable of inhibiting the activation of LPS-induced NF $\kappa$ B in macrophages and pre-B cells [61]. This study supports the evidence that IL-10 mediates anti-inflammatory effects by inhibiting the up-stream NF $\kappa$ B transcription factor, an essential secondary messenger required for inducing proinflammatory cytokine gene expression.

In the pathogenesis of IBD, the potent immunosuppressive effects of IL-10 have been highlighted in several studies. The IL-10 knockout mouse model has successfully portrayed spontaneous development of chronic inflammatory enteritis, a condition similar to IBD in humans, suggesting that endogenous IL-10 is a central regulator of the mucosal immune response [63, 64]. Further dysregulation of the ratio of pro/anti-inflammatory cytokines, IL-1 $\beta$ /IL-1ra, has been associated with IL-10 administration in mucosal biopsies of UC patients [65]. In addition, *in vitro* analysis by Schreiber et al. (1995) also demonstrated that IL-10 downregulates the

enhanced production of proinflammatory cytokines from IBD mononuclear phagocytes [66]. Thus, low production of IL-10 anti-inflammatory cytokine in the mucosa of IBD patients has been regarded as an important factor in the pathogenesis of IBD. Such data in the pathogenesis of mucositis lacks and investigation is warranted. Essentially recognised as an inflammatory condition, the highly complex and interactive nature of mucositis pathobiology strictly limits our approach towards targeting an appropriate molecular pathway. This evidence strongly supports the notion that IL-10 is in fact a crucial cytokine with anti-inflammatory properties that remains to be investigated in the setting of chemotherapy-induced mucositis.

**5.3. Interleukin-11 (IL-11).** IL-11 is a well-known pleiotropic cytokine. Physiological levels of IL-11 expression is identified in a wide range of normal adult murine tissues including thymus, spleen, bone marrow, heart, lung, small and large intestine, kidney, brain, testis, ovary, and uterus [67, 68]. IL-11 functions to control inflammation, ameliorate tissue damage, and maintain cytokine haemostasis during infection by acting on various cell types including hematopoietic precursor cells, macrophages, adipocytes, epithelial, and T cells [69, 70]. IL-11 mediates its biological activities by

binding to its low affinity IL-11 $\alpha$  receptor (IL-11R $\alpha$ ) and subsequently complexing with the gp130 signal transduction subunit commonly shared with IL-6 receptor [70–72].

There is much evidence highlighting the anti-inflammatory properties of IL-11 in duration of an inflammatory response. Trepicchio et al. (1996) used mouse peritoneal macrophages and effectively demonstrated that rhIL-11 reduced the production of a wide spectrum of proinflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-12, and NO [73]. Furthermore, administration of rhIL-11 in mice reduced proinflammatory cytokine production during a systemic inflammation [73]. In a follow-up study to elucidate the molecular mechanisms of IL-11 activity, *in vitro* analysis in LPS-stimulated peritoneal macrophages by Trepicchio et al. (1997) illustrated that rhIL-11 blocked nuclear translocation of NF $\kappa$ B transcription factor and showed increase cytoplasmic levels of the NF $\kappa$ B inhibitory proteins, I $\kappa$ B- $\alpha$  and I $\kappa$ B- $\beta$  [74]. In addition, mRNA detection of the IL-11R $\alpha$  and gp130 complex in human and murine CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes suggests that IL-11 enhances the direct production of T-cell-derived anti-inflammatory cytokines such as IL-4 and IL-10 [76]. Together, these findings clearly establish the immunoregulatory pleiotropic properties of IL-11 in substantiating inflammatory responses during cytokine-derived tissue injury.

Due to its potent anti-inflammatory nature, IL-11 has been under thorough investigation in several animal models exhibiting inflammatory conditions such as gut microorganism-induced sepsis [77], chronic IBD [78], and ischemic bowel necrosis [79]. As a multifunctional cytokine, IL-11 acts to attenuate the production of proinflammatory cytokine in correlation with this ability to reduce inflammation [75]. Flow cytometric analysis by Trepicchio and Colleagues revealed that administration of rhIL-11 *in vitro* successfully attenuated the production of IL-1 $\beta$  and TNF- $\alpha$  from activated peritoneal macrophages [73]. Furthermore, rhIL-11 treatment also reduced serum levels of LPS-induced IFN- $\gamma$ , a well-known proinflammatory cytokine that enhances inflammation through further activation of macrophages [73, 80].

