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PROSPECT

Is HMGB1 an Osteocyte Alarmin?

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Abstract The death of osteocytes, the terminally differentiated cells of the osteoblast lineage that are embedded in bone and regulate remodeling, is significant to both normal and pathological bone resorption. Apoptotic osteocytes putatively release a clarion signal that enhances the development of the bone-resorbing osteoclasts and targets their migration to the breach in the osteocyte network. This phenomenon is thought to underlie normal repair of bone microdamage and contribute to the etiologies of inflammatory bone loss. The chromatin protein high mobility group box 1 protein (HMGB1) has been identified as an ''alarmin'' in other tissues. An alarmin is an endogenous molecule released by dead and dying cells that alert the innate immune system to damage and the need for tissue repair. Wang and colleagues presented evidence in a landmark 1999 study showing that released HMGB1 is a lethal mediator of sepsis. Extracellular HMGB1 is a ligand for the toll-like receptors (TLRs) and for the receptor for advanced glycation end products (RAGE) all of which amplify inflammation. Recent studies by our lab and others have shown that HMGB1 is a bone-active cytokine. It is released by apoptotic osteoblasts in vitro, including the MLO-Y4 osteocyte-like cells. Extracellular HMGB1 enhances the expression of RANKL, TNFα, and IL6 in osteoblastogenic bone marrow stromal cell cultures, and it is chemotactic to osteoclasts. In this prospectus we will review HMGB1 activity at the immune-bone interface and propose a role for HMGB1 as an osteocyte alarmin and mediator of normal remodeling and inflammatory bone loss. J. Cell. Biochem. 103: 1671–1680, 2008.

Key words: apoptosis; chemotaxis; glucocorticoid-induced osteoporosis; inflammation; necrosis; osteoimmunology

THE DYING OSTEOCYTE

The osteocyte is the terminally differentiated cell of the osteoblast lineage that is embedded within the bone lacunae and the most abundant cell type in bone [Kogianni and Noble, 2007]. These entombed cells form a syncitial network that acts as a bone mechanosensor, is responsive to hormones and cytokines, and regulates remodeling [Noble, 2003; Kogianni and Noble, 2007 and references therein].

Dying osteocytes, more specifically the apoptotic ones, play a significant role in both normal and pathological bone resorption, and putatively release an alarm signal or signals that both activate and target osteoclasts to breaches in the osteocyte network [Noble, 2003]. Apoptotic osteocytes are typically found in regions of bone resorption [Noble, 2003; Kogianni and Noble, 2007 and references therein] and massive apoptotic osteocyte death has been associated with glucocorticoid treatment and the subsequent induced osteoporosis [O'Brien et al., 2004]. This osteocytic network appears critical to bone health for as the human skeleton ages osteocyte lacunar density typically declines and is associated with an increase in bone porosity, microcrack damage, and a decrease in remodeling [Noble, 2003; Kogianni and Noble, 2007 and references therein].

The identity of the reputed osteocyte death rattle remains unknown and few candidates have been put forward. However it remains an attractive therapeutic target for regulating bone resorption [Bonewald, 2007].

ALARMINS SIGNAL TISSUE DAMAGE

Pattern recognition receptors (PPRs) mediate innate immune cell detection of invading pathogens via binding of specific parts of the invaders known as pathogen-associated molecular pattern

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molecules (PAMPs) [Bianchi, 2007]. Examples of PAMPs include bacterial lipopolysaccharide (LPS), undermethylated CpG motifs characteristic of bacterial and viral DNA, and viral double-stranded RNA. Toll-like receptors (TLRs), the mannose receptor (MR), and the NOD-like receptors (NLRs) are all examples of PPRs. Engagement of these receptors with PAMPS induces the release of proinflammatory cytokines, which trigger inflammation. This rapid innate response comprises the first line of host defense against invading pathogens and can initiate the acquired immune response to eliminate the intruders if they ultimately evade the innate arm of defense [Lin, 2006].

Alarmins are endogenous molecules that are released by dead and dying cells altering the innate immune system to tissue damage and these disgorged molecules are ligands to some of the PPRs [Bianchi, 2007]. At present the emerging characteristics of alarmins include their (i) rapid release by necrotic but not apoptotic cells, (ii) release by living immune cells, (iii) capacity to recruit and activate cells of the innate immune system including dendritic cells (DCs), and (iv) capacity for ultimately initiating tissue repair [Bianchi, 2007]. Putative alarmins include the S100 proteins, heat shock proteins, and uric acid [see Bianchi, 2007 for a complete list]. The endogenous alarmins and exogenous PAMPs are subgroups of a larger class of molecules collectively known as DAMPs (damage-associated molecular patterns) that convey a similar message and elicit a similar response from the innate immune system [Bianchi, 2007].

A TRANSCRIPTION FACTOR WITH TEETH

High mobility group box 1 (HMGB1) protein, a.k.a. amphoterin, expressed in nearly all cells and one of the most abundant non-histone chromatin proteins is perhaps the most intensely studied of these newly identified alarmins. As an architectural transcription factor, HMGB1 bends or loops DNA thereby altering and/or stabilizing the interactions between other transcription factors within nucleoprotein complexes known as enhanceosomes [Mitsouras et al., 2002].

In a landmark paper by Wang et al. [1999] HMGB1 was identified as a late mediator of lethality in sepsis. In this study they observed that HMGB1 was released by cultured macrophages 8 h after stimulation with endotoxin, a constituent of Gram-negative bacteria, tumor necrosis factor alpha (TNFα), or interleukin-1 (IL1). Additionally, delayed administration of HMGB1 antibodies attenuated endotoxin lethality in mice, and administration of HMGB1 itself was lethal [Wang et al., 1999]. Finally, septic patients who died of infection had increased HMGB1 serum levels [Wang et al., 1999].

HMGB1's bite derives from its capacity to induce the release of inflammatory cytokines from macrophages, monocytes, and neutrophils [Andersson et al., 2000; Park et al., 2003], in addition to itself being released by these cells in response to pro-inflammatory stimuli, thus amplifying the inflammatory response. Likewise, HMGB1 upregulates the expression of adhesion molecules and pro-inflammatory cytokines in human microvascular endothelial cells [Fiuza et al., 2003].

Since the Wang report appeared a prodigious number of studies have been published on the activities of extracellular HMGB1 in the immune and tumor microenvironments and its potential functional roles in arthritis, cancer, Alzheimer's and ischemia-reperfusion injury [see Ellerman et al., 2007 and references therein]. Numerous excellent reviews on HMGB1 have appeared in the literature and we will not recapitulate all of the information provided in these essays but instead focus on those aspects of HMGB1 biology that may be particularly significant to osteocyte death and the resultant impact on bone remodeling.

HMGB1 VITAL STATISTICS

HMGB1 is comprised of 215 amino acids and has a molecular weight of approximately 30 kDa. This transcription factor/cytokine has three functional domains. The DNA-binding activity of HMGB1 is localized within the positively charged A and B boxes (Fig. 1). The B-box also contains the cytokine activity, which can be antagonized by the A box [see Ellerman et al., 2007 for review]. The negatively charged carboxyl terminus or acidic tail is necessary for HMGB1 target binding to nucleosome linker DNA and transcription stimulation [Ueda et al., 2004].

HMGB1 postranslational modifications include phosphorylation, ADP ribosylation, acetylation, and methylation [see Ellerman et al., 2007 and references therein]. Acetylation regulates

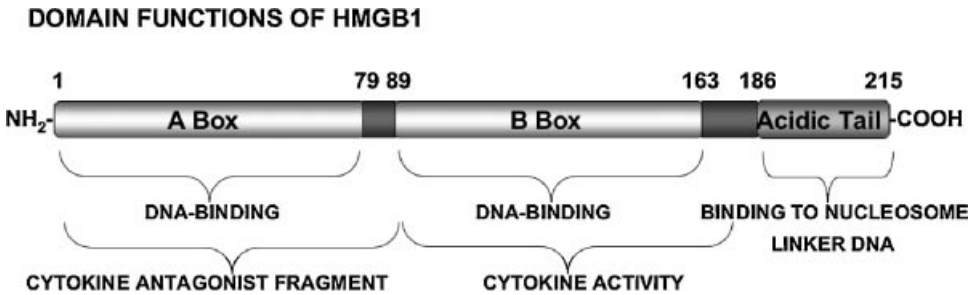

Fig. 1. Functional domains of HMGB1. This chromatin protein/cytokine (not drawn to scale) is highly conserved between human, bovine, and rodent. The A box and B box are positively charged basic domains that mediate DNA-binding. The B box also contains the cytokine activity whereas the A box is antagonistic to this cytokine function. The negatively charged carboxyl terminus acidic tail mediates binding to the nucleosome linker DNA and enhances transcriptional activity of this protein. [See Ellerman et al., 2007 and references therein for review].

HMGB1 entry into the nucleus and likely influences its activity as a cytokine [Dumitriu et al., 2005]. Secreted HMGB1 is hyperacetylated whereas necrotic cells release the non-acetylated molecule [Bonaldi et al., 2003; Dumitriu et al., 2005].

HMGB1 is a ligand for the PRRs TLR2 and TLR4 [Yu et al., 2006]. HMGB1/TLR4 signaling mediates PMN NAD(P)H oxidase activation induced by hemorrhagic shock/resuscitation and underlies PMN-mediated inflammation and organ injury after hemorrhage [Fan et al., 2007]. HMGB1 is also a ligand for non-PPRs including the receptor for advanced glycation end products (RAGE) and the triggering receptor expressed on myeloid cells-1 (TREM-1) [Lotze and Tracey, 2005; El Mezayen et al., 2007]. RAGE, a member of the Ig superfamily, has multiple ligands including, as its name implies, advanced glycation end products, select members of the S100 family of proteins, and amyloid-beta peptide [Herold et al., 2007]. Depending on the context of activation, RAGE mediates the amplification of inflammation leading either to tissue repair or injury and is a significant player in both wound healing and tissue damage associated with chronic disease [Herold et al., 2007]. The TREM receptors are expressed broadly on myeloid cells as well as osteoclasts and function primarily as modulators of cellular response and appear to set thresholds for cytokine response [Klesney-Tait et al., 2006]. A recent study has shown that TREM-1 mediates HMGB1-induced cytokine expression in human THP-1 monocytic cells [El Mezayen et al., 2007]. Blocking TREM-1 on THP-1 cells attenuated cytokine production whereas simultaneously blocking TLR4 and TREM-1 abolished the HMGB1-induced release of cytokines suggesting that full response to HMGB1 requires a synergistic interaction between TREM-1 and TLR4 [El Mezayen et al., 2007]. Similarly, a recent study has demonstrated a synergistic interaction between HMGB1, RAGE, and TLR9 in mediating IFN-α production in plasmacytoid DCs response to class A CpG oligodeoxynucleotides [Tian et al., 2007] and it has been proposed that HMGB1 binding of TLR2/4 and RAGE may act to re-enforce the mobilization of their respective signaling cascades thus contributing to inflammation of certain chronic disease states [Lin, 2006].

The HMGB1/TLR2 & 4 and HMGB1/RAGE signaling axes ultimately induce the nuclear translocation of NF-kappaB, a hallmark of the pro-inflammatory signal transduction cascade [Lin, 2006]. Signaling through RAGE also mobilizes the p38 and ERK pathways. HMGB1 activation of TLR2 and TLR4 triggers NF-kappaB nuclear translocation via the MyD88 pathway (myeloid differentiation primary-response protein 88) [see Lotze and Tracey, 2005; Lin, 2006 for review].

HMGB1 IS RELEASED FROM THE QUICK AND THE DEAD

Living cells actively release HMGB1, typically, although not exclusively, as an immunomodulatory molecule [Lotze and Tracey, 2005]. HMGB1 is a leaderless cytokine that is not processed through the Golgi apparatus for release but instead is released via the endolysomal compartment like the haematopoietic cytokines IL-2, -4, and -7 [Lotze and Tracey, 2005 and references

therein]. In monocytes and macrophages, HMGB1 is a nucleocytoplasmic shuttling transcription factor that upon acetylation, cannot re-enter the nucleus but instead is stored in the secretory lysosomes whereupon activation of the immune cell, the acetylated HMGB1 is secreted into the microenvironment [Bonaldi et al., 2003]. The phosphorylation of HMGB1 near the two nuclear localization signals also appears to contribute to redirecting this chromatin protein toward secretion in these cells [Youn and Shin, 2006]. In neutrophils, HMGB1 is monomethylated at Lys42, which attenuates its DNA binding activity, allowing it to passively diffuse into the cytoplasm, but how it then exits the living neutrophil is not clear [Ito et al., 2007].

Until recently, necrotic but not apoptotic cells have been considered the source of HMGB1 from dying or damaged tissue. Earlier studies suggest that apoptotic-mediated hypoacetylation of chromatin acts to retain HMGB1 within the remains of the dead nucleus [Rovere-Querini et al., 2004 and references therein].

However, recent studies from Pisetsky and colleagues [Bell et al., 2006; Jiang et al., 2007] and our own laboratory [Yang et al., 2007] demonstrate release of HMGB1 by apoptotic cells. Treatment of Jurkat, HeLa, Panc-1, and U937 cells with staurosporine, etoposide, or camptothecin, inducers of apoptosis resulted in release of HMGB1 into the culture medium [Bell et al., 2006]. Furthermore, HMGB1 release was attenuated by Z-VAD-fmk, a pan-caspase inhibitor, and confocal microscopy demonstrated the HMGB1 and DNA alter their nuclear location in apoptotic Jurkat cells [Bell et al., 2006]. Similarly, LPS- and polyinosinic-polycytidylic acid (poly(I:C))-induced HMGB1 release by RAW 264.7 and primary murine macrophages correlated with the incidence of apoptosis [Jiang et al., 2007].

We have reported that treatment of MLO-Y4 osteocyte-like cells, MC3T3-E1 osteoblast-like cells, and primary murine bone marrow-derived osteoblasts release HMGB1 into the medium upon induction of apoptosis with TNFα and cycloheximide (CHX) [Yang et al., 2007]. The time course of HMGB1 release into the medium by the MLO-Y4 cells correlated with the appearance of caspase-3 cleavage, a mediator of apoptosis, in the harvested cells lysates [Yang et al., 2007]. In related experiments we showed that the anti-apoptotic action of parathyroid hormone (PTH), a chief regulator of bone remodeling and osteoprogenitor/osteoblast apoptosis, was correlated with a decrease in HMGB1 release by MC3T3-E1 cells under serum withdrawal [Yang et al., 2007]. Exposing MC3T3-E1 cells to serum withdrawal for 24 h elevated baseline apoptosis as measured by the appearance of cytoplasmic histone-associated-DNA-fragments and was accompanied by HMGB1 release into the medium. Treating these cells with PTH during serum withdrawal reduced the appearance of cytoplasmic histone-associated DNA fragments in these cells by 50% but completely abrogated HMGB1 release, suggesting that a subset of apoptotic MC3T3-E1 osteoblasts were responsible for releasing HMGB1 [Yang et al., 2007].

HMGB1 IMMUNE AND NON-IMMUNE ACTIVITIES

The action of HMGB1 the cytokine has been most intensely studied within the context of the immune microenvironment. As previously indicated, HMGB1 is a potent pro-inflammatory cytokine and evokes the release of TNFα and IL6 from macrophages and monocytes, just as TLR ligands induce the release of HMGB1 from these cells thus acting to amplify the inflammatory response [Andersson et al., 2000; Park et al., 2003]. Additionally, extracellular HMGB1 acts as an autocrine/paracrine factor that supports DC maturation [Dumitriu et al., 2005] and HMGB1/RAGE signaling is required for the chemokine-mediated migration of these maturing DCs [Dumitriu et al., 2007]. HMGB1 is also a chemoattractant to monocytes and neutrophils within the immune microenvironment [Orlova et al., 2007; also see Ellerman et al., 2007 and references therein]. Interestingly, a recent study has shown that HMGB1 suppresses plasmacytoid DC cytokine secretion and maturation in response to TLR9 agonists including hyomethylated oligodeoxynucleotide CpG- and DNA-containing viruses [Popovic et al., 2006]. These investigators speculate that the observed differences in DC response to HMGB1 in these aforementioned studies may be due, in part, to potential differences in RAGE-mediated signaling between actively secreted (hyperacteylated) and passively released (non-acetylated) forms of HMGB1 [Popovic et al., 2006]. Secreted HMGB1 is also necessary for the survival, proliferation, and polarization of naïve $CD4^+$ T cells

subsequent to activation by allogeneic DCs [Dumitriu et al., 2005].

Extracellular HMGB1 makes its presence felt outside the confines of the immune microenvironment and appears to have a role in cardiovascular disease. As a cytokine, HMGB1 induces chemotaxis and cytoskeleton reorganization in rat smooth muscle cells [Degryse et al., 2001]. Activated vascular smooth muscle cells release HMGB1 in human advanced artherosclerotic lesions, which in turn acts as an autocrine/paracrine factor inducing the expression of C-reactive protein and matrix metalloproteinase (2, 3, and 9) via RAGE signaling [Inoue et al., 2007]. Additionally, cultured smooth muscle cells were observed to actively secrete HMGB1 after cholesterol loading and in response to this extracellular HMGB1 these cells initiated cell division, migration, and the secretion of more HMGB1 [Porto et al., 2006]. Thus HMGB1 produced by activated smooth muscle cells appears to contribute to the etiology/progression of human artherosclerotic lesions and restenosis [Porto et al., 2006; Inoue et al., 2007].

In the nervous system, HMGB1 appears to assume a variety of roles and can act as a neurotrophic factor, a pro-inflammatory cytokine, or as an enhancer of neurodegeneration [Pedrazzi et al., 2006 and references therein]. For example, HMGB1/RAGE signaling mediates neurite outgrowth and migration in vitro [Chou et al., 2004]. HMGB1 levels are significantly increased in the brains of Alzheimer patients and this protein has been detected in association with senile plaques [Takata et al., 2003]. Additionally, the co-injection of HMGB1 in rats delayed the clearance of amyloid-β1-42 (Aβ42) and accelerated neurodegeneration in these Aβ42-injected rodents [Takata et al., 2004]. The investigators speculate that HMGB1 released from dying neurons may inhibit Aβ42 clearance and enhance the latter's neurotoxicity [Takata et al., 2004]. Extracellular HMGB1 is a stimulator of glutamate/aspartate release from glial plasmalemmal vesicles and it has been proposed that extracellular HMGB1 in certain areas of the brain may promote a local increase in excitotoxicity [Pedrazzi et al., 2006].

HMGB1 has a role in tumorigenesis both as a transcription factor and as a cytokine [see Ellerman et al., 2007 for review]. HMGB1/RAGE signaling mediated the invasion, migration, and the growth and spread of implanted C6 gliomas in athymic nude mice [Taguchi et al., 2000]. Blockade of HMGB1/RAGE signaling by administering soluble, extracellular ligand-binding domain of RAGE (sRAGE) suppressed migration of the glioma, forcing the tumor to undergo prolonged dormancy, as well as attenuated proliferation, invasion, and MMP activity [Taguchi et al., 2000]. HMGB1 was identified as an over-expressed gene in a suppression subtractive hybridization assay of colorectal carcinoma tissue and distant normal colon tissue [van Beijnum et al., 2006]. In this same study, HMGB1 antibodies had a significant inhibitory effect on endothelial tube formation in an in vitro collagen gel-based sprout formation assay [van Beijnum et al., 2006]. Additionally, the HMGB1 antibodies inhibited angiogenesis by 45% in the chorioallantoic membrane of the embryonic chick, thus supporting the hypothesis that HMGB1 may support tumorigenic angiogenesis in colorectal cancer [van Beijnum et al., 2006].

HMGB1 AND BONE

HMGB1 plays a significant role in endochondral but not intramembraneous ossification [Taniguchi et al., 2007]. HMGB1 knockout mice ