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COMPLICATIONS OF TREATMENT

# The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: Pathobiology, animal models and cytotoxic drugs

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**Summary** Alimentary tract (AT) mucositis can be a major problem for patients undergoing cancer treatment. It has significant clinical and economic consequences and is a major factor that can compromise the provision of optimal treatment for patients. The pathobiology of AT mucositis is complex and the exact mechanisms that underlie its development still need to be fully elucidated. Current opinion considers that there is a prominent interplay between all of the compartments of the mucosa involving, at a molecular level, the activation of transcription factors, particularly nuclear factor-κB, and the subsequent upregulation of pro-inflammatory cytokines and inflammatory mediators. The purpose of this review is to examine the literature relating to what is currently known about the pathobiology of AT mucositis, particularly with respect to the involvement of pro-inflammatory cytokines, as well as currently used animal models and the role of specific cytotoxic chemotherapy agents in the development of AT mucositis.


## Introduction

Mucositis is a major problem for patients undergoing treatments such as chemotherapy and radiotherapy. Mucositis

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occurs throughout the alimentary tract (AT)[1,2] and causes a spectrum of clinical signs and symptoms ranging from intractable and debilitating oral pain as a result of ulceration through to, and including, gastrointestinal symptoms such as abdominal bloating, vomiting and diarrhoea.[3,4] It has recently also been suggested that other mucosal surfaces throughout the body, such as the genitourinary and respiratory mucosae, may also be affected.[5]

Other side effects of cancer treatment, such as severe nausea or potentially life threatening events, such as neutropenia, are now relatively well managed.[6] Mucositis however, remains an important dose-limiting factor in a patient's cancer treatment.[6,7] The debilitating effects of mucositis can result in unplanned treatment interruptions or even premature cessation of treatment. The risk of systemic infections and even death is increased in patients with mucositis. For patients undergoing radiotherapy, doses of treatment are limited by the proximity of the mouth to critical anatomical structures such as the brain and spinal cord and therefore increases in dose may not be feasible. However with respect to chemotherapy, effective treatment of mucositis may lead to increased maximum tolerated doses of treatment and improved quality of life for cancer patients during and after treatment. Conceivably, this would also translate to an increased likelihood for cancer remission.

Mucositis increases the morbidity of patients undergoing cancer treatment, results in extended hospital stays, and increases re-admission rates.[4,8] Hospitalisation of patients for supportive care and pain management due to mucositis has significant economic consequences.[8,9] As well as being important clinically and economically, patient perceptions of mucositis and its impact on their treatment and quality of life are also important. A study investigating patient reported complications of bone marrow transplantation clearly identified mucositis as the single most debilitating side effect of treatment.[6] Furthermore, opioid analgesics used the management of mucositis had secondary effects on the quality of life of patients because of adverse drug reactions such as decreased mental acuity and hallucinations.[6]

The prevalence of mucositis is variable and appears to be dependent on the type of treatment given as well as the disease that is being treated. For example, mucositis occurs in 80–100% of patients undergoing so-called ''high-risk'' regimens such as radiotherapy to the head and neck or high dose chemotherapy and stem cell (or bone marrow) transplantation.[3,4] Furthermore, specific cytotoxic chemotherapy agents, such as 5-fluorouracil (5-FU), are associated with more severe mucositis.[10] In regimens considered to be ''low-risk'' for the development of mucosal toxicity, the prevalence of mucositis may be as low as 10–15%, however given the numbers of people receiving chemotherapy, this still represents a significant number of patients that are affected by mucositis.[10]

It is important, therefore, that the pathobiology of mucositis is determined and fully described so that effective targeted treatment strategies can be developed. The most recent hypothesis for the pathogenesis of mucositis has implicated an important role for transcription factors and pro-inflammatory cytokines in the development of mucositis.[11,12] The focus of this review is to examine the literature relating to the role that pro-inflammatory cytokines might play in the pathobiology of mucositis, particularly in the context of chemotherapy-induced mucositis (radiation-induced mucositis has been adequately reviewed elsewhere[13]). The review will also examine animal models that are currently used to study mucositis pathobiology and, finally, discuss the role of specific chemotherapy drugs in the pathobiology of mucositis.

## Mucositis pathobiology

Historically, the main mechanism behind the development of mucositis was considered to be the result of direct cytotoxic effects of chemotherapy or radiotherapy on the basal cells of the epithelium which line the AT.[14,15] It was thought that the epithelial cells were particularly vulnerable to the effects of treatment because of their high cell turnover rate. Following the development of an appropriate animal model to study mucositis,[16] it became clear that the pathobiology was more complex and involved an interplay between all compartments of the mucosa including the connective tissue elements as well as the epithelium. For instance, subsequent researchers investigating intestinal damage that occurred following radiation, found that the primary damage response occurred in endothelial cells.[17–20] Such damage has also been demonstrated to occur in the oral mucosa following exposure to radiation.[21] Sonis et al. demonstrated that this primary damage to endothelial cells occurred well before any detectable changes were apparent in the epithelium.[21]