In the context of gastrointestinal inflammation, IL-11 has been of particular interest due to its anti-inflammatory and mucosal protective effects. In a rat model of colitis, Peterson et al. (1998) demonstrated that rhIL-11 treatment ameliorated the development of colitis by downregulating the production of proinflammatory cytokines as well as maintaining the trophic structure of the gastrointestinal epithelium [81]. Recombinant human IL-11 enhances recovery from mucosal injury after cancer chemotherapy treatment. Gibson and Colleagues investigated the effect of IL-11 on ameliorating mucositis in a rat model implanted with syngeneic breast cancer following chemotherapy [49]. It was concluded that IL-11 has significant protective trophic effects on the intestinal epithelium as chemotherapy-related damage of the villous atrophy and crypt length damage was less severe. Also, IL-11 did not produce protective effects on the breast cancer tissue in this rat model of mucositis further highlighting its efficacy and safety upon administering an effective dose [49]. In 1995, Sonis and Colleagues primarily

established that IL-11 decreases the severity and duration of mucosal inflammation by protecting the intestinal epithelium and connective tissue using a hamster model of oral mucositis [82]. Liu et al. (1996) also demonstrated that IL-11 significantly increased villus height and the rate of crypt cell mitosis in the rat model of short bowel syndrome [83]. The IL-11 receptor- $\alpha$  (IL-11R $\alpha$ ) is expressed within the gastrointestinal epithelium and colonic epithelial cells of the mucosa. *In vitro* studies have confirmed that IL-11 directly interacts on untransformed IEC-18 epithelial cells to inhibit cellular proliferation [84, 85]. Although IL-11 is well researched in a number of different inflammatory conditions, current literature lacks to understand the direct mechanism of IL-11 on the intestinal epithelium relating to ameliorating chemotherapy-induced mucositis.

**5.4. Interleukin-1 Receptor Antagonist (IL-1ra).** IL-1 receptor antagonist is a naturally synthesised and secreted 23–25-kDa glycosylated protein produced primarily by monocytes, macrophages, neutrophils, microglial cells, hepatocytes, and many other cells in response to tissue injury, infection, and inflammation [86, 87].

Extensive molecular research has established the central biological role of IL-1ra as a highly competitive antagonist of its functional proinflammatory ligands, IL-1 $\alpha$  and IL-1 $\beta$ . For many years, these isoforms of the IL-1 cytokine family have been recognised to participate in initiating and amplifying inflammation upon induced tissue injury and infection. Amongst its various pleiotropic localised and systemic effects, IL-1 cytokines are known to promote inflammatory cell infiltration at site of tissue injury, induce fever and vascular dilation, promote NO, COX-2, and prostaglandin E<sub>2</sub> production, and induce production of other cytokine mediators such as IL-6 [88].

Evidence from previous independent studies have identified that IL-1 cytokines, in particular IL-1 $\beta$ , play crucial roles in the pathogenesis of various gastrointestinal tract associated inflammatory conditions such as IBD, ischemic-reperfusion injury, chronic enteritis, and irritable bowel syndrome (IBS) [89].

An *in vitro* study by Al-Sadi and Ma (2007) has additionally highlighted the role of IL-1 $\beta$  on gastrointestinal tract epithelial obliteration by successfully showing that at a dose of 10 ng/mL, IL-1 $\beta$  effectively increased tight-cell junction permeability in the Caco-2 cell line 48 hours following treatment [89]. This data provides strong evidence that elevated IL-1 $\beta$  levels during intestinal injury further amplifies inflammation by disrupting epithelial barrier and increasing paracellular permeation of toxic luminal agents into the mucosa [89].

Furthermore, Andus et al. (1997) proposed a lower IL-1ra to IL-1 ratio in inflamed mucosa samples from patients with Crohn's Disease (CD) highlighting the importance of localised tissue IL-1ra presence to downregulate excessive inflammation [90]. IL-1ra animal models have also been utilised in previous investigations to establish a clear understanding of the role of IL-1ra during inflammation. *IL-1ra* gene knockout mice are known to be highly susceptible to

suffering from endotoxemia and develop spontaneous joint inflammation, arthritis thus resulting growth deficit [91].

In recent years, scientific research has shifted focus towards determining the anti-inflammatory role of IL-1ra during mucositis development. Preliminary microarray data provided by Wu et al. (2011) identified the *IL-1ra* gene to be highly expressed in correlation with increased serum levels of IL-1ra after 5-FU treatment [92]. In a tumour-bearing mouse model of 5-FU-induced mucositis, exogenous application of IL-1ra significantly reduced intestinal crypt cell apoptosis and severity of diarrhoea without affecting 5-FU-induced tumour regression [93]. Further comprehensive analysis of the role of IL-1ra is necessary to validate its protective anti-inflammatory effects in the context of chemotherapy-induced mucositis prior to its application in a clinical setting.

## 6. Tregs and Cancer

Regulatory T cells, more commonly referred to as Tregs, are responsible for the induction and maintenance of peripheral tolerance, a critically important function enabling the body to suppress immune responses by influencing different cell type activity [94]. Tregs make up approximately 10% of thymus-derived CD4+ T cells, coexpressing the CD25 antigen (IL-2R  $\alpha$ -chain), and requiring Fox3p transcription factor expression for suppressive phenotype [95]. Two main types of Tregs exist, natural and peripherally-induced, with natural Tregs primarily responsible for controlling immune responses to autoantigens [94], and induced Tregs inhibiting inflammation [94, 96]. Both types of Tregs contribute to overall tumour tolerance in many cancers including, but not limited to pancreatic, [97] ovarian [98], melanoma [99], and renal cell carcinoma [96, 100]. Given the importance of Tregs in tumour tolerance, Tregs, have gained increased recognition in cancer patients and treatments. Wolf and Colleagues (2003) examined the Treg levels in peripheral blood samples of 42 cancer patients and 34 healthy controls and found cancer patients had increased numbers of Tregs compared with controls. Importantly, these increases were associated with immunosuppression. The authors suggested that the increase in Tregs in cancer patients may negatively impact the effectiveness of immunotherapies including monoclonal antibody therapy [101].

Most relevant to this paper, one of the immune suppressive mechanisms by which Tregs maintain immune homeostasis is through secretion of the anti-inflammatory cytokines IL-10 and TGF $\beta$  [102]. Importantly, IL10 is required to maintain immune homeostasis in the gut, where Treg-specific deletion of IL-10 leads to colitis in mice [103]. Since evidence shows that Th1 immune responses simultaneously stimulate Treg induction [102], prevention of inflammation should theoretically be an effective means of limiting Treg-produced anti-inflammatory cytokines.

*6.1. Therapy Options for Tregs.* A number of clinical studies have been reported in recent years examining the potential use of Tregs as a specific marker of treatment response in a

number of different cancer types. Kaufman and Colleagues conducted a small clinical study to examine the effectiveness of interleukin-2 on Treg responses in metastatic renal cell carcinoma. They enrolled 25 patients and found that although Tregs were elevated in all patients prior to treatment, in patients who achieved stable disease state a 50% reduction in the Tregs was seen. These findings suggest that interleukin-2 therapy is effective in reducing Tregs, although the study was extremely small [100]. These findings are in agreement with an earlier clinical study, whereby 11 patients with renal cell carcinoma vaccinated with DAB<sub>389</sub>IL-2 (recombinant IL-2 diphtheria toxin) had their Tregs eliminated from peripheral cells [95].

Ascierto et al. [99] evaluated the Treg levels in 22 melanoma patients following IFN- $\alpha$  2b therapy and reported that prior to therapy, Treg levels were significantly higher ( $P < 0.001$ ) in melanoma patients compared to healthy controls. Following therapy, Treg levels decreased although this did not reach significance. The authors suggested that it was impossible to draw any conclusions regarding Tregs as a marker to cancer treatment response due to small sample size [99].

There are many other papers which suggest a variety of ways in which Tregs can be “managed” in the context of cancer. These include histone deacetylase inhibitors (which would augment the suppressive function of Tregs) [104], retinoids [105], Dendritic cell vaccines [106], and blocking either Treg effector functions [107] or Treg differentiation [108]. This list is by no means exhaustive, but a more detailed analysis is beyond the scope of this paper.

## 7. Conclusions

In the past decade, active research has been conducted to define the pathogenesis of mucositis and the role of proinflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Evidence of the upregulation of these proinflammatory cytokines coordinated with the extent of mucosal injury in mucositis proves to be valuable. This subclass of cytokines is recognised to play an enormous role during inflammation and tissue damage in response to cytotoxic therapy. There remains, however, a huge gap in the knowledge to recognise whether anti-inflammatory cytokines such as IL-4, IL-10, IL-11, and IL-1ra are essential tools in downregulating the inflammatory response associated with mucositis. Lack of this knowledge which ties pro- and anti-inflammatory cytokines together within the complex yet interesting cytokine milieu leaves an incomplete image of immune response associated with mucositis. Furthermore, there is no evidence in literature that interprets the net balance of the subclass of cytokines in accordance with different phases of mucositis development. Moreover, the underlying mechanisms of action of these anti-inflammatory cytokines in chemotherapy-induced mucositis remain underresearched. Taking into consideration the lack of knowledge of anti-inflammatory cytokines in the setting of chemotherapy-induced mucositis, it is obligatory for future cytokine studies of inflammation to base their research on identifying and interpreting the



interrelationships of anti-inflammatory cytokines in the pathogenesis of mucositis.

## Acknowledgment

Miss M. Sultani was supported by an Australian Postgraduate Scholarship; Drs. A. Stringer and J. Bowen are recipients of NHMRC Postdoctoral Training Fellowships; Dr. R. Gibson received financial support from Cure Cancer and Cancer Australia.

## References

- [1] D. M. K. Keefe, J. Brealey, G. J. Golland, and A. G. Cummins, "Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans," *Gut*, vol. 47, no. 5, pp. 632–637, 2000.
- [2] D. M. K. Keefe, R. J. Gibson, and M. Hauer-Jensen, "Gastrointestinal mucositis," *Seminars in Oncology Nursing*, vol. 20, no. 1, pp. 38–47, 2004.
- [3] D. M. K. Keefe, A. G. Cummins, B. M. Dale, D. Kotasek, T. A. Robb, and R. E. Sage, "Effect of high-dose chemotherapy on intestinal permeability in humans," *Clinical Science*, vol. 92, no. 4, pp. 385–389, 1997.
- [4] A. M. Stringer, R. J. Gibson, J. M. Bowen, R. M. Logan, A. S. J. Yeoh, and D. M. K. Keefe, "Chemotherapy-induced mucositis: the role of gastrointestinal microflora and mucins in the luminal environment," *Journal of Supportive Oncology*, vol. 5, no. 6, pp. 259–267, 2007.
- [5] A. S. J. Yeoh, J. M. Bowen, R. J. Gibson, and D. M. K. Keefe, "Nuclear factor  $\kappa$ B (NF $\kappa$ B) and cyclooxygenase-2 (Cox-2) expression in the irradiated colorectum is associated with subsequent histopathological changes," *International Journal of Radiation Oncology Biology Physics*, vol. 63, no. 5, pp. 1295–1303, 2005.
- [6] S. T. Sonis, "Pathobiology of mucositis," *Seminars in Oncology Nursing*, vol. 20, no. 1, pp. 11–15, 2004.
- [7] D. M. Keefe, M. M. Schubert, L. S. Elting et al., "Updated clinical practice guidelines for the prevention and treatment of mucositis," *Cancer*, vol. 109, no. 5, pp. 820–831, 2007.
- [8] R. M. Logan, R. J. Gibson, J. M. Bowen, A. M. Stringer, S. T. Sonis, and D. M. K. Keefe, "Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis," *Cancer Chemotherapy and Pharmacology*, vol. 62, no. 1, pp. 33–41, 2008.
- [9] R. M. Logan, A. M. Stringer, J. M. Bowen et al., "The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs," *Cancer Treatment Reviews*, vol. 33, no. 5, pp. 448–460, 2007.
- [10] S. Silverman Jr., "Diagnosis and management of oral mucositis," *Journal of Supportive Oncology*, vol. 5, no. 2, pp. 13–21, 2007.
- [11] R. J. Gibson, J. M. Bowen, E. Alvarez, J. Finnie, and D. M. K. Keefe, "Establishment of a single-dose irinotecan model of gastrointestinal mucositis," *Chemotherapy*, vol. 53, no. 5, pp. 360–369, 2007.
- [12] D. M. K. Keefe, "Gastrointestinal mucositis: a new biological model," *Supportive Care in Cancer*, vol. 12, no. 1, pp. 6–9, 2004.
- [13] S. T. Sonis, "A biological approach to mucositis," *Journal of Supportive Oncology*, vol. 2, no. 1, pp. 21–32, 2004.
- [14] J. M. Bowen, R. J. Gibson, A. G. Cummins, and D. M. K. Keefe, "Intestinal mucositis: the role of the Bcl-2 family, p53 and caspases in chemotherapy-induced damage," *Supportive Care in Cancer*, vol. 14, no. 7, pp. 713–731, 2006.
- [15] A. Yeoh, R. Gibson, E. Yeoh et al., "Radiation therapy-induced mucositis: relationships between fractionated radiation, NF- $\kappa$ B, COX-1, and COX-2," *Cancer Treatment Reviews*, vol. 32, no. 8, pp. 645–651, 2006.
- [16] A. M. Stringer, R. J. Gibson, R. M. Logan et al., "Irinotecan-induced mucositis is associated with changes in intestinal mucins," *Cancer Chemotherapy and Pharmacology*, vol. 64, no. 1, pp. 123–132, 2009.
- [17] J. M. Bowen, R. J. Gibson, A. G. Cummins, A. Tyskin, and D. M. K. Keefe, "Irinotecan changes gene expression in the small intestine of the rat with breast cancer," *Cancer Chemotherapy and Pharmacology*, vol. 59, no. 3, pp. 337–348, 2007.
- [18] S. T. Sonis, "Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity," *Oral Oncology*, vol. 34, no. 1, pp. 39–43, 1998.
- [19] C. Scully, J. Epstein, and S. Sonis, "Oral mucositis: a challenging complication of radiotherapy, chemotherapy, and radiochemotherapy: part 1, pathogenesis and prophylaxis of mucositis," *Head and Neck*, vol. 25, no. 12, pp. 1057–1070, 2003.
- [20] S. T. Sonis, L. S. Elting, D. Keefe et al., "Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients," *Cancer*, vol. 100, supplement 9, pp. 1995–2025, 2004.
- [21] S. T. Sonis, "Pathobiology of oral mucositis: novel insights and opportunities," *Journal of Supportive Oncology*, vol. 5, no. 9, supplement 4, pp. 3–11, 2007.
- [22] T. Criswell, K. Leskov, S. Miyamoto, G. Luo, and D. A. Boothman, "Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation," *Oncogene*, vol. 22, no. 37, pp. 5813–5827, 2003.
- [23] T. Lawrence, "The nuclear factor NF-kappaB pathway in inflammation," *Cold Spring Harbor perspectives in biology*, vol. 1, no. 6, Article ID a001651, 2009.
- [24] P. D. Hall, H. Benko, K. R. Hogan, and R. K. Stuart, "The influence of serum tumor necrosis factor- $\alpha$  and interleukin-6 concentrations on nonhematologic toxicity and hematologic recovery in patients with acute myelogenous leukemia," *Experimental Hematology*, vol. 23, no. 12, pp. 1256–1260, 1995.
- [25] C. Ferrà, S. De Sanjosé, D. Gallardo et al., "IL-6 and IL-8 levels in plasma during hematopoietic progenitor transplantation," *Haematologica*, vol. 83, no. 12, pp. 1082–1087, 1998.
- [26] B. Perfetto, G. Donnarumma, D. Criscuolo et al., "Bacterial components induce cytokine and intercellular adhesion molecules-1 and activate transcription factors in dermal fibroblasts," *Research in Microbiology*, vol. 154, no. 5, pp. 337–344, 2003.
- [27] S. T. Sonis, K. E. O'Donnell, R. Popat et al., "The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis," *Oral Oncology*, vol. 40, no. 2, pp. 170–176, 2004.
- [28] K. A. Papadakis and S. R. Targan, "Role of cytokines in the pathogenesis of inflammatory bowel disease," *Annual Review of Medicine*, vol. 51, pp. 289–298, 2000.
- [29] C. A. Dinarello, "Proinflammatory cytokines," *Chest*, vol. 118, no. 2, pp. 503–508, 2000.

- [30] C. A. Feghali and T. M. Wright, "Cytokines in acute and chronic inflammation," *Frontiers in Bioscience*, vol. 2, pp. d12–d26, 1997.
- [31] C. Chen, L. C. Edelstein, and C. G elinas, "The Rel/NF- $\kappa$ B family directly activates expression of the apoptosis inhibitor BCL-x(L)," *Molecular and Cellular Biology*, vol. 20, no. 8, pp. 2687–2695, 2000.
- [32] S. T. Sonis, "The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy," *Critical Reviews in Oral Biology and Medicine*, vol. 13, no. 5, pp. 380–389, 2002.
- [33] S. D. Catz and J. L. Johnson, "Transcriptional regulation of bcl-2 by nuclear factor  $\kappa$ B and its significance in prostate cancer," *Oncogene*, vol. 20, no. 50, pp. 7342–7351, 2001.
- [34] R. M. Logan, R. J. Gibson, S. T. Sonis, and D. M. K. Keefe, "Nuclear factor- $\kappa$ B (NF- $\kappa$ B) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy," *Oral Oncology*, vol. 43, no. 4, pp. 395–401, 2007.
- [35] T. Huxford, S. Malek, and G. Ghosh, "Structure and Mechanism in NF- $\kappa$ B/I $\kappa$ B signaling," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 64, pp. 533–540, 1999.
- [36] P. P. Tak and G. S. Firestein, "NF- $\kappa$ B: a key role in inflammatory diseases," *Journal of Clinical Investigation*, vol. 107, no. 1, pp. 7–11, 2001.
- [37] R. M. Logan, A. M. Stringer, J. M. Bowen, R. J. Gibson, S. T. Sonis, and D. M. K. Keefe, "Serum levels of NF $\kappa$ B and pro-inflammatory cytokines following administration of mucotoxic drugs," *Cancer Biology and Therapy*, vol. 7, no. 7, pp. 1139–1145, 2008.
- [38] R. M. Logan, A. M. Stringer, J. M. Bowen, R. J. Gibson, S. T. Sonis, and D. M. K. Keefe, "Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered?" *Cancer Chemotherapy and Pharmacology*, vol. 63, no. 2, pp. 239–251, 2009.
- [39] M. A. Brach, R. Hass, M. L. Sherman, H. Gunji, R. Weichselbaum, and D. Kufe, "Ionizing radiation induces expression and binding activity of the nuclear factor  $\kappa$ B," *Journal of Clinical Investigation*, vol. 88, no. 2, pp. 691–695, 1991.
- [40] N. Mohan and M. L. Meltz, "Induction of nuclear factor  $\kappa$ B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway," *Radiation Research*, vol. 140, no. 1, pp. 97–104, 1994.
- [41] S. J. Lee, A. Dimtchev, M. F. Lavin, A. Dritschilo, and M. Jung, "A novel ionizing radiation-induced signaling pathway that activates the transcription factor NF- $\kappa$ B," *Oncogene*, vol. 17, no. 14, pp. 1821–1826, 1998.
- [42] Z. Y. Ong, R. J. Gibson, J. M. Bowen et al., "Pro-inflammatory cytokines play a key role in the development of radiotherapy-induced gastrointestinal mucositis," *Radiation Oncology*, vol. 5, no. 1, article no. 22, 2010.
- [43] S. M. Opal and V. A. DePalo, "Anti-inflammatory cytokines," *Chest*, vol. 117, no. 4, pp. 1162–1172, 2000.
- [44] C. A. Gogos, E. Drosou, H. P. Bassaris, and A. Skoutelis, "Pro-versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options," *Journal of Infectious Diseases*, vol. 181, no. 1, pp. 176–180, 2000.
- [45] F. Shanahan and S. Targan, "Medical treatment of inflammatory bowel disease," *Annual Review of Medicine*, vol. 43, pp. 125–133, 1992.
- [46] P. Isom aki and J. Punnonen, "Pro- and anti-inflammatory cytokines in rheumatoid arthritis," *Annals of Medicine*, vol. 29, no. 6, pp. 499–507, 1997.
- [47] H. K. De Jong, T. Van Der Poll, and W. J. Wiersinga, "The systemic pro-inflammatory response in sepsis," *Journal of Innate Immunity*, vol. 2, no. 5, pp. 422–430, 2010.
- [48] R. J. Gibson, J. M. Bowen, M. R. B. Inglis, A. G. Cummins, and D. M. K. Keefe, "Irinotecan causes severe small intestinal damage, as well as colonic damage, in the rat with implanted breast cancer," *Journal of Gastroenterology and Hepatology*, vol. 18, no. 9, pp. 1095–1100, 2003.
- [49] R. J. Gibson, D. M. K. Keefe, F. M. Thompson, J. M. Clarke, G. J. Goland, and A. G. Cummins, "Effect of interleukin-11 on ameliorating intestinal damage after methotrexate treatment of breast cancer in rats," *Digestive Diseases and Sciences*, vol. 47, no. 12, pp. 2751–2757, 2002.
- [50] K. Nelms, A. D. Keegan, J. Zamorano, J. J. Ryan, and W. E. Paul, "The IL-4 receptor: signaling mechanisms and biologic functions," *Annual Review of Immunology*, vol. 17, pp. 701–738, 1999.
- [51] F. Letzelter, Y. Wang, and W. Sebald, "The interleukin-4 site-2 epitope determining binding of the common receptor  $\gamma$  chain," *European Journal of Biochemistry*, vol. 257, no. 1, pp. 11–20, 1998.
- [52] R. P. Donnelly, M. J. Fenton, J. D. Kaufman, and T. L. Gerrard, "IL-1 expression in human monocytes is transcriptionally and posttranscriptionally regulated by IL-4," *Journal of Immunology*, vol. 146, no. 10, pp. 3431–3436, 1991.
- [53] E. Vannier, L. C. Miller, and C. A. Dinarello, "Coordinated antiinflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 9, pp. 4076–4080, 1992.
- [54] J. Ohara and W. E. Paul, "Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage," *Nature*, vol. 325, no. 6104, pp. 537–540, 1987.
- [55] O. Hultgren, M. Kopf, and A. Tarkowski, "Staphylococcus aureus-induced septic arthritis and septic death is decreased in IL-4-deficient mice: role of IL-4 as promoter for bacterial growth," *Journal of Immunology*, vol. 160, no. 10, pp. 5082–5087, 1998.
- [56] J. K. Lacki and K. E. Wiktorowicz, "Biological properties of interleukin 10," *Postepy Higieny i Medycyny Doswiadczalnej*, vol. 48, no. 4, pp. 363–370, 1994.
- [57] K. Asadullah, W. Sterry, and H. D. Volk, "Interleukin-10 therapy—review of a new approach," *Pharmacological Reviews*, vol. 55, no. 2, pp. 241–269, 2003.
- [58] K. W. Moore, R. De Waal Malefyt, R. L. Coffman, and A. O'Garra, "Interleukin-10 and the interleukin-10 receptor," *Annual Review of Immunology*, vol. 19, pp. 683–765, 2001.
- [59] R. De Waal Malefyt, J. Abrams, B. Bennett, C. G. Figdor, and J. E. De Vries, "Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes," *Journal of Experimental Medicine*, vol. 174, no. 5, pp. 1209–1220, 1991.
- [60] D. F. Fiorentino, A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra, "IL-10 inhibits cytokine production by activated macrophages," *Journal of Immunology*, vol. 147, no. 11, pp. 3815–3822, 1991.
- [61] C. J. Clarke, A. Hales, A. Hunt, and B. M. Foxwell, "IL-10-mediated suppression of TNF-alpha production is independent of its ability to inhibit NF kappa B activity," *European Journal of Immunology*, vol. 28, no. 5, pp. 1719–1726, 1998.
- [62] C. Gerard, C. Bruyins, A. Marchant et al., "Interleukin 10 reduces the release of tumor necrosis factor and prevents

- lethality in experimental endotoxemia," *Journal of Experimental Medicine*, vol. 177, no. 2, pp. 547–550, 1993.
- [63] R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, and W. Muller, "Interleukin-10-deficient mice develop chronic enterocolitis," *Cell*, vol. 75, no. 2, pp. 263–274, 1993.
- [64] M. C. Li and S. H. He, "IL-10 and its related cytokines for treatment of inflammatory bowel disease," *World Journal of Gastroenterology*, vol. 10, no. 5, pp. 620–625, 2004.
- [65] K. Ishizuka, K. Sugimura, T. Homma et al., "Influence of interleukin-10 on the interleukin-1 receptor antagonist/interleukin-1 $\beta$  ratio in the colonic mucosa of ulcerative colitis," *Digestion*, vol. 63, no. 1, pp. 22–27, 2001.
- [66] S. Schreiber, T. Heinig, H. G. Thiele, and A. Raedler, "Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease," *Gastroenterology*, vol. 108, no. 5, pp. 1434–1444, 1995.
- [67] A. J. Davidson, S. A. Freeman, K. E. Crosier, C. R. Wood, and P. S. Crosier, "Expression of murine interleukin 11 and its receptor  $\alpha$ -chain in adult and embryonic tissues," *Stem Cells*, vol. 15, no. 2, pp. 119–124, 1997.
- [68] X. Du, E. T. Everett, G. Wang, W. H. Lee, Z. Yang, and D. A. Williams, "Murine interleukin-11 (IL-11) is expressed at high levels in the hippocampus and expression is developmentally regulated in the testis," *Journal of Cellular Physiology*, vol. 168, no. 2, pp. 362–372, 1996.
- [69] A. J. Dorner, S. J. Goldman, and J. C. Keith Jr., "Interleukin-11," *BioDrugs*, vol. 8, no. 6, pp. 418–429, 1997.
- [70] U. S. Schwertschlag, W. L. Trepicchio, K. H. Dykstra, J. C. Keith, K. J. Turner, and A. J. Dorner, "Hematopoietic, immunomodulatory and epithelial effects of interleukin-11," *Leukemia*, vol. 13, no. 9, pp. 1307–1315, 1999.
- [71] D. J. Hilton, A. A. Hilton, A. Raicevic et al., "Cloning of a murine IL-11 receptor  $\alpha$ -chain; Requirement for gp130 for high affinity binding and signal transduction," *The EMBO Journal*, vol. 13, no. 20, pp. 4765–4775, 1994.
- [72] T. Taga, "The signal transducer gp130 is shared by interleukin-6 family of haematopoietic and neurotrophic cytokines," *Annals of Medicine*, vol. 29, no. 1, pp. 63–72, 1997.
- [73] W. L. Trepicchio, M. Bozza, G. Pedneault, and A. J. Dorner, "Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production," *Journal of Immunology*, vol. 157, no. 8, pp. 3627–3634, 1996.
- [74] W. L. Trepicchio, L. Wang, M. Bozza, and A. J. Dorner, "IL-11 regulates macrophage effector function through the inhibition of nuclear factor- $\kappa$ B," *Journal of Immunology*, vol. 159, no. 11, pp. 5661–5670, 1997.
- [75] W. L. Trepicchio, L. Wang, M. Bozza, and A. J. Dorner, "IL-11 regulates macrophage effector function through the inhibition of nuclear factor- $\kappa$ B," *Journal of Immunology*, vol. 159, no. 11, pp. 5661–5670, 1997.
- [76] M. Bozza, J. L. Bliss, A. J. Dorner, and W. L. Trepicchio, "Interleukin-11 modulates Th1/Th2 cytokine production from activated CD4+ T cells," *Journal of Interferon and Cytokine Research*, vol. 21, no. 1, pp. 21–30, 2001.
- [77] X. X. Du, C. M. Doerschuk, A. Orazi, and D. A. Williams, "A bone marrow stromal-derived growth factor, interleukin-11, stimulates recovery of small intestinal mucosal cells after cytoablative therapy," *Blood*, vol. 83, no. 1, pp. 33–37, 1994.
- [78] J. C. Keith, L. Albert, S. T. Sonis, C. J. Pfeiffer, and R. G. Schaub, "IL-11, a pleiotropic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology," *Stem Cells*, vol. 12, pp. 79–89, 1994.
- [79] X. Du, Q. Liu, Z. Yang et al., "Protective effects of interleukin-11 in a murine model of ischemic bowel necrosis," *American Journal of Physiology*, vol. 272, no. 3, pp. G545–G552, 1997.
- [80] M. A. Farrar and R. D. Schreiber, "The molecular cell biology of interferon- $\gamma$  and its receptor," *Annual Review of Immunology*, vol. 11, pp. 571–611, 1993.
- [81] R. L. Peterson, L. Wang, L. Albert, J. C. Keith, and A. J. Dorner, "Molecular effects of recombinant human interleukin-11 in the HLA-B27 rat model of inflammatory Bowel disease," *Laboratory Investigation*, vol. 78, no. 12, pp. 1503–1512, 1998.
- [82] S. Sonis, A. Muska, J. O'Brien, A. VanVugt, P. Langer-Safer, and J. Keith, "Alteration in the frequency, severity and duration of chemotherapy-induced mucositis in hamsters by interleukin-11," *European Journal of Cancer B*, vol. 31, no. 4, pp. 261–266, 1995.
- [83] Q. Liu, X. X. Du, D. T. Schindel et al., "Trophic effects of interleukin-11 in rats with experimental short bowel syndrome," *Journal of Pediatric Surgery*, vol. 31, no. 8, pp. 1047–1051, 1996.
- [84] C. Booth and C. S. Potten, "Effects of IL-11 on the growth of intestinal epithelial cells in vitro," *Cell Proliferation*, vol. 28, no. 11, pp. 581–594, 1995.
- [85] R. L. Peterson TW, M. M. Bozza, L. Wang, and A. J. Dorner, "G1 growth arrest and reduced proliferation of intestinal epithelial cells induced by rhIL-11 may mediate protection against mucositis," *Blood*, vol. 86, p. 311a, 1995.
- [86] W. P. Arend and C. J. Guthridge, "Biological role of interleukin 1 receptor antagonist isoforms," *Annals of the Rheumatic Diseases*, vol. 59, no. 1, pp. i60–i64, 2000.
- [87] W. P. Arend, H. G. Welgus, R. C. Thompson, and S. P. Eisenberg, "Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist," *Journal of Clinical Investigation*, vol. 85, no. 5, pp. 1694–1697, 1990.
- [88] C. A. Dinarello, "The biological properties of interleukin-1," *European Cytokine Network*, vol. 5, no. 6, pp. 517–531, 1994.
- [89] R. M. Al-Sadi and T. Y. Ma, "IL-1 $\beta$  causes an increase in intestinal epithelial tight junction permeability," *Journal of Immunology*, vol. 178, no. 7, pp. 4641–4649, 2007.
- [90] T. Andus, R. Daig, D. Vogl et al., "Imbalance of the interleukin 1 system in colonic mucosa—association with intestinal inflammation and interleukin 1 receptor agonist genotype 2," *Gut*, vol. 41, no. 5, pp. 651–657, 1997.
- [91] E. Hirsch, V. M. Irikura, S. M. Paul, and D. Hirsh, "Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 20, pp. 11008–11013, 1996.
- [92] Z. Wu, X. Han, S. Qin et al., "Interleukin 1 receptor antagonist reduces lethality and intestinal toxicity of 5-Fluorouracil in a mouse mucositis model," *Biomedicine and Pharmacotherapy*, vol. 65, no. 5, pp. 339–344, 2011.
- [93] Z. Q. Wu, X. D. Han, Y. Wang et al., "Interleukin-1 receptor antagonist reduced apoptosis and attenuated intestinal mucositis in a 5-fluorouracil chemotherapy model in mice," *Cancer Chemotherapy and Pharmacology*, vol. 68, no. 1, pp. 87–96, 2011.
- [94] W. Zou, "Regulatory T cells, tumour immunity and immunotherapy," *Nature Reviews Immunology*, vol. 6, no. 4, pp. 295–307, 2006.
- [95] J. Dannull, Z. Su, D. Rizzieri et al., "Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells," *Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3623–3633, 2005.

- [96] G. Zhou and H. I. Levitsky, "Natural regulatory T cells and de novo-induced regulatory T cells contribute independently to tumor-specific tolerance," *Journal of Immunology*, vol. 178, no. 4, pp. 2155–2162, 2007.
- [97] S. Hinz, L. Pagerols-Raluy, H. H. Oberg et al., "Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer," *Cancer Research*, vol. 67, no. 17, pp. 8344–8350, 2007.
- [98] D. Wolf, A. M. Wolf, H. Rumpold et al., "The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer," *Clinical Cancer Research*, vol. 11, no. 23, pp. 8326–8331, 2005.
- [99] P. A. Ascierto, M. Napolitano, E. Celentano et al., "Regulatory T cell frequency in patients with melanoma with different disease stage and course, and modulating effects of high-dose interferon- $\alpha$  2b treatment," *Journal of Translational Medicine*, vol. 8, article no. 76, 2010.
- [100] H. L. Kaufman, B. Taback, W. Sherman et al., "Phase II trial of Modified Vaccinia Ankara (MVA) virus expressing 5T4 and high dose Interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma," *Journal of Translational Medicine*, vol. 7, article no. 2, 2009.
- [101] A. M. Wolf, D. Wolf, M. Steurer, G. Gastl, E. Gunsilius, and B. Grubeck-Loebenstein, "Increase of regulatory T cells in the peripheral blood of cancer patients," *Clinical Cancer Research*, vol. 9, no. 2, pp. 606–612, 2003.
- [102] S. Wing, "Multiple Treg suppressive modules and their adaptability," *Frontiers in Immunology*, vol. 3, article 178, 2012.
- [103] Y. P. Rubtsov, J. P. Rasmussen, E. Y. Chi et al., "Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces," *Immunity*, vol. 28, no. 4, pp. 546–558, 2008.
- [104] R. Tao, E. F. De Zoeten, E. Özkaynak et al., "Deacetylase inhibition promotes the generation and function of regulatory T cells," *Nature Medicine*, vol. 13, no. 11, pp. 1299–1307, 2007.
- [105] S. G. Kang, H. W. Lim, O. M. Andrisani, H. E. Broxmeyer, and C. H. Kim, "Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells," *Journal of Immunology*, vol. 179, no. 6, pp. 3724–3733, 2007.
- [106] J. Y. Liu, Y. Wu, X. S. Zhang et al., "Single administration of low dose cyclophosphamide augments the antitumor effect of dendritic cell vaccine," *Cancer Immunology, Immunotherapy*, vol. 56, no. 10, pp. 1597–1604, 2007.
- [107] M. Kurooka and Y. Kaneda, "Inactivated Sendai virus particles eradicate tumors by inducing immune responses through blocking regulatory T cells," *Cancer Research*, vol. 67, no. 1, pp. 227–236, 2007.
- [108] B. Li, S. J. Saouaf, A. Samanta, Y. Shen, W. W. Hancock, and M. I. Greene, "Biochemistry and therapeutic implications of mechanisms involved in FOXP3 activity in immune suppression," *Current Opinion in Immunology*, vol. 19, no. 5, pp. 583–588, 2007.