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Mucosal barrier injury: biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview

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Summary:

Mucositis is an inevitable side-effect of the conditioning regimens used for haematopoietic stem cell transplantation. The condition is better referred to as mucosal barrier injury (MBI) since it is primarily the result of toxicity and is a complex and dynamic pathobiological process manifested not only in the mouth but also throughout the entire digestive tract. A model has been proposed for oral MBI and consists of four phases, namely inflammatory, epithelial, ulcerative and healing phases. A variety of factors are involved in causing and modulating MBI including the nature of the conditioning regimen, the elaboration of pro-inflammatory and other cytokines, translocation of the resident microflora and their products, for example, endotoxins across the mucosal barrier, exposure to antimicrobial agents and whether or not the haematopoietic stem cell graft is from a donor. Neutropenic typhlitis is the most severe gastrointestinal manifestation of MBI, but it also influences the occurrence of other major transplant-related complications including acute GVHD, veno-occlusive disease and systemic infections. The pathobiology, clinical counterparts and the means of measuring MBI are discussed together with potential approaches for prevention, amelioration and, perhaps, even cure. *Bone Marrow Transplantation* (2000) **25,** 1269–1278.
Keywords: mucositis; mucosal barrier injury; diagnosis; risk factors; treatment

Mucositis is an inevitable side-effect of the intensive conditioning therapy used for haematopoietic stem cell transplantation[1] and usually refers to the mucosal ulceration of mouth and throat. However, it is generally accepted that oral mucositis is in reality the most obvious manifestation of damage or injury elsewhere particularly that of the gut. Hence, mucosal barrier injury (MBI) may be a more appropriate term for this biological process. There exists no clear definition of MBI which is defined by a constellation of signs and symptoms that vary in their clinical expression. Oral MBI is reported to affect 60% to 100% of transplant recipients[2,3] and is characterised by pain, oedema, erythema, lesions, pseudomembrane formation, excessive mucous production, reduced saliva and bleeding, all of which reduce the patient's ability to eat and drink. In contrast, there are no reliable data on the incidence of gut MBI although intestinal symptoms affect almost every transplant recipient to some extent and include nausea, vomiting, abdominal cramping and watery diarrhoea occasionally accompanied by macroscopic blood loss. The exact course and severity of bowel symptoms of MBI are also difficult to ascertain because many patients are in such pain due to oral MBI that they only gain relief from narcotic analgesia which induces constipation as a result of reduced gut motility. There are also a number of scoring systems for oral MBI[4] although none is universally accepted and all lack standardisation. As yet, there is no system for registering gut MBI although there are published definitions for grading toxicity of individual signs and symptoms. Consequently, much more is known about the course of oral MBI than its intestinal counterpart. Oral MBI is known to begin around the time conditioning therapy is completed, and has been shown to worsen until a peak is reached after which it declines gradually until resolving completely. The onset and duration of mucositis has also been shown to mirror the course of neutropenia[5] (Figure 1). This phenomenon may not be peculiar to any one specific regimen. It would therefore be of considerable interest were gut MBI shown to follow a similar course to oral MBI.

MBI is a complex process that diminishes the quality of life and can predispose to more serious clinical complications including disseminated infection, veno-occlusive disease, acute graft-versus-host disease (GVHD) and even death. However, it seems more likely that gut MBI rather than oral MBI will present a greater risk to the patient even though it goes largely unrecognised.

A pathobiological model for oral mucositis has recently been proposed which attempts to incorporate and explain all that is currently known about oral MBI.[6] This model describes four successive phases: (1) an inflammatory phase followed by (2) an epithelial phase leading to (3) an ulcerative/bacteriological phase and ultimately resolving in (4) the healing phase. This model could also be applicable to the gut as a whole even though it is a more complex organ having a dynamic epithelial border with different functions and unique interactions with immune system and luminal microflora[7] (Figure 2).

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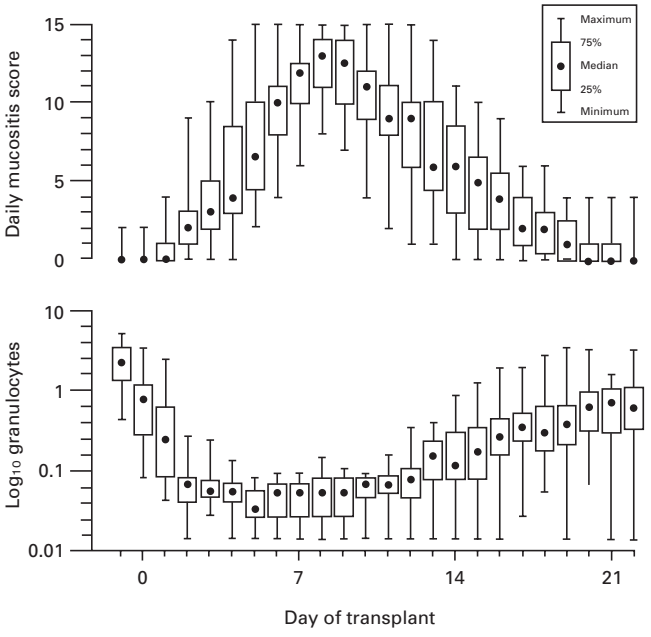

Figure 1 The relationship between oral mucositis and neutrophil counts of allogeneic haematopoietic stem cell transplant recipients. Twenty-eight patients received idarubicin, cyclophosphamide and TBI 9 Gy as conditioning therapy for an allogeneic haematopoietic stem cell transplant. The course of mucositis closely mirrors that of neutropenia. Donnelly *et al*[5] 1992.

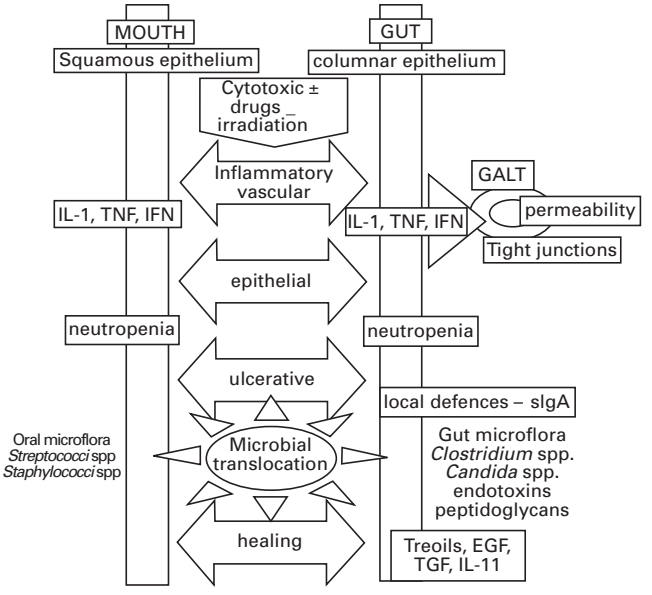

Figure 2 Mucosal barrier injury. Mucosal barrier injury occurs in four phases. The first phase is the inflammatory/vascular phase and is characterised by the induction of pro-inflammatory cytokines IL-1, TNF-alpha and IFN-gamma by cytotoxic drugs and irradiation while the epithelial cells are still intact. The second phase is the epithelial phase when cells cease dividing and die. This coincides with neutropenia. The third phase is when necrosis and ulceration occur and is when the resident microbial flora and their products, eg endotoxin translocate into the bloodstream. Moreover, impaired local defences and lower levels of secretory IgA may allow local infection to develop. The final phase is when healing takes place and involves the action of naturally occurring substances including trefoils, EGF and TGF. The events that take place in the gut are almost certainly more complicated than those occurring in the oral cavity since the gastrointestinal tract is intrinsically more complex in terms of its function, it possesses the specialised gastrointestinal-associated lymphoid tissue (GALT) system, and its resident microflora are more numerous and varied.

Pathobiology

The inflammatory phase

Radiation and cytotoxic drugs induce the systemic release of pro-inflammatory cytokines, interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) from activated macrophages and monocytes. Ionising radiation also induces cytokine gene expression directly.[8] TNF-α and its receptors are activated and suggest that resident tissue macrophages and monocytes rather than circulating polymorphonuclear (PMN) cells are the main target *in vivo*.[9] Tissue macrophages are not eliminated by conditioning therapy and may persist up to 4 months after transplantation. In the gut, macrophages reside in the gastrointestinal-associated lymphoid tissue (GALT) which houses the vast majority of total circulating lymphocytes as well as other members of the lymphoreticular system such as monocytes and intraepithelial lymphocytes. Once released into the circulation, the cytokines increase expression of HLA histocompatibility antigens and critical adhesion molecules that amplify local tissue injury by inviting PMN cells and activated lymphocytes to invade.[10] This results in increased vascularity and probably higher local levels of cytotoxic agents. In an animal model, exposure to bleomycin or 5-fluorouracil (5-FU) resulted in increased cellularity of subepithelial oral tissue, vascular dilation and leukocyte margination within 24 h.[11] The generation of cytokines is self-limited during autologous transplantation and resolves within 7–10 days.[10]

The inflammatory response may be specific to different classes of chemotherapeutic agents and to the particular sequence of preparative regimens used in haematopoietic stem cell transplantation since a variety of different profiles of cytokine release have been reported.[12–14] For instance, elevated TNF-α levels were found in 13 (24%) of 56 patients given either cyclophosphamide and total body irradiation or cyclophosphamide and busulphan and these levels were predictive for transplant-related complications within the first 6 months post BMT.[14] Serum levels of TNF-α and IL-1β have also been shown to be markedly higher with higher doses of TBI 1 week after transplant.[13] In contrast, busulphan, VP-16 and cyclophosphamide induced interferon-gamma production directly.[12,15] Moreover, intestinal damage manifest by villous blunting, apoptosis and brush border loss (the surface area of villous cells amplified by numerous finger-like microvilli) correlated well with cytokine levels.[13]

In a clinical phase I/II study, use of the monoclonal antibody MAK 195F diminished the release of TNF-α but it was also observed that the kinetics of TNF–anti-TNF complexes were different after conditioning therapy with cyclophosphamide and TBI compared with cyclophosphamide and busulphan, which actually induced less TNF-α release.[16] Elevated cytokine levels detected as early as 1 week after transplantation might be related to engraftment in the absence of complications or to infectious disease,

non-infectious events or GVHD rather than MBI.[17] Moreover, other investigators have failed to find elevated levels of cytokines during or shortly after conditioning therapy.[12,17,18]

Before total cell destruction, TNF-α, IFN-γ and IL-1 induce major changes in the functionality, permeability, brush border transport, glutamine utilisation (glutamine is the main source of energy for intestinal cells) and mucosal cell integrity.[19–22] IFN-γ and TNF-α induce dose-related cellular exfoliation, leading to the formation of a mucoid cap in a vain attempt to protect the mucosa.[23]

Epithelial cells are also capable of producing and secreting TNF-α and IL-1α.[24] In an H-2-incompatible transplanted SCID mice model, colonic TNF-α, IL-1α and IL-6 appeared 4 h after TBI and peaked by 24 h. If no transplantation followed, TNF-α and IL-1α levels decreased rapidly 3–5 days later.[17,18,25] Epithelial cells are also capable of mounting an immune host response, and of taking up, processing and presenting soluble antigens as well as expressing MHC class II molecules.[26] Thus, taken together, these data support the view that the primary step in MBI is an inflammatory response.

The epithelial phase

Cytotoxic drugs and radiation interfere with rapidly dividing cells and the kinetics of proliferating mucosal cells influence their sensitivity to these agents. Normally, cell renewal takes place continuously in crypts from a proliferating pool of clonal undifferentiated stem cells and cell division is completed in about 24 h.[27] Younger cells migrate up to the villous tips and slough off into the lumen at the extrusion zone. Anti-metabolites, for example cytarabine, are cell cycle-dependent and interfere with the synthesis of DNA in dividing cells whereas the intercalating agents such as the anthracyclines are more effective during the G2 phase after mitosis is complete when the cell has time to restore errors. In contrast, alkylating agents such as cyclophosphamide generate lethal DNA lesions even in resting cells by forming cross-links between DNA strands while ionising radiation exerts its main effect during mitosis. Various chemotherapeutic drugs such as adriamycin, bleomycin and 5-FU increase cellular sensitivity to radiation in a synergistic manner.[28] This has also been observed clinically when idarubicin was given at the same time as cyclophosphamide and TBI.[29]

Normally, the entire epithelium is renewed in 4–6 days, but decreased cell renewal is thought to lead to mucosal atrophy, thinning and necrosis although in rats, sublethal doses of alkylating agents mainly induced lower absorption rather than villous atrophy.[30]

The ulcerative-bacteriological phase

Increased redness and swelling of the mucosa and underlying tissue are usually the first signs of oral MBI, mainly due to increased vascularity and vascular permeability (inflammatory phase) and thinning of epithelium (epithelial phase). This process usually culminates in the ulcerative phase within about 14 days of starting chemotherapy.[1] It is also during this phase that the resident microflora are assumed to play a role. Normally, these microorganisms contribute to maintaining the integrity of the integument and prevent pathogenic microbes from gaining a foothold. The ecological system tries to maintain its balance but once the mucosa is damaged, microbes may infect the submucosal tissue. Non-pathogenic streptococci specifically bind to, and use, the glycoproteins in the dental plaque that develops in the absence of normal food intake and saliva production. The non-cellular defence depends on amount and quality of mucus and saliva produced which contain diverse host defence peptides (defensins), lactoferrin, lysozyme and immunoglobulins.[31] Secretory IgA inhibits bacterial adherence, specifically of oral streptococci, neutralises toxin and virus, prevents antigen uptake and possesses anti-inflammatory activity.[32] Several classes of host defence peptides can be found in saliva and on surfaces each possessing rapid lytic activity against the membranes of Gram-positive and Gram-negative bacteria as well as yeasts.[33] During conditioning therapy and after transplantation, the salivary immunoglobulins (IgA, IgG, IgM) have been shown to be lower than normal and the elimination of T cells from engrafted bone marrow results in less initial capacity for immunoglobulin production and secretion.[34] Thus, the local immune defences of the oral cavity are impaired.

It is common practice to administer antimicrobial agents, particularly the fluoroquinolones and local antiseptics such as chlorhexidine, to haematopoietic stem cell transplant recipients, leading inevitably to marked shifts in the resident oral flora towards the more resistant species particularly the viridans (alpha-haemolytic) streptococci. This shift is more profound in patients with overt oral mucositis.[35] There has also been a corresponding increase in bacteraemia due to these streptococci with oral mucositis being an important risk factor in autologous haematopoietic stem cell transplant recipients.[36] Similarly, Donnelly *et al*[37] reported a higher incidence of viridans streptococcal bacteraemia due to the marked mucositis associated with treatment intensification. One particular species, *Streptococcus mitis*, is apparently associated with sepsis and adult respiratory distress syndrome (ARDS), mainly after high-dose cytarabine.[38,39] This syndrome could be provoked by changes in the pulmonary endothelium and lung macrophages induced by cytotoxic chemotherapy which, in turn, induces cytokine production perhaps triggered by infection with *Streptococcus mitis*.[40] The stomach or small intestine could also be a portal of entry if colonisation with these streptococci occurs as a result of the achlorhydria induced by H_2 histamine antagonists and proton pump inhibitors since the use of these agents has been noted as a risk factor for the so-called 'alpha-strep syndrome'.[38] Obviously, MBI is itself a risk factor for viridans streptococcal bacteraemia but it might not always indicate systemic infection since transient bacteraemia also occurs in healthy persons after dental manipulation.[41] Moreover, these bacteria do not elaborate exotoxins nor are they professional pathogens. Thus, viridans streptococcal bacteraemia might simply signal the presence of mucosal barrier injury rather than infection.

Although, to some extent, similar to the oral cavity, the gut harbours a much more complex ecosystem comprising

a greater variety of aerobic and anaerobic bacteria that share a symbiotic relationship with the host. This relationship plays an important role in maintaining the gut's histological structure and also provides so-called 'colonisation resistance', ie the ability of the gut to repel foreign bacteria. The intestinal microflora depends on prebiotics, the fibrous nutrients[42] that enhance probiotic bacteria, like bifidobacteria, lactobacilli and *Clostridium* species. These are the species that are thought to provide the colonisation resistance by elaborating antibacterial compounds and competing for nutrients so preventing overgrowth by potentially pathogenic bacteria.[43] These probiotic bacteria also produce nutrients for mucosal cells. Certain antimicrobial agents, particularly those that affect cell wall synthesis, exert a major impact on the gut's ecosystem by destroying the 'protective' anaerobic flora particularly the probiotic bacteria. When the gut epithelium is disrupted, bacterial translocation occurs and pro-inflammatory bacterial oligopeptides, especially endotoxin (lipopolysaccharide or LPS) readily gain access.[44] In the normal host (whether animal or human) pathogenic bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* penetrate the mucosa and migrate to extra-intestinal sites such as the mesenteric lymph nodes, spleen and liver. The GALT system, together with the Kupffer cells of liver and spleen serve as a backup to trap endotoxins and kill bacteria. The rate of translocation of enterobacteria like *E. coli* and other gram-negative bacilli such as *Pseudomonas aeruginosa* is strongly associated with the degree of neutropenia.[45] Microbial translocation is exacerbated by irradiation[46] and chemotherapy[47] as microorganisms can be cultured in extra-intestinal sites as well as in blood.[48] Different modes of translocation exist and occur even before any histological damage is apparent. Anaerobic non-pathogenic bacteria rarely translocate but yeasts such as *Candida albicans* can do so more easily when disruption has occurred.[49] Endotoxin can be transported through the lymphatic channels, bypass the liver or enter the peritoneal cavity directly and can cause systemic endotoxaemia.[50] Endotoxin can also increase intestinal permeability directly[51] or by stimulating primed macrophages to release an excessive amount of cytokines, mostly TNF-α, thereby inducing mucosal inflammation and increasing permeability.[52] Higher levels of circulating endotoxin are obtained after giving intensive TBI containing regimens[13] suggesting that persistent low-grade endotoxaemia or the inflammation associated with MBI induce fever of unknown origin since endotoxaemia and gut mucosal damage occurred in 44 (70%) of 63 HSC transplant recipients (both allogeneic and autologous) all of whom developed fever that could not be explained by infection.[53]

Peptidoglycan (the major component of the cell wall of Gram-positive bacteria) may play a similar role as endotoxin as it is also biologically active in tissues and may induce a pro-inflammatory response.[54] Although much less potent than endotoxin gram-for-gram, large amounts of peptidoglycan may well be released into the circulation when gut MBI is present simply because there are many more Gram-positive than Gram-negative bacteria in the gut. Exposure to antibiotics that cause lysis will also liberate cell wall fragments.

Neutropenic typhlitis, a paradigm for gut MBI

Typhlitis, also called neutropenic enterocolitis, necrotising enterocolitis or ileocaecal syndrome, is a caecitis often extending to both the proximal and distal caecum that may be primarily a severe manifestation of gut MBI. Indeed, all factors that contribute to the development of MBI are present clinically. First, typhlitis occurs after the administration of cytotoxic drugs, particularly high-dose cytarabine, etoposide and anthracyclines at the nadir of neutropenia and thrombocytopenia. Secondly, prolonged exposure to antibiotics results in a marked shift in the gut microflora towards toxin producing bacteria[55] such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Clostridium septicum*.[56,57] In fact, *Clostridium* species are now more likely to predominate for reasons which are poorly understood so that bacteraemia due to *C. tertium* or *C. septicum* is almost pathognomonic for typhlitis. Antimicrobial pressure also predisposes to intestinal overgrowth by *Clostridium difficile* in transplant recipients.[58] Necrosis of the mucosal surface of the ileocaecal region probably provides a favourable environment for the spores of *Clostridium* species to germinate and may be their portal of entry into the bloodstream. The pathogenesis of typhlitis would therefore seem to require various elements to be present simultaneously, namely gut MBI, a perturbed resident microflora and profound neutropenia. Typhlitis is not only a paradigm for MBI but, because of the high mortality rate, it is also the most severe clinical form of MBI and deserves more attention both in terms of developing techniques for early diagnosis as well as in evolving strategies for prevention and treatment. Consequently, we can expect to encounter more cases of typhlitis as chemotherapeutic regimens become more intense.

The healing phase

In general, the repair of oral MBI parallels haematological reconstitution as peripheral blood counts return to normal[3,5,59] with complete resolution occurring within 2–3 weeks.[60] In contrast, gut function does not return to normal for several more weeks, since malabsorption and diminished enzyme activity still persist even after structural repair. The healing of mucosal damage probably occurs in two phases commencing with the restitution of mucosal integrity and then remodelling of the mucosal architecture. The mucosal repair process depends on the severity of damage since superficial injury can be repaired rapidly by epithelial cell migration without mitosis.[61] However, proliferation in conjunction with angiogenesis is necessary for deep lesions, involving large areas of necrosis, to recover.[62] Trefoil peptides (mucin-associated peptides) are secreted by epithelial cells, with each region of the gut probably having its own variant.[62] These peptides act as rapid response molecules to injury by promoting cell migration, cell differentiation and wound healing.[63] Epidermal growth factor, transforming growth factor alpha, interleukin-11 and fibroblast growth factor also appear to promote epithelium repair and regeneration.[64] In contrast, these agents appear to play only a limited role in the healing of the oral mucosa.

Acute graft-versus-host disease

Gut MBI may evolve into acute GVHD since tissue damage caused by conditioning regimens plays a role in both conditions. Any mature donor T lymphocytes within the allograft that recognise host-antigens are activated by endotoxin and pro-inflammatory cytokines.[10,13] Animal studies[25] and some studies in humans[13,14] suggest that high levels of pro-inflammatory cytokines predispose to acute GVHD, although others could not confirm this observation.[12,15,17,65] The early administration of the anti-TNFα monoclonal antibody MAK 195F changes the nature of the inflammatory response, reduces the number of febrile episodes and delays the onset and severity of acute GVHD.[16] The microflora might also play a role in triggering acute GVHD because less disease was observed in decontaminated murine chimeras.[66,67] Intestinal decontamination with metronidazole also significantly reduced the severity of acute GVHD in HLA-identical sibling transplants.[68] Taken together, these data suggest a role for MBI in triggering acute GVHD because of either the release of cytokines induced by conditioning regimens or the translocation of microbial toxins.

Diagnostic tools

Intestinal permeability

Permeability refers to the property possessed by a epithelium that enables passage of a solute by unmediated diffusion.[69] Permeability can be measured *in vivo* by means of the urinary excretion of test substances or by detecting their presence in blood. Lactulose, various polymers of polyethylene glycol (PEG) or ^{51}Cr-labelled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) have all been used, but the results are markedly influenced by extraneous factors such as bowel transit time, gastric emptying and renal function. Nonetheless, permeability to ^{51}Cr-EDTA is increased as soon as 2 days after starting conditioning therapy and continues to increase until shortly after BMT, about 12 days later.[70] Others have shown that the intestinal toxicity induced by melphalan can be monitored using ^{51}Cr-EDTA thus allowing the effectiveness of various treatments for reducing the intestinal toxicity to be assessed.[71] Unfortunately, ^{51}Cr-EDTA is radioactive and not suitable for routine use.

The uptake of antibiotics such as gentamicin and tobramycin may provide a safer means of determining increased permeability since such drugs are normally excluded by the intact gut but can be detected in plasma during mucositis when given by mouth.[72] Studies of epithelial cell handling of cytotoxic drugs, radiation and antimicrobial agents could offer new possibilities for documenting MBI.[73,74]

Sugar absorption tests

The principal features of gut MBI are a loss of epithelial surface and a change in the permeability.[69] This can also be measured if at least two different probes are used at once since the extraneous factors equally affect the pre- and post-mucosal determinants and the urinary excretion ratio becomes an index of intestinal permeability. For example, monosaccharides such as mannitol and rhamnose are absorbed through aqueous pores in the cell membrane whilst disaccharides like lactulose gain access through the tight junctions located at the upper end of adjacent epithelial cells. Tight junctions are dynamic structures exerting physiologic control over the flow of solutes through paracellular spaces and play an important role in gut permeability. Reduction of urinary monosaccharide excretion represents a loss of epithelial cell surface area, while increased urinary disaccharide excretion indicates damage to the tight junctions. Sugar absorption tests (SAT) have proved their value in intestinal diseases but they lack diagnostic specificity.[75] SATs offer an easy, reliable means of assessing the onset, duration and severity of gut MBI in patients treated with cytotoxic agents. Absorption is increased after only 2 days treatment with chemotherapy[76] suggesting that cytokines might interfere with the tight junctions (see *inflammatory phase*) rather than directly inhibiting cell proliferation, which tends to occur later.[77] Altered permeability continues to progress until reaching a peak about 7 days after conditioning therapy has been completed[78] and returns to normal about 4 weeks later.[79] This mirrors the oral MBI and neutropenia (Figure 1).

It should be possible to discriminate patients at risk of developing serious toxicity to therapy from those not at risk by using these SATs since a positive correlation was found between progressive non-oral clinical toxicity and increased permeability in transplant recipients.[70,71] Bow *et al*[80] also found that the absorption of D-xylose was at its lowest 2–3 weeks after remission induction treatment had been started in patients who developed systemic candidosis. Malabsorption of D-xylose was also found to be an independent predictor of neutropenic enterocolitis and hepatosplenic candidosis and also correlated well with bacteraemia.[80,81] As yet, there are no objective means of determining gut MBI in use routinely and none has been validated for use in clinical trials to assess gut toxicity although the data available suggest SATs may be useful for helping adapt supportive care regimens for selected patients in order to reduce morbidity and perhaps mortality.

A consensus in the way MBI is measured in clinical practice analogous to the validated scoring system of oral mucositis of the Mucositis Study Group[82] is a prerequisite for such studies. There is also a pressing need for much simpler tests of each phase of MBI. Data from chemotherapy-induced cytokine expression of epithelial cells in single-cell testing or cell lines, quantitation of cytokine profiles in saliva or stools[83] and the results of basal cell kinetic studies like grading of epithelial cell viability by trypan blue dye exclusion obtained by oral washings[84] should be incorporated in clinical research and care. It should be possible to demonstrate overt mucositis using radionuclide imaging techniques, such as indium-labelled leukocytes[85] and technetium-labelled diphytanoylphosphatidylcholine liposomes but there are only a few anecdotal reports and their clinical feasibility is expected to be minimal.

Intervention and treatment of mucosal barrier injury

Nutrition

Enteral nutrition stimulates gut-responsive hormones, prevents mucosal atrophy, improves mucosal blood flow and gastrointestinal motility, stimulates mucus formation and secretion of sIgA and reduces bacterial translocation.[86] In children without severe MBI, enteral and parenteral nutrition were equally effective in maintaining the nutritional status[87] and a diet containing lactose and bovine milk protein appeared to be well tolerated.[88] Oral administration of short-chain fatty acids (SCFAs) typically produced by the anaerobic flora of the gut reduces the inflammation and necrosis induced by cytarabine in mice.[89] These SCFAs are normally produced by the fermentation of dietary fibre and unabsorbed starch by the same gut microflora and are the preferred fuel of enterocytes.

Total parenteral nutrition (TPN) is advocated for those patients who are either malnourished or who are expected to have inadequate oral intake for a prolonged period (usually 7–10 days) to restore the negative nitrogen and caloric balance. These patients are typically those with MBI that is sufficiently severe that it impedes adequate enteral nutrition leading to malnutrition, weight loss, malabsorption and micronutrient deficiencies. TPN does help to reduce the morbidity of malnourished patients completing a course of myeloablative therapy,[1] but at the same time it promotes villous atrophy, increases intestinal permeability, reduces luminal sIgA content and enhances bacterial translocation.[90] Nevertheless, the long-term outcome for allogeneic HSC transplant recipients is better with TPN, even when they are well-nourished[91] whereas autologous HSC transplant recipients gain little or no benefit.[92]

Glutamine

Glutamine has attracted a lot of attention because it is the primary fuel for intestinal epithelia and the cornerstone of protein and nucleic acid synthesis but mucosal cells cannot synthesise enough themselves making glutamine conditionally essential during stress.[93] Administering glutamine to animals after irradiation and chemotherapy prevented mucosal atrophy and reduced bacterial translocation, endotoxaemia and infections.[94–96] It is much less clear whether glutamine given orally prevents human oral MBI,[97,98] although patients treated with high-dose chemotherapy experienced less diarrhoea.[99] Glutamine supplementation given to HSC transplant recipients parenterally helps to preserve hepatic function, reduces the length of stay in hospital, improves the nitrogen balance and lowers the infection rate[100,101] but has no influence on the occurrence of mucositis or fever.[102,103] However, nothing is known about the effect of glutamine on gut integrity or function since permeability tests were not performed.

Cytoprotectants

Direct cytoprotectants such as sucralfate and diphenhydramine do not ameliorate oral MBI[1,104–106] whereas indirect cytoprotectants, like transforming growth factor β3 and epidermal growth factor, interfere with epithelial cell replication in animals and are being tested in clinical trials for their efficacy and safety in modulating oral MBI.[107,108] Recombinant-human GM-CSF given as a mouthwash shortened the duration of severe oral MBI[109,110] but the mechanism of action remains unclear. GM-CSF might have a direct pleiotropic effect on epithelial cell kinetics. Alternatively, the effect may be indirect as a result of the first neutrophils produced by haematopoietic progenitor cells migrating to the oral mucosa and thereby reducing local infection.[111] Other clinical trials exploring the effects of recombinant growth factors such as transforming growth factor-β1 or TGF-β3 and others on MBI are coming.[112–114] A clearly different approach consists of delivering monoclonal antibodies that bind and inactivate doxorubicin (MAD11) in intestinal cells.[115]

Antimicrobial agents

It is common practice to try to reduce the bioburden of gram-negative bacilli in the oral cavity by giving antimicrobial agents, and maintaining good oral hygiene and also to provide remedial dental treatment when necessary to reduce oral complications.[116] Antibiotic lozenges containing tobramycin, polymyxin and amphotericin B reduce oral MBI[117] but the effect of chlorhexidine is unclear.[118–121]

There have been no formal studies of the effect of antimicrobial agents whether given for prophylaxis or treatment on MBI although it is usually assumed that they are beneficial. If MBI is not primarily the result of infection as seems to be the case, treatment with antimicrobial agents is unlikely to be of benefit and may even prove harmful in exerting selective pressure on the resident flora. Probiotics may help restore the balance of gut flora in cancer patients[122] but trials of sufficient size are lacking. IgM-enriched immunoglobulin has been shown to reduce endotoxaemia and febrile episodes in transplant recipients.[53]

Future directions

Mucosal barrier injury is far more than simply a toxicological side-effect of cytotoxic regimens. Enough evidence exists to indicate that MBI is a complex and dynamic pathological process but it is essential to understand its nature more fully. A model for oral MBI already exists and shows that it is the net result of an almost complete breakdown of the epithelium initiated by the release of pro-inflammatory cytokines induced by the cytotoxic drugs followed by an arrest of the mucosal cell cycle and inhibition of repair leading to apoptosis. Infection, if it plays any role at all, is largely secondary. This model may go some way to explain the corresponding phenomenona in the gut although gut MBI is likely to be much more complex and more difficult to unravel mainly because the damage cannot be seen and the signs and symptoms are too imprecise.

Since gut permeability increases very soon after exposure to chemotherapy and irradiation it seems logical to pursue tests such as the SATs further and to look for other chemical probes. Certainly, a means of objectively

monitoring MBI is necessary before drug products can be formally tested for their effects on MBI.

At this moment there are several products ranging from cytokines and defensins to nutrients and probiotics which look promising. For example the growth factor interleukin-11 (IL-11) by reducing pro-inflammatory cytokine expression and secretion by macrophages (phase I), prevents apoptosis of intestinal crypt cells partially by inhibiting proliferation (phase II) and promotes recovery of these crypt cells while remodelling connective tissue (phase IV). Defensins, trefoil peptides and even sIgA-antibodies could offer additional tools to tackle hostile microbes, for example, IgA-IgG administered orally has been shown to reduce gut MBI in patients undergoing intensive cytotoxic therapy.[123]

With the means of reliably detecting and monitoring gut MBI at our disposal, the process will graduate from being an expected although unpleasant side-effect with few therapeutic options to a condition that might actually be preventable.

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