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THE CALCIUM-SENSING RECEPTOR AS A MEDIATOR OF INFLAMMATION

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Abstract

The teleologic link between increased production of pro-inflammatory cytokines resulting from a systemic inflammatory response to a burn injury and consequent stimulation of bone resorption is unclear. While it is known that cytokines can stimulate osteocytic and osteoblastic production of the ligand of the receptor activator of NF κ B, or RANKL, it is not certain why this occurs. It was therefore hypothesized that the subsequent osteoclastic bone resorption liberates calcium from the bone matrix and somehow affects the inflammatory response. In this paper we show how the cytokine-mediated inflammatory response following severe burn injury in children results in simultaneous increase in bone resorption and up-regulation of the parathyroid calcium-sensing receptor. The acute bone resorption leads to release of calcium from the bone matrix with consequent calcium accumulation in the circulation. The up-regulation of the parathyroid calcium-sensing receptor suppresses the release of parathyroid hormone resulting in a lowering of blood calcium concentration. The simultaneous occurrences of these processes could regulate blood calcium concentration and if calcium concentration affects the inflammatory response, then the calcium-sensing receptor could, at the very least, modulate the inflammatory response by adjusting the blood calcium concentration. We describe in vitro studies in which we demonstrated that peripheral blood mononuclear cells in culture produce the chemokines MIP-1 α and RANTES in proportion to the medium calcium concentration and they produce the chemokine MCP-1 in quantities inversely related to medium calcium concentration. CD14⁺ monocytes in culture will also produce MIP-1 α in direct relationship to media calcium concentration but the correlation coefficient is markedly reduced compared to that with peripheral blood mononuclear cells. These monocytes, which possess the calcium-sensing receptor, do not produce MCP-1 in either direct or inverse relationship to media calcium concentration. Therefore, it is possible that other peripheral blood mononuclear cells are primarily responsible for the production of chemokines in relation to calcium concentration but these cells have not yet been defined.

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Keywords

calcium sensing receptor (CaSR); inflammation; chemokines; burns; peripheral blood mononuclear cells; calcium

1. Introduction

There are numerous reports regarding the association of inflammatory cytokines with bone resorption. The basic mechanism appears to be by stimulating osteoblasts and osteocytes to produce increasing quantities of the ligand (L) for the receptor activator (RA) of the transcription factor NF κ B (NK), or RANKL (1,2). RANKL in turn stimulates bone marrow stem cells to differentiate into osteoclasts resulting in increased bone resorption. The pro-inflammatory cytokines that have been implicated in this activity include interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF) α . The cause and effect relationship has been demonstrated nicely in Crohn's disease in which the use of infliximab, the monoclonal antibody directed against TNF α , has resulted in improved bone formation (3). Rheumatoid arthritis, a chronic inflammatory condition affecting large joints, has also been associated with inflammatory bone loss (1). Also, not only systemic inflammation but local inflammation as well can result in resorptive bone loss. To illustrate this statement work from Guise's laboratory (4) indicates that in breast cancer, bony metastases can produce a local inflammatory response leading to the release of transforming growth factor (TGF) β from bone. In turn the released TGF β may be transported to muscle where it contributes to the pathogenesis of cachexia. Thus, both systemic and local inflammation are capable of producing cytokine-mediated bone resorption. Inasmuch as there is no clear mechanism that associates the inflammatory response to bone loss the critical question presented is why should this mechanism occur? Does this cytokine-mediated bone resorption make teleologic sense?

The aim of this article is to attempt to summarize what information is available that may begin to explain why this action occurs. In order to approach this question systematically the example of burn injury will be utilized. The importance and the consequences of inflammation following burns will be discussed and its relationship to bone will be clarified. This paper will also attempt to make the case that the calcium-sensing receptor may be involved in the modulation of the inflammatory process, and the critical role of burn injury in the regulation of the parathyroid calcium-sensing receptor will be shown.

2. The Relevance of Burn Injury

To begin with, children suffering a burn injury of 40% total body surface area damage the primary barrier to the entry of bacterial organisms into the body, the skin. Thus they suffer immediate wound sepsis and generate a vigorous systemic inflammatory response well within the first 24 hours after injury. This response includes a three-fold elevation of blood concentrations of interleukin (IL) 1 β and a hundred-fold elevation of blood levels of IL-6 at two weeks post-burn (5). While serum concentrations of TNF α have been reported to be elevated in certain studies they have been normal at two weeks post-burn in our studies (5). Moreover, these same patients have been shown to lose up to 7% of the lumbar spine bone

mineral density by six weeks post-burn and 3% of their total body bone mineral content by six months post-burn (6).

While we have shown the temporal association of post-burn inflammation and bone loss, what evidence is there that the bone loss is resorptive, a known effect of inflammation? In a well –developed sheep model of severe burn injury, urinary excretion of the C-telopeptide of type I collagen (CTx), is two-fold increased within the first 24 hours following the burn injury and by five days following the burn there is evidence of iliac crest bone surface scalloping as shown by backscatter scanning electron microscopy (7). This is a hallmark of bone resorption. Thus we now have a coincidence in time of systemic inflammation and bone resorption.

