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INIVITED MEDICAL REVIEW

New thoughts on the initiation of mucositis

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It has been slightly more than a decade since the classic mechanistic paradigm that defined the pathogenesis of mucositis was revised. A five-stage sequence of linked biological events forms the basis for our current understanding of how regimen-related mucosal injury occurs. The first stage is the initiation phase, although the gateway to toxicity has been the least studied. This essay proposes new thoughts on the phase's components, how they might interact, and how they present new opportunities for treatment interventions and mucositis risk prediction.

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Introduction

Mucositis remains one of the most common and troubling side effects of antineoplastic radiation and drug therapy (Sonis, 2009). Virtually, every patient with an oral cancer who receives chemoradiation can expect to develop the confluent painful and deep mucosal ulcerations that characterize the condition. And, the patient's health insurer can expect to add almost $18 000 to the cost of treatment to support direct and indirect management of the condition (Nonzee *et al*, 2008). Mucositis has been most tenacious in its ability to avoid effective prevention or cure. But that could change soon. The recognition that mucositis evolves, not only through direct cell injury mediated by chemotherapy or radiation, but more significantly as a consequence of a complex cascade of biological events, has provided numerous targets for intervention that are now being exploited. Lest we get too cocky or complacent though, we need to understand that we still have ways to go to fully map each mechanistic step. And furthermore, we have yet to take advantage of mechanistic clues to develop an effective algorithm to predict patient risk and ultimately develop treatments.

It has been 11 years since the conventional pathogenic paradigm for mucositis was first challenged (Sonis, 1998). Prior to 1998, dogma dictated that radiation- and chemotherapy-induced epithelial damage was solely the consequence of nonspecific injury targeted at rapidly dividing basal stem cells (Lockhart and Sonis, 1979). I should point out that our use of 'stem cells' has been incorrect as they do not have the pluripotential capabilities that characterize true stem cells (Smith *et al*, 2009). Nevertheless, we thought that radiation or chemotherapy destroyed the basal cells, they stopped dividing, epithelium no longer was renewed, atrophy developed, and was followed shortly thereafter by ulceration – a nice neat package. But some things did not add up. The extent of epithelial breakdown could not be explained kinetically based only on the consequences of basal cell death. Endothelial damage was seen very soon after chemoradiation challenge (Paris *et al*, 2001). The organization of submucosal connective tissue was disrupted and early monocyte/macrophage infiltration was noted (Etiz *et al*, 2000; Handschel *et al*, 2001; Bonan *et al*, 2007). Pro-inflammatory cytokines were overly expressed by cells in the submucosa (Sonis *et al*, 2000; Logan *et al*, 2007). Within minutes of radiation, a range of genes with diverse functional ramifications were expressed throughout the mucosa (Sonis *et al*, 2002). And probably most perplexing, all of these things happened before any epithelial damage was seen. Experiments were designed to answer specific questions and, as data accumulated, a framework emerged that described the biological sequence of mucositis.

This sequence (Sonis, 2004) has been described as a five-phase process beginning with clonogenic cell death and the release of reactive oxygen species, progressing through a series of steps in which biological pathways are activated and amplified, culminating in ulcer development, and then finishing with healing. In the intervening time between 1998 and 2009, we have learned a lot about the process, both directly and serendipitously. Investigations targeted at mucositis have confirmed the importance of specific transcription factors (i.e. NF-κB) (Sonis, 2002; Logan *et al*, 2007; Yeoh *et al*, 2007),

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cytokines (i.e. TNF-α)(Sonis *et al*, 2000; Logan *et al*, 2008), and inflammatory mediators (i.e. COX-2)(Sonis *et al*, 2004; Logan *et al*, 2007; Lopes *et al*, 2009; Lalla *et al*, 2010), and described physiologic molecules that modify mucosal response to chemoradiation challenges (i.e. KGF) (Dorr *et al*, 2005). A series of toxicity-related canonical pathways have been described based on the expression of genes in patients receiving cancer treatment (Sonis *et al*, 2007). And more information has been gleaned from studies of radiation- and chemotherapy-mediated non-mucositis injury. The bystander effects of radiation in which damage to one cell influences a neighbor was consistent with hypotheses describing mucosal injury (Mothersill and Seymour, 2004). The story gets more complete as time goes on. But we still have a long way to go.

One of the most perplexing things about regimen-related toxicities is the apparent randomness with which they affect individuals. Out of a hundred patients receiving identical standard chemotherapy for breast cancer, 20% will develop ulcerative mucositis in cycle 1, but the remaining patients will endure treatment with no oral ulceration. And when a patient does develop one toxicity, it is highly likely that he/she will develop others, and develop them in a pattern that is relatively predictable (Aprile *et al*, 2008). So although we are not good at predicting if many of our patients will or will not develop mucositis, once they get it, we know that they will probably have other issues as well.

PAMPS, DAMPS, and now CRAMPS

Initiation of radiation- or chemotherapy-induced injury is a critical first step in the development of mucositis. In the current mechanistic model, the two most noted components of this stage are clonogenic cell death and the production of reactive oxygen species by injured cells. The initiation phase is a gatekeeper. Delay it or stop it and we can prevent or minimize regimen-related injury. Understand its genetic control and we can predict mucositis risk. Maybe the local environment even has an impact on initiation rate. Although we (the collective 'we') have spent the bulk of our investigational effort in understanding the later stages of mucositis, it seems like some additional thinking around the initiation phase makes sense.

What in the initiation phase triggers downstream response and how does it happen?

The body's response to external and internal threats has long been on immunologists' agenda. Building on Janeway's hypothesis (Janeway and Medzhitov, 2002) that the body's first line of defense against pathogens consists of the recognition of an ubiquitous conserved molecular pattern displayed by pathogens (pathogen-associated molecular pattern, PAMP), Matzinger (2002) proposed a model in which injured tissues released factors (damage-associated molecular pattern molecules, DAMPs) to induce a cascade of pathways that ultimately affected the extent of damage and repair. Subsequent studies have proposed that radiation- and chemotherapy-treated tumor cells release DAMPs and that these may play a role in cancer progression and metastases (Lotze *et al*, 2007; Srikrishna and Freeze, 2009).

It also seems highly probable that normal cells made apoptotic or necrotic by chemotherapy or radiation may release endogenous damage-associated pattern molecules that could play an integral role in initiating toxicity (hereafter referred to as CRAMPs). A potential example of the CRAMP class is the alarmin high-mobility group box 1 (HMGB1; Bianchi, 2007). In healthy cells, HMGB1 is located in the nucleus where it facilitates DNA assembly. However, upon necrotic cell death, HMGB1 is passively released. What is more, chemotherapy and radiation therapy can induce pulsatile HMGB1 release from apoptotic cells (Srikrishna and Freeze, 2009). Once outside the cell, HMGB1 has the potential to create havoc.

We can hypothesize that, in a manner similar to that described for PAMPs and DAMPs, HMGB1 binds to pathogen recognition receptors (PRRs; Palm and Nedzhitov, 2009) such as Toll-like receptors (TLRs; Park *et al*, 2004) or receptors for advanced glycation end products (RAGEs; Schmidt *et al*, 1996) to promote NF-κB signaling and the expression of proinflammatory cytokines that are known to be important in the development of mucositis. The importance of PRRs as intermediates in the response of cells and tissues to microbial pathogens has been well established. TLRs and RAGEs perform a bridging function to communicate extracellular signals (i.e. microbial cell wall products, DAMPs, and teleologically CRAMPs) intracellularly to induce a protective response. The presence of TLRs on oral epithelial cells (Bahri *et al*, 2010) and their role in tissue response to oral microorganisms have been suggested by a number of investigators (Zunt *et al*, 2009). Similarly, cells in tissues of the submucosa such as endothelial cells and fibroblasts express TLRs.

Why is this important for mucositis? Let us assume that radiation or chemotherapy causes direct injury to epithelial cells (top down for radiation, bottom up for chemotherapy). Some cells die (necrosis or apoptosis), some cells are injured, and some cells escape. At the same time, cells in the submucosa undergo the same fate, but to a differing extent. For example, in the very early phases of radiation treatment, the cumulative dose of radiation is pretty low (10 Gy typically at the end of week 1). On the other hand, patients getting chemotherapy have a whole dose of drugs coursing through their circulation in short order after infusion and that is when the drugs are most active. In the early radiation case, one could argue that the cells most likely to be damaged are the ones closest to the radiation source's target – most are now surface sparing. For the chemotherapy model, the cells of the endothelium seem to be at most immediate risk. So the cells are damaged and release CRAMPs that go looking for an inviting receptor. PRRs are conveniently expressed by all of the cells in the vicinity – epithelial and endothelial cells and fibroblasts. The CRAMPs bind to the PRRs, the on-switch is pulled, NF-κB is activated, and you are off to the mucositis races.

But wait, there is more. The pro-inflammatory cytokines programmed by NF-κB production effectively ramp up DAMP expression (as in the amplification phase of mucositis). And cell wall products from the local microbial flora (mouth) provide PAMPs.

PRRs and CRAMPs: risk prediction and treatment opportunities

As I already mentioned, we are not very good at predicting mucositis risk for the majority of patients. Of course, we know that it is a pretty safe bet that a patient being treated with a standard chemoradiation regimen for a tongue cancer is going to end up with a mouth looking like it has been worked over by a rotary lawnmower. But patients with head and neck cancer represent only 15% of patients who develop mucositis in a given year. What about the patient with colorectal cancer, breast cancer, or lung cancer?

There has been a flurry of activity identifying differences in the expression of genes that impact drug metabolism as an approach to predict toxicity risk among patients being treated with chemotherapy (Pullarkat *et al*, 2001; Lecomte *et al*, 2004; Jakobsen *et al*, 2005; Schwab *et al*, 2008). From an applications standpoint though, the genes that impact drug metabolism are relatively small potatoes in the big picture and are applicable to risk prediction of only a small group of patients – maybe 5%. The authors of a large, recently completed randomized trial in patients being treated for advanced colorectal cancer reached a similar conclusion (Braun *et al*, 2009).

However, the continuing identification of the biological pathways that play roles in mucositis development also provides us with opportunities to evaluate differences in gene expression that contribute to risk. For example, TNF polymorphisms have been described, which, when present, increase the relative risk of non-hematological toxicities by a factor of 17 (Bogunia-Kubik *et al*, 2003). But the bar by which an individual SNP imparts risk is high, especially when it is associated with a single cytokine. On the other hand, what if there were multiple layers of SNP-risk predictors: some associated with the ease with which CRAMPs were produced and released, others that impacted PRRs, and still others that were associated with individual pathways? We know that genes control PAMP and DAMP activity – why not CRAMPs? The impact of TLR-associated polymorphisms to affect mucosal disease risk has already been demonstrated in inflammatory bowel disease. And genes expressed by canonical pathways associated with mucositis (of both the mouth and gut) have been clearly identified. So now one of our goals should be to identify genes in each group, create a hierarchy with respect to importance, figure out how they interact, and then apply them for risk determination – a career.

The identification of a CRAMP route for initiation also opens up some therapeutic opportunities. As early as 2004, HMGB1 was identified as a compound of potential clinical interest (Andersson and Tracey, 2004). Pilot studies using anti-HMGB1 antibody as a prototype antagonist have been efficacious in animal models of sepsis. PRR targeted therapy has also garnered followers. A recent review of TLRs and nod-like receptors identifies nine companies evaluating the use of TLR agonists as interventions for a range of clinical conditions ranging from malignancies to allergic rhinitis (Fukata *et al*, 2009). A CRAMP route inhibitor might be an approach that would attenuate regimen-related toxicities before they ever got started and certainly deserves some thought.

It's not over 'til it's over

We have come a long way in our understanding of mucositis. We can now talk knowledgably about its initiation, development, resolution, and impact on cost. Sadly though, when we develop treatment guidelines, our choices are exasperatingly sparse. Palifermin remains the only approved mucositis treatment and is applicable to a scant 4% of the at-risk population. Incredibly, mucositis is among the best studied and understood of the regimen-related toxicities, and we now appreciate how infrequently it occurs in isolation.

As frustrating as the quest for effective treatments has been, I think that we have turned the corner. The increasing identification of potential targets, the development of innovative delivery platforms, and the realization that mucositis provides an archetypal model that relates, not only to other toxicities, but also to other mucosal diseases (i.e. IBD), and its commercial potential have created a 'time is ripe' environment for the generation of successful interventions.

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