In 1998 Sonis proposed a four-stage model that described four stages involved in the development of oral mucositis.[11] This model was subsequently modified to comprise five continuous overlapping phases (Fig. 1).[11,12,22] The first of these phases is described as an *initiation phase*. This occurs immediately following exposure to cytotoxic agents and results in direct tissue damage to mucosal components as a result of the production of reactive oxygen species. This is followed by an *upregulation and message generation phase*, an important element of which is the activation of transcription factors, in particular nuclear factor-κB (NF-κB). This transcription factor is activated in response to chemotherapy and radiotherapy and is responsible for the upregulation of up to 200 genes that have an effect on mucosal integrity by inducing clonogenic cell death, apoptosis and tissue injury throughout the mucosa, not limited to the epithelium.[23] NF-κB activation results in the production of pro-inflammatory cytokines, including tumour necrosis factor (TNF, formerly referred to as TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6).[24] In the context of mucositis, these cytokines have all been demonstrated in the mucosa as well as in the peripheral blood of patients undergoing cancer treatment.[25,26] The third phase involves *signal amplification* and occurs as a consequence of the pro-inflammatory cytokines acting via positive feedback mechanisms causing further activation of NF-κB and subsequent increased production of cytokines. Other biologically active proteins or pro-inflammatory mediators, such as cyclo-oxygenase-2 (COX-2),[24] are upregulated and initiate an inflammatory cascade leading to activation of matrix metalloproteinases whose production elicit further tissue damage.[27] The *ulcerative phase* develops where, clinically,

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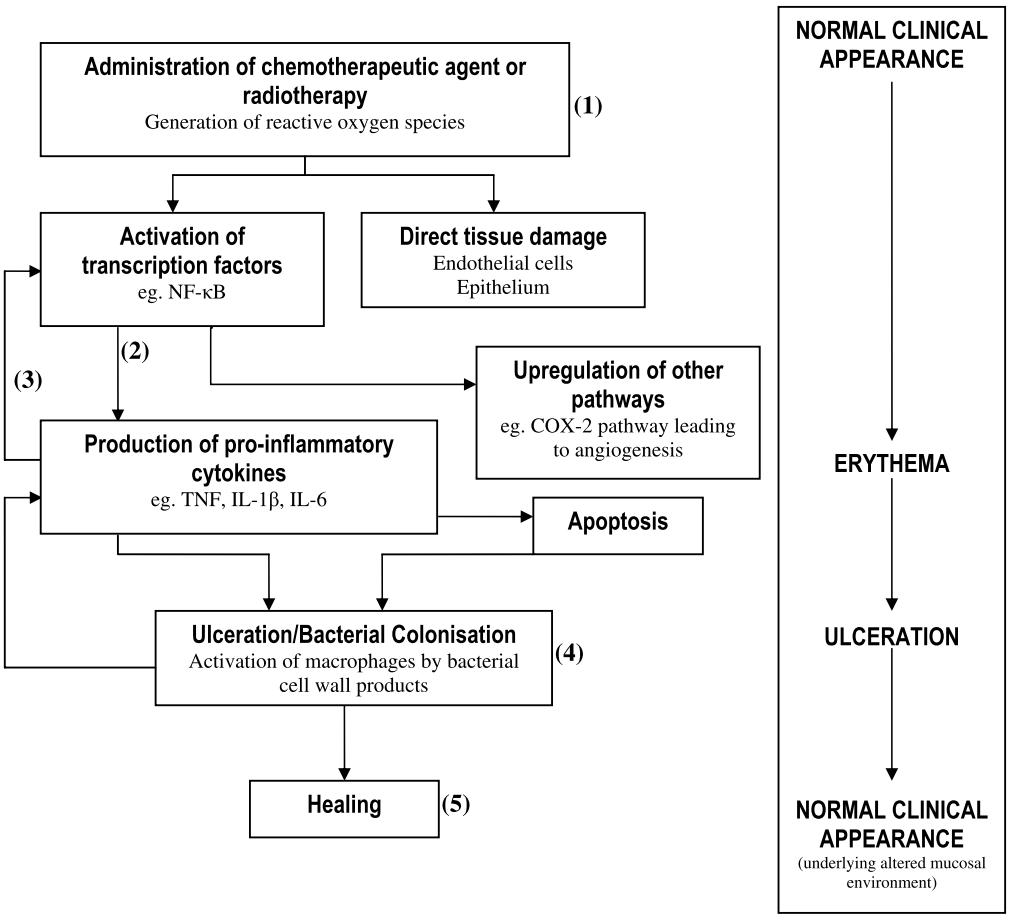


**Figure 1** Diagram illustrating mucosal and clinical changes that occur leading to mucositis according to the current hypothesis.[22] The five overlapping stages are demonstrated (1) initiation; (2) upregulation and message generation; (3) signalling and amplification; (4) ulceration; (5) healing.

there is a breach of the epithelium accompanied by bacterial colonisation. It is not until this stage that mucositis becomes clinically evident. The patient will experience significant pain and possibly abdominal symptoms; the risk of systemic infection is increased, particularly if the patients are immunosuppressed. Furthermore, bacterial products can stimulate further amplification of cytokine production leading to further potentiation of tissue injury.[11] Following cessation of the cancer treatment, the final *healing phase* occurs. This phase results in the restoration of normal mucosal appearance at the clinical level. Re-epithelialisation of the mucosa due to signals from the extracellular matrix is observed histologically. The healing phase is probably the least well understood and studied with respect to mucositis pathobiology.

It is important to reiterate that the clinical manifestations of mucositis are not apparent until the ulcerative phase develops. A normal clinical appearance prior to this phase belies the fact that a complex myriad of biological events is taking placed subclinically. Elucidation of the exact mechanisms involved could potentially reveal therapeutic targets to enable the cessation of mucositis development before clinical signs and symptoms, such as ulceration, occur. Furthermore, it has also been highlighted that although healing does occur, ultrastructural and histological evidence indicates that the structure of the mucosa is altered for an extended period following the completion of treatment.[28,29] The ability of the mucosa to withstand further trauma or insult may therefore be compromised.

A recent review by Anthony et al. highlighted the fact that there are various factors that impact on the risk or likelihood that a patient will develop mucositis.[30] That review divided these ''drivers'' of mucotoxicity into either global factors or tissue specific factors, including those at a cellular or molecular level. It was postulated that it was the presence and interaction of these factors which led to an increased risk of mucositis and may explain the increased propensity for mucositis that is apparent in some individuals compared to others who undergo comparable treatment regimens. This review also highlighted the fact that additional work needs to be completed to determine mucosal susceptibility to injury subsequent to cancer treatment.

## Evidence for the role of NF-κB and pro-inflammatory cytokines in mucositis

Cytokines are inducible chemical messengers which are produced by a variety of cells throughout the body. They are low-molecular weight glycoproteins and are involved in both

the inflammatory and immune responses.[31] The role of cytokines in the pathogenesis of mucositis has been investigated by various studies. Sonis et al. found that cytokines that targeted epithelial proliferation such as epidermal growth factor[32] and transforming growth factor-β3[33] were able to modify the course of mucositis. Antin and Ferrara described dysregulation of TNF and IL-1 production following conditioning regimens that included radiation.[34]

The recent advent of more targeted therapy for mucositis in specific patient populations using Palifermin (recombinant keratinocyte growth factor-1), has provided further support for the role cytokines play in the development of mucosal toxicity.[35,36] One of the effects of Palifermin is to cause alteration of cytokine profiles resulting, amongst other things, in downregulation of TNF.[36]

As indicated previously, NF-κB is thought to play an important role in the pathobiology of mucositis, particularly with respect to the upregulation and subsequent expression of the pro-inflammatory cytokines TNF, IL-1β and IL-6.

## Nuclear factor-κB

A thorough review of the role of NF-κB in diseases and its potential involvement in the pathology of mucositis has been previously published.[24] Collectively the NF-κB family comprises a group of five different members (NF-κB1 (p50/p105), NF-κB2 (p52/p100), p65 (Rel A), Rel 3 and c-Rel) which have a diverse range of biological effects.[24,37] This is indicated by the large number of target genes that are influenced by NF-κB activation. These target genes include various cytokines (including, as already mentioned TNF, IL-1β and IL-6), immunoreceptors, cell adhesion molecules, acute phase proteins, stress response genes and cell-surface receptors.[24] Generally NF-κB plays important roles in inflammatory and immune responses as well as the development of haematopoietic cells, keratinocytes and lymphoid structures. With respect to inflammation, NF-κB can have both pro- and anti-inflammatory effects depending at which stage during the inflammatory process the pathway is stimulated.[38] As mentioned previously, activation of NF-κB can be facilitated by various factors including both radiation and chemotherapy[36] as well as infectious agents, physiological stress and inflammatory cytokines.[24]

The evidence for NF-κB's role in mucositis is based on various observations as outlined by Sonis.[24] Cell death that results from cytotoxic chemotherapy is attributed to programmed cell death (PCD) or apoptosis — this occurs in both normal cell populations and in neoplastic cells. NF-κB plays an important role in the process of apoptosis.[24] A recent study provided data on morphological and ultrastructural changes in the oral mucosa following cytotoxic chemotherapy.[28] This study demonstrated that the level of apoptosis occurring in the oral mucosa peaked at 3 days following chemotherapy at 400 times the level seen in the healthy controls. A further study, conducted within the same patient group, demonstrated that tissue levels of NF-κB were also elevated following to chemotherapy.[29] It has also been postulated that reactive oxygen species produced in tissues by ionising radiation may cause activation of NF-κB in normal alimentary mucosa and cause increased apoptosis, manifesting clinically as mucosal damage.[13] Further circumstantial evidence for the role of NF-κB in the pathogenesis of mucositis is that the onset and severity of mucositis are associated with its activation and that inhibition of pro-inflammatory cytokines and reduction in bacterial load leads to decreased levels of NF-κB, which clinically translates to a reduction in mucositis.[21,39]

As well as having pro-apoptotic properties NF-κB can, in some situations demonstrate anti-apoptotic properties.[24,40] Studies have demonstrated that generally NF-κB has a pro-apoptotic effect on normal cells and an anti-apoptotic or cytoprotective effect on tumour or neoplastic cells.[24] The reason for this dichotomy of action is unclear. Sonis suggested various explanations for this. First, stimulation of different Bcl-2 genes occurs via NF-κB activation.[24] The Bcl-2 family of genes can have either pro-apoptotic or anti-apoptotic actions. In the rat and in humans, increased expression of the Bcl-2 members Bax and Bak has been demonstrated in the small intestine following cytotoxic chemotherapy.[41] These Bcl-2 family members have been demonstrated to promote apoptosis.[42] Alternative explanations for the duality of NF-κB's action include alternative pathway mediation between neoplastic and normal cells resulting in amplified tissue damage in normal cell populations compared to neoplastic populations. Other factors such as the oral environment and specific features such as epithelial type, local microbial flora and underlying pathology may also be important influences.[24,30]