Both inflammation (8) and sepsis (8) have been documented to cause a tissue build-up of oxygen free radicals. The effect of oxidative stress on bone cells has, in animal studies, been shown to result in an up-regulation of the family of transcription factors known as forkhead box O, or FOXO (9). Up-regulation of FOXO in bone has been demonstrated to displace β -catenin from the cell nucleus in osteoblast precursors (9), thus inhibiting osteoblastogenesis and consequently reducing bone formation. In osteoclast precursors FOXO accumulation prevents the build-up of hydrogen peroxide in the mitochondria, promoting the process of osteoclastogenesis (10). This same pathway may also be shared by endogenous glucocorticoids , resulting in a similarity of activity associated with cytokines and glucocorticoids. Thus we may be able to explain why inflammatory cytokines may stimulate bone resorption and inhibit bone formation. However, we are no closer to understanding the teleology of these actions.

2.1 Animal Models

We now need to return to the sheep model of burn injury to understand an apparently unrelated set of events that occur at the same time as the resorptive bone loss. These involve the up-regulation of the parathyroid calcium sensing receptor (CaSR). This issue is devoted in part to the CaSR and thus the description of this membrane-bound G protein-coupled receptor will be left to other articles. In vitro studies by Nielsen et al (11) demonstrated that incubation of bovine parathyroid cells with IL-1 β resulted in increased CaSR mRNA and decreased PTH mRNA. This finding was duplicated by Toribio et al (12) using equine parathyroid slices. Their observations also included a reduction in PTH mRNA secretion when these parathyroids were incubated with IL-6. Finally, Cannaff and Hendy (13) demonstrated a reduction in CaSR when exposed to IL-6.

In collaboration with Brown's laboratory, our studies of burned sheep revealed a 50% up-regulation of the parathyroid CaSR by 48 hours following burn injury under anesthesia (14). Furthermore, immunoperoxidase staining of the parathyroid chief cells demonstrated in a semi-quantitative fashion greater staining of the cell membranes from the burned sheep than from controls undergoing sham injury (14).

The up-regulation of the parathyroid CaSR results in the reduction of the concentration of circulating calcium necessary to suppress PTH secretion (15), thus leading to hypocalcemia and urinary calcium wasting. This is precisely what happens in pediatric burn victims. They

develop hypocalcemic hypoparathyroidism with urinary calcium wasting (16). The post-burn hypocalcemia in these children can last up to one year post-burn (17). Thus we observe in our sheep model of burn injury the simultaneous occurrence of bone resorption, which liberates calcium into the blood, and up-regulation of the parathyroid CaSR, which results in the lowering of blood calcium concentration. Therefore, at the very least, up-regulation of the parathyroid CaSR may serve to protect the burn victim from the effects of the hypercalcemia brought about by the pro-inflammatory cytokine-mediated bone resorption. However, while we may partially understand how inflammation may produce resorptive bone loss, we still do not know whether the bone loss produced is coincidental or whether there is a true teleology behind this set of reactions.

3. Clinical Relevance

One thing we do know is that severely burned children exhibit a clinical expression consistent with up-regulation of the parathyroid CaSR. Thus children with 30% of their total body surface area burned are manifestly hypocalcemic, hypercalciuric, and hypoparathyroid (16). Moreover, subcutaneous injection of parathyroid hormone into pediatric burn victims results in blunted renal responses including reduced urinary cyclic AMP and phosphate excretion (16). This clinical picture cannot be explained by magnesium depletion because even though all severely burned children are fluid resuscitated using magnesium-free Ringer's Lactate solution, magnesium repletion fails to correct the hypocalcemic hypoparathyroidism (18).

4. In Vitro Studies

The missing part to the puzzle is whether blood calcium concentrations have any influence on the inflammatory response. An abstract published by Castro et al and presented at the 2003 meeting of the American Society for Bone and Mineral Research attempted to address this question. Peripheral blood mononuclear cells (PBMC) from normal adult volunteers were cultured in RPMI medium varying only in the concentration of calcium for up to 5 days. PBMC production of the chemokine macrophage inflammatory protein (MIP) 1 α , currently known as CCL3, and the chemokine Regulated on Activation Normal T Cell Expressed and Secreted (RANTES), currently known as CCL5, correlated highly with the concentration of calcium in the media (Figures 1 and 2), $r=0.854$, $p<0.0001$ and $r=0.933$, $p<0.0001$ respectively. In contrast, monocyte chemotactic protein (MCP)-1, currently known as CCL2, varied inversely with higher medium calcium concentration, $r=-0.911$, $p<0.0001$ (Figure 3). Furthermore, when flow cytometry isolated CD 14-positive peripheral blood monocytes, that is, those containing the extracellular CaSR, the same relationship held for MIP 1 α , $r=0.578$, $p=0.0095$, suggesting that the peripheral monocyte CaSR may be a mediator of the chemokine production associated with increasing media calcium concentration. However, while with peripheral blood mononuclear cells nearly 73% of the changes in chemokine concentrations could be explained by changes in medium calcium concentration, in the CD14+ monocytes only 33% of the changes in chemokine concentration could be so explained. Moreover, the concentration of MCP-1 in the culture of CD14+ monocytes was not significantly inhibited by increasing concentrations of calcium,

$r=0.423$, $p=0.171$, suggesting that other cell types in the peripheral blood mononuclear cell family may be exerting an influence on chemokine production (Figure 4).

5. Discussion

Chemokines are proteins produced by peripheral blood mononuclear cells that attract additional inflammatory cells to sites of inflammation. Thus by increasing chemokine production extracellular calcium may serve to either sustain or to intensify an inflammatory response. This scenario raises the possibility that blood calcium concentration may mediate the intensity or duration of an inflammatory response by influencing chemokine production by peripheral blood mononuclear cells.

Therefore, the cytokines produced by the systemic inflammatory response resulting from severe burn injury can stimulate bone resorption in order to liberate calcium which, in turn, can stimulate chemokine production sufficient to sustain or intensify the specific inflammatory response. Furthermore, cytokine-mediated up-regulation of the parathyroid CaSR may represent an effort to fine tune the intensity or duration of the inflammatory response by excreting some of the liberated calcium into the urine, thereby serving as a buffer for the calcium liberated by cytokine-mediated bone resorption. Figure 5 illustrates how this balancing act could work following severe burn injury.

It should be noted that our data followed the publication of a report by Olszak et al in 2000 (19), which demonstrated that the chemokine MCP-1 was capable of stimulating monocyte chemotaxis in peripheral blood mononuclear cells containing activated CaSR and that this same CaSR was capable of up-regulating MCP-1. Moreover, monocyte chemotaxis increased directly as the concentration of extracellular calcium in those cells that exhibited the CaSR while monocyte chemotaxis did not change in response to calcium concentration for cells that did not exhibit the CaSR. Inasmuch as chemokine function is mediated via G protein coupled trans-membrane receptors (20) of which the CaSR is one, involvement of the CaSR in the inflammatory process is reasonable to postulate

The stimulation of the CaSR by MCP-1 as reported by Olszak et al (19) and the inhibition of monocyte MCP-1 production by high concentrations of media calcium in our study () may be less meaningful than the stimulation of either monocyte chemotaxis or selective monocyte chemokine production by increasing concentrations of media calcium. This variability may be due to differences in experimental design as well as culture media. However, these studies mark the earliest evidence of CaSR involvement in the inflammatory process

6. Conclusion

Thus, the systemic inflammatory response can stimulate production of pro-inflammatory cytokines such as IL-1 β and IL-6, which in turn can stimulate osteoblast and osteocyte production of RANKL. RANKL can stimulate marrow stem cell osteoclastogenesis and increased bone resorption. The bone resorption would then liberate calcium into the blood. The increment in blood calcium concentration could then result in peripheral blood mononuclear cell production of MIP-1 α and RANTES, which would help sustain the

inflammatory response. Depending perhaps on the amount of cytokine production by the inflammatory response, the parathyroid CaSR could be up-regulated resulting in hypocalcemic hypoparathyroidism and urinary calcium wasting, thus lowering blood calcium concentrations and consequently chemokine production by the peripheral blood mononuclear cells. While we do not have data definitively demonstrating that this role of the CaSR is in fact real, some of the data support the existence of this kind of modulatory function for the parathyroid CaSR and studies must be performed to attempt to confirm this function. Some of the possible studies could involve the use of calcimimetics to up-regulate the CaSR and evaluate its effect on inflammation, or of calcilytics to evaluate the down-regulation of the CaSR.

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Figure 1.

The media concentration of the chemokine MIP-1 α produced by cultured peripheral blood mononuclear cells in response to increasing media concentrations of calcium.

Figure 2.

The media concentration of the chemokine RANTES produced by cultured peripheral blood mononuclear cells in response to increasing media concentrations of calcium.

Figure 3.

The media concentration of the chemokine MCP-1 produced by cultured peripheral blood mononuclear cells in response to increasing media concentrations of calcium.

Figure 4.

The media concentration of the chemokine MIP-1α produced by cultured CD14+ monocytes in response to increasing media concentrations of calcium.

SNAPSHOT: 24 HR POST-BURN

IL-6 drives myelopoiesis in marrow; IL-6, IL-1 β drive bone resorption and raise CaSR; Blood Ca drives inflammatory response; up-regulated CaSR is adaptive response, lowers blood Ca ?? Modifies intensity of inflammatory response?

Figure 5.

A schematic diagram of metabolic alterations produced by the inflammatory and stress responses to burn injury at 24 hr post-burn and the putative roles of blood calcium concentrations and the parathyroid calcium-sensing receptor.