## Tumour necrosis factor

Tumour necrosis factor is a pleiotropic protein that was initially isolated from mouse serum following exposure to bacterial endotoxin.[43] It was demonstrated that TNF replicated endotoxin's ability to cause haemorrhagic necrosis in methylcolantrene-induced sarcomas.[43] Subsequent to this discovery, it has become evident that this protein belonged to a larger group, or family, of proteins that have both beneficial, as well as potentially damaging, effects throughout the body.[44] The beneficial roles played by members of the TNF family include inflammatory and protective immune responses as well as being important factors in the organogenesis of secondary lymphoid organs and lymphoid structure maintenance.[44] Conversely, TNF has also been shown to have a host damaging role in the context of sepsis and autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease (IBD).[44,45]

Tumour necrosis factor is predominantly expressed by activated macrophages, NK cells and T lymphocytes.[46] The two receptors for TNF are expressed either on all cell types (TNF-R1) or only on immune or endothelial cells (TNF-R2).[46] TNF through the interaction with TNF-R1 causes various cellular events including activation of the caspase cascade which leads to apoptosis. TNF interaction with TNF-R1 also leads to activation of NF-κB. TNF-R2 signalling is less well characterised, however it is known that this receptor does not possess a death domain and can therefore not directly precipitate apoptosis. The role of NF-κB activation leading to apoptosis via TNF-R2 signalling is unclear.[46] In addition to causing the ''classical'' caspase-dependent form of apoptosis or PCD, TNF has also been demonstrated to induce necrosis-like caspase-independent PCD.[46]

Clinically it has been demonstrated that increased serum levels of TNF occur in patients who have undergone bone

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marrow transplantation and that this event precedes the development of major transplant related complications.[47,48] Other researchers have demonstrated elevated TNF levels occurring in association with non-haematological toxicities.[25,26,49,50] Inhibition of TNF using agents such as pentoxifylline reduced these non-haematological toxicities.[48] With respect to mucositis, various animal and human studies have shown a decrease in the occurrence or severity of mucositis following administration of TNF inhibitors.[48,51,52] Interestingly, Orlicek et al. demonstrated that isolates from viridans streptococci were able to induce TNF production by murine macrophages.[53] These organisms are normal commensal flora in the mouth and respiratory tract, the induction of TNF by these bacteria therefore is important in the context of mucositis development. This may be particularly so in the ulcerative phase of the tissue damage process resulting in further amplification of pro-inflammatory cytokine production and subsequent further tissue damage. Lima et al. demonstrated, using a hamster model of 5-FU induced mucositis, that administration of pentoxifylline and thalidomide, both of which inhibit cytokine synthesis, had a protective effect.[52] These authors concluded that this indicated an important role for TNF in the pathobiology of 5-FU induced oral mucositis.

## Interleukin-1β

IL-1β is a multifunctional cytokine that has an affect on a wide variety of cell types as well as interacting with many other cytokines.[54] IL-1β is part of a family of cytokines which also include IL-1α and IL-1 receptor antagonist (IL-1Ra). The latter molecule binds to each of the two IL-1 receptors.

IL-1β has multiple biologic effects which have been demonstrated in in vitro and in vivo situations including systemic reactions such as fever and increased gene expression of a range of genes including pro-inflammatory cytokines and pro-inflammatory mediators. IL-1β production can be stimulated by both microbiological and non-microbiological factors. The latter includes, among many things, other cytokines and irradiation.[54] Along with TNF, IL-1β is an important cytokine that is involved in the activation of the NF-κB pathway. In fact IL-1β and TNF have been reported to have a synergistic effect, for example causing induction of endothelial adhesion molecules essential for the initial phases of the inflammatory response.[31]

Local tissue levels of IL-1β and TNF have been shown to increase markedly in animal models of radiation-induced oral mucositis concurrently with the development of mucositis.[21] IL-1β may also have a role to play in the healing phase of mucositis development.[55] There is, however, a paucity of data in the literature about the exact role that IL-1β plays in the context of mucositis pathobiology.

## Interleukin-6

Like TNF and IL-1β, IL-6 has a broad range of biological activities on a range of target cells. It is particularly involved in the immune response and in the pathogenesis of inflammatory diseases such as rheumatoid arthritis, Castleman's disease and Crohn's disease.[56,57] Initially, it was identified as a factor that induced B-cells to produce immunoglobulins.[56,58] It is produced by a variety of cell types including T and B lymphocytes, fibroblasts, keratinocytes, endothelial cells and some tumour cells.[58,59]

IL-6 production is induced by TNF. Conversely, TNF is strongly inhibited by IL-6 thereby forming an effective negative feedback loop which inhibits the activation or hyperactivation of the pro-inflammatory cytokine cascade.[26] IL-6 therefore, can have anti-inflammatory as well as pro-inflammatory effects.[60]

With respect to intestinal damage, IL-6 has been demonstrated to be elevated in both the serum and tissues of patients with IBD and in addition, the levels of IL-6 correspond with the severity of the disease.[61] In this context, epithelial cells and mononuclear cells in the lamina propria are the major source of this cytokine.[61] It has been demonstrated that IL-6 can induce activation of NF-κB in the intestinal epithelia.[61] It has been postulated that it is via NF-κB activation that IL-6 exerts its biological effects leading to inflammatory diseases such as IBD and rheumatoid arthritis.[61] Again, as is the case with IL-1β, there is little data on the specific role of IL-6 in the pathobiology of mucositis.

## Animal models

Various animal models have been developed to investigate oral mucositis with respect to morphological changes that occur in mucosae subsequent to the administration of cytotoxic agents and to determine the effects of antimucotoxic medication. The choice of animal model depends on various factors such as accessibility to the animal, cost and, of course, what is being investigated. In addition, many of these animal models have been used to study radiation-induced mucositis; these, however, are beyond the scope of this review.

## Hamsters

The first animal model to investigate chemotherapy-induced mucositis involved the use of Golden Syrian hamsters that were administered 5-FU.[16] Clinical and histological evaluation indicated that the changes that occurred in the mucosa were similar to that seen in humans with mucositis. In addition, the changes were also influenced by the degree of myelosuppression experienced by the animals. Subsequent studies using this model, or modified versions of it, have been used to investigate various aspects of mucositis including pathobiology and treatment as well as mucotoxicity of cytotoxic medication.[21,39,52,62–74]

Some of the first studies using the hamster model investigated the roles of epidermal growth factor (EGF),[32] transforming growth factor-β3 (TGF-β3)[33,64] and recombinant human interleukin-11 (rhIL-11)[63,65,66] in the pathobiology of mucositis.

Early studies using the hamster as a model for mucositis investigated features relating to the biology of mucositis and the susceptibility of mucosa to injury. EGF is a protein that stimulates the growth and differentiation of epithelial cells.[75] Early on it was considered that susceptibility to mucosal damage may be related to the rate of epithelial cell replication. Sonis et al., demonstrated that, by stimulating epithelial basal cell turnover rate by administration of EGF, mucosal injury was increased, supporting the concept that

the epithelial turnover affects the susceptibility of the mucosa to chemotherapy.[32]

TGF-β is a regulatory growth factor that has the ability to reversibly arrest proliferating cells in the G1 phase of the cell cycle. Sonis et al., demonstrated that topical application of TGF-β3 to the oral mucosa of hamsters prior to administration of 5-FU reduced various parameters related to mucositis including incidence, severity and duration of mucositis as well as other factors such as weight loss attributed to chemotherapy.[64] Conversely, survival in this animal model was increased. Again, this study demonstrated that mucosal susceptibility to chemotherapeutic agent may be affected by epithelial cell turnover. A subsequent study that demonstrated the proliferation of basal cells in the epithelium by measuring proliferating cell nuclear antigen (PCNA) confirmed that topical application of TGF-β3 significantly reduced basal cell replication in the oral epithelium of the hamster.[33]

IL-11 is a pleiotropic cytokine that causes stimulation of bone marrow proliferation and has been demonstrated to ameliorate mucosal injury following 5-FU administration in a hamster model of mucositis.[63] Subsequent studies further demonstrated that the administration of rhIL-11 had beneficial effects with respect to frequency, severity and duration of mucositis as well as weight loss.[65,66] The animal model developed by Sonis et al.[16] was modified to investigate radiation-induced mucositis, specifically relating to the mechanism behind the action of IL-11 on the progression of mucosal damage.[21] rhIL-11 was demonstrated to attenuate mucositis resulting from radiation in the hamster model. This was attributed to the effect of IL-11 in maintaining mucosal integrity as well as causing a reduction in pro-inflammatory cytokine expression by inflammatory cells within the mucosa. This study provided further evidence for the interplay that occurs between epithelial and connective tissue compartments of the mucosa in the development of mucosal injury.

Other studies that used the hamster model to investigate various aspects of mucositis pathology have included Sonis et al., who investigated COX-2 expression in experimental radiation-induced mucositis[68] and Leitão et al. who investigated the role of nitric oxide (NO) on the pathogenesis of 5-FU-induced oral mucositis.[76] Both of these studies demonstrated the complexities of mucositis pathogenesis and the multiple pathways that may be involved in the development of mucosal injury.

Studies have also employed the hamster model to described potential treatment strategies for mucositis. The use of fibroblast growth factor-20 (FGF-20) or Velafermin has been demonstrated to be effective in helping to maintain mucosal integrity in the context of experimental radiation- and combined chemotherapy/radiation-induced mucositis.[69] The TNF-inhibiting effects of pentoxifylline and thalidomide were investigated for the management of experimental oral mucositis using the hamster model.[52] As well as demonstrating that these drugs had a protective effect for the development of mucositis in the animals, this study also demonstrated the potentially important role for TNF in the pathogenesis of mucositis as already described in this review. Patients undergoing cancer treatment are susceptible to increased oral infections, the most common of which are due to *Candida albicans*, a commensal organism in the mouth. Various studies have demonstrated that infections may exacerbate the severity of mucositis, although the role of antimicrobial and antifungal agents in the treatment of mucositis is debatable.[77] Regardless of this, the use of a suitable delivery mechanism to effectively administer antifungal agents may improve oral health in some patients who develop secondary infections. Aksungur et al. used a hamster model, based on that developed by Sonis et al., to develop a medication delivery system for nystatin for the treatment and prophylaxis of oral mucositis.[71] This study demonstrated a beneficial effect of the use of topical nystatin with respect to mucositis scores and survival. The use of topical granulocyte-macrophage colony stimulating factor (GM-CSF) has also been evaluated using the hamster model of oral mucositis.[73] As well as having a beneficial effect on mucositis, this study provided additional support for the role of pro-inflammatory cytokines in the development of mucositis. GM-CSF administration appeared to cause a decrease in the expression of the pro-inflammatory cytokines TGF-β, IL-2, TNF, IL-1β and β-actin in the oral mucosa of the hamsters.

The hamster model developed by Sonis et al. has also been used in drug development.[78] Protegrins are molecules with antimicrobial activity against gram-positive and negative bacteria as well as yeasts. Chen et al. described the development and selection of an appropriate protegrin for the potential treatment of mucositis using the hamster model.[78]

## Mice

The murine model represents another commonly used animal model to investigate aspects of mucositis. Again the diversity of studies is wide and encompasses investigation into the treatment of mucositis[79–86] and pathobiology.[87–90]

Murine models have been used to test treatment strategies related to toxicities associated with specific drugs. Administration of doxorubicin has been described to cause apoptosis in the intestinal epithelium in rats resulting in gastrointestinal toxicity.[91] Using mice, Morelli et al. and Balsari et al. investigated the effect of systemic and topical applications of an anti-doxorubicin monoclonal antibody for the treatment and prevention of mucositis.[84,85] They demonstrated reduced signs of gastrointestinal toxicity in mice following the oral administration of the anti-doxorubicin antibody.[84] In the oral mucosa, in the murine model, epithelial apoptosis due to doxorubicin was eliminated by the topical application of the anti-doxorubicin antibody.[85]

The development of Palifermin or keratinocyte growth factor (KGF) for clinical use was preceded by numerous pre-clinical studies, many of which involved murine models to determine any adverse effects of KGF as well as to determine the effectiveness of KGF in preventing mucosal toxicity. Early investigations using murine models of chemotherapy-induced injury where KGF was administered prior to chemotherapy using 5-FU or methotrexate demonstrated that survival was improved as well as histological parameters in the small intestine such as increased villus height and crypt depth.[82] Subsequent studies confirmed this and looked at other parameters such as cell proliferation markers as well as radiation-induced and radiochemotherapy-induced mucositis.[79,86,92–96]

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Other studies that have investigated treatment strategies for mucositis using murine models have included the addition of short-chain fatty acids to the diet of mice treated with Ara-C (cytarabine).[83] This study demonstrated that histological features of the small intestinal mucosa such as villus height were increased and that inflammation and necrosis was decreased. Woo et al., demonstrated in a mouse model that mucositis due to cyclophosphamide could be reduced by the administration of clarithromycin.[90] Huang et al. investigated the role of EGF administration in the treatment of chemotherapy-induced intestinal injury in mice.[81] Transgenic mice that over-express EGF were administered 5-FU – these mice did not demonstrate any reduced effects of mucosal damage compared to control mice, for example, features such as loss of mucosal histological architecture and weight loss were no different between the groups. Likewise mice treated with exogenous EGF after receiving 5-FU did not demonstrate a beneficial effect. These results appear to concur with those of the early studies done in hamsters as outlined previously.[32]

Mice have also been used in studies investigating aspects of mucositis pathobiology. Kang et al. investigated the role of caspase activation, particularly caspase-11, in the context of mucosal injury subsequent to melphalan administration in mice.[87] Caspase-11 is a murine caspase which is 60% homologous with human caspase-4.[97] Caspase-11 is an important regulator of apoptosis in various pathological conditions.[97] In the context of mucositis however, gastrointestinal damage due to melphalan appeared to be independent of caspase-11 and therefore this study indicated that other pathways leading to intestinal apoptosis might be involved.[87]

Beck et al. investigated the role of trefoil factors in chemotherapy- and radiotherapy-induced mucositis in mice.[89] They found that mice deficient in intestinal trefoil factor were more susceptible to mucosal damage following chemotherapy or radiotherapy compared to their non-deficient counterparts. In addition, supplementary intestinal trefoil factor included in the diets of mice reduced the severity of intestinal mucositis following chemotherapy or radiotherapy. This study demonstrated the important role of protective factors of the AT in the maintenance of mucosal integrity.

## Rats

Rats have also been used widely in studies investigating mucositis pathobiology[41,89,98–100] and treatment.[101–105] In addition, there have been studies which have been designed to determine non-invasive methods for the detection of intestinal mucositis.[106,107]

One of the most extensively used models to investigate chemotherapy-induced mucositis is that that employs the use of the female Dark Agouti rat.[41,98,99,105,108] This model has been demonstrated to effectively parallel the development of mucositis that occurs in humans. This model has the added benefit of the rats bearing tumours; the ''tumour effect'' can be studied as well as mucosal damage. Various cytotoxic chemotherapy agents have been investigated with this model including irinotecan, methotrexate and 5-FU.

The effect of KGF administration on small intestinal mucositis and tumour growth following administration of methotrexate was investigated by Gibson et al.[105] This study found that although KGF administration increased intestinal growth prior to chemotherapy, it provided no benefit with respect to mucositis. KGF administration appeared to reduce tumour growth; this is an important factor when an agent is considered as a potential therapeutic option. A subsequent study by Gibson et al. investigated another potential therapeutic agent, IL-11.[108] The administration of this pleiotropic cytokine to rats with breast carcinomas given methotrexate resulted in attenuation of mucositis measured by maintenance of intestinal weight and morphometrical features. This appeared to occur via the induction of compensatory crypt cell proliferation rather than by inhibiting apoptosis. Furthermore, the administration of IL-11 did not increase the growth of the tumours in the rats.

The DA rat model has also been used to characterise the small intestinal damage that occurs following the administration of specific drugs.[98] Irinotecan causes severe diarrhoea, the mechanism by which this occurs is poorly characterised. Gibson et al. demonstrated that irinotecan administration in tumour-bearing rats resulted in apoptosis and hypoproliferative changes in the small and large intestines. In addition, goblet cell number and mucus secretion were affected in the large intestine. The diarrhoea that is induced by irinotecan was attributed to these factors collectively. The effect of Palifermin, or KGF, on diarrhoea and survival following irinotecan administration was also investigated by Gibson et al.[101] It was found that, in tumour-bearing rats, diarrhoea was reduced and survival increased following Palifermin administration without promoting tumour growth.[101]

Bowen et al. further characterised the effect of irinotecan administration in tumour-bearing DA rats by investigating changes in gene expression in the small intestine.[99] It was found that irinotecan resulted in differential regulation of various genes associated with the mitogen-activated protein kinase (MAPK) signalling pathway which is involved in the caspase-cascade, the activation of which ultimately results in apoptosis.

Obviously animal models do not always accurately replicate what happens in humans in a true clinical situation; however these studies are important in determining events that occur in the mucosa following the administration of chemotherapy or radiation. With respect to oral mucositis, many animal studies employ mechanical irritation to induce ulceration of the mucosa.[16,52,62,66,67,70–74,76] This adds a further complicating issue to these studies in that they appear to be based on the historical paradigm that considers mucositis to be a predominantly epithelial phenomenon and can only be said to occur when there is clinical evidence of ulceration, that is, loss of epithelium. If mucositis is considered to be a true *mucosal* phenomenon, then clinical evidence of ulceration should not be strictly necessary. Different types of epithelium are considered to be more resilient and resistant to injury, however the events occurring in the underlying connective tissue should conceivably be consistent regardless of whether they lead to frank ulceration. It is conceivable that induction of ulceration by mechanical irritation of the mucosa may initiate biological events which might complicate or even mask the histological and molecular changes induced by chemotherapeutic agents or radiation.

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To date, all animal models have focussed predominantly on one area of the AT, particularly the oral cavity. There have been no studies at this stage, which have compared the changes that occur in different sites of the AT. Studies are currently underway using the female DA rat model to investigate the changes that occur along the AT comparing the effects of different cytotoxic agents.

## Specific cytotoxic chemotherapeutic agents

In the literature, the development of mucositis has largely been assumed to be independent of the agent causing it. This idea, however, is simplistic in that different cytotoxic agents obviously have different modes of action and, in themselves, affect different molecular pathways in both neoplastic and normal cells. In addition, many chemotherapeutic agents are given in combination; a fact that further complicates the characterisation of the pathways affected by these agents. It is therefore conceivable that, although the clinical outcomes may be similar, the process of mucositis development may also differ between cytotoxic agents. If this is the case, there would therefore be important implications for the effective treatment of mucositis.

### Irinotecan

Irinotecan hydrochloride is a chemotherapy agent that has been used to treat various types of solid tumours. It exerts its cytotoxic effect by inhibiting DNA topoisomerase 1; this requires conversion of irinotecan to its active metabolite SN-38 by carboxylesterase.[109] Irinotecan causes severe side effects which limit the dose of drug that can safely be delivered to patients. These side effects include myelosuppression and severe diarrhoea.[110,111] The features of irinotecan-induced damage to the small intestine are well documented and include an increase in apoptosis in the crypts as well as effacement of the villi in the small intestine.[98] Increased apoptosis within the crypts of the colon mucosa have also been described.[98] Clinically this damage manifests as diarrhoea, abdominal bloating and pain.[1,3,4,112]

The diarrhoea that results following irinotecan administration occurs in two phases. The first occurs within 24 h of administration of the drug and is considered to be a result of its cholinergic effects. This diarrhoea is treated with atropine and, in most cases, is not severe.[110] The second phase of diarrhoea develops 5–11 days following administration of irinotecan.[110] This phase of diarrhoea is more prolonged and can be potentially life-threatening due to severe dehydration and resulting electrolyte imbalance.[110,113] With respect to the late onset diarrhoea, the toxic effects of irinotecan are thought to be potentiated by factors related to the intestinal microflora.[110] Following conversion by carboxylesterase to its active metabolite SN-38 in the liver, detoxification occurs which involves glucuronidation to form the inactive SN-38G. SN-38G is secreted into the bile and, from there, is emptied into the small intestine. Within the lumen of the small intestine, it has been demonstrated that bacterial β-glucuronidase can convert SN-38G back to the active SN-38, where it causes direct damage to the epithelial cells of the intestinal mucosa. Antimicrobial strategies to alter the intestinal microflora have been trialled in order to reduced the levels of bacterial β-glucuronidase in the intestine and therefore reduce the degree of mucosal damage.

The cytotoxic effects of irinotecan are also potentiated by the fact that TNF production is increased by the drug.[114] The DNA damage that is caused by irinotecan, in addition to elevated TNF, activates NF-κB thereby effecting tumour cell death via pro-apoptotic pathways. This also has important implications in the pathobiology of mucositis. However, as already mentioned, NF-κB activation can have antiapoptotic effects with respect to neoplastic cells. It is thought that tumour resistance to irinotecan may be enhanced by pathways promoting cell survival and also counteract the effect of TNF activation.[115]

### Methotrexate

Methotrexate (MTX) is a commonly used drug for the treatment of various neoplastic diseases as well as some inflammatory diseases (for example, rheumatoid arthritis).[116] It belongs to a class of drug that acts by antagonising folate, although its exact mode of action is not fully understood.[117] MTX has anti-inflammatory effects that are a result of various molecular events including decreased gene expression of pro-inflammatory cytokines IL-1, 2, 6 and interferon-γ[118]. It also inhibits COX-2 synthesis and chemotaxis of neutrophils.[118] In addition, Majumdar and Aggarwal demonstrated that, in Jurkat cells, MTX caused suppression of NF-κB that was induced by inflammatory factors such as TNF.[117] All of this evidence helps to explain the beneficial effect of MTX in treatment of inflammatory diseases, however does not indicate how the development of mucositis might occur in the AT associated with MTX administration. It is well documented that MTX causes oral mucositis[116] and damage to the small intestinal mucosa.[108] In spite of its reported anti-inflammatory effects, MTX has been demonstrated to have other actions that may explain its associated toxicity, particularly in the gastrointestinal mucosa. For example, MTX administration causes apoptosis of intestinal epithelial cells in rat models of mucositis.[119] MTX-associated apoptosis specifically occurs in the intestinal crypts and causes villous atrophy.[108] In addition, damage to goblet cells in the gastrointestinal epithelium as a result of MTX administration may reduce the natural, non-specific defences of the epithelium by reducing mucin secretion and other proteins such as the trefoil factors that have important protective roles in the gut.[57,88] Also, in the context of mucositis following MTX administration, production of TNF by mucosal T cells and macrophages is increased in response to LPS derived from commensal gut flora.[120] This indicates that the immune cells within the mucosa may, in themselves, contribute to mucositis development.[120]

### 5-Fluorouracil

5-FU is a commonly used chemotherapeutic agent and well documented to cause oral mucositis. This drug is a uracil analogue and its major effect is to inhibit nucleotide metabolism. 5-FU has been demonstrated to inhibit NF-κB activation.[121,122] It is therefore likely that the pathways through which mucosal damage is mediated is different from the other, previously described cytotoxic drugs. Pritchard

et al.[123] demonstrated in a murine model that the intestinal toxicity of 5-FU results due to the combined effects of apoptosis and inhibition of cell proliferation. These events led to reduced cellularity in both the intestinal crypts and villi. Furthermore, the same authors demonstrated that the changes that occurred in the intestinal mucosa in mice subsequent to 5-FU administration were p53 dependent.[123] Leitão et al. investigated the role of nitric oxide (NO) in the pathogenesis of 5-FU-induced experimental oral mucositis using a hamster model.[76] NO is known to induce apoptosis in various cell types and is associated with p53 and changes in the expression of members of the Bcl-2 family.[124] Therefore, whilst NF-κB may not be a significant factor in 5-FU associated mucositis, alternative pathways leading to apoptosis appear to be involved in causing mucosal damage. The exact mechanism, however, behind the development of 5-FU induced mucositis remains to be fully determined.

### Combined chemotherapy and radiotherapy

The treatment of haematological diseases has used radiation, in the form of total body irradiation, is combined with chemotherapy for myeloablation prior to bone marrow or stem cell transplantation. More recently combined modality treatments have been investigated for other forms of neoplastic disease. In the case of head and neck squamous cell carcinoma, for example, standard treatment of surgery plus or minus radiotherapy and/or chemotherapy has an overall 4-year survival rate of 30–40%.[125] In the specific case of tongue cancer for example, a recent study which looked at data collected between 1987 and 2004 found that 5-year survival rates were between 30% and 50%.[126] Moreover, this survival rate had not improved during the 18-year period studied. Such statistics have prompted the investigation of other methods or combinations of treatment to try and improve survival rates.[125] Furthermore, surgery can be disfiguring (particularly in the head and neck region), affect function and consequently result in significant problems in the quality of life for patents following treatment. Treatment strategies that preserve organ function and minimise the need for invasive and disfiguring surgery have led to the use of combined modality treatments employing both chemotherapy and radiotherapy.[127] Whilst treatment outcomes may be improved by more aggressive treatment strategies, this benefit comes at a cost of increased risk of toxicity.

As mentioned elsewhere in this review, it is well established that both radiotherapy and chemotherapy can cause mucositis and, when combined, the prevalence of mucositis is increased. Woo et al. demonstrated that, in the context of bone marrow transplantation, oral ulceration occurred in over 75% of patients.[128] Numerous studies reporting various combinations of combined chemoradiotherapy or induction chemotherapy followed by different schedules of radiotherapy have been reported in the literature.[125,129–131] Comparison between these various studies is complicated because of the number of variables involved. In addition many single-institution studies have limited numbers of patients enrolled in the trials making conclusive judgements about the value of the results difficult. The prevalence of mucositis in the studies however is consistently high. It should be noted that there are also difficulties in comparing rates of mucositis due to the variability in the clinical assessment of mucositis and grading scales that are used by respective studies. One study, investigated the treatment of 127 patients with advanced squamous cell carcinoma of the head and neck cancer, Hanna et al. reported that 64% of patients developed mucositis, and 33% had grade 3–4 mucositis.[127] All of the patients in the study received standard fractionation radiotherapy concurrently with at least 2 cycles of cisplatin and 5-FU. Other gastrointestinal symptoms reported in the study that could be attributed to AT mucositis included severe nausea, dehydration and electrolyte imbalance which required intravenous or enteral fluid replacement.[127] Other studies have reported similar results.[125,129–131] The benefit in survival as a result of these new treatment regimens is variable.

## Conclusions

Whilst there has been a great deal of work conducted into the pathogenesis of mucositis, the role of pro-inflammatory cytokines needs to be further defined. No studies have investigated the role of pro-inflammatory cytokines and the development of mucositis along the entire AT. Furthermore, the complex interplay between the cytokines themselves and the cytokines and treatment modality needs to be elucidated. Certainly it is clear that different cytotoxic drugs activate different molecular pathways and, whilst the clinical outcomes may be similar, the routes leading to those outcomes may be vastly different. This has important implications for the development of targeted treatment for AT mucositis. Further characterisation of the biological events occurring in the context of mucositis will inevitably lead to improved treatment outcomes and quality of life for patients undergoing cancer treatment.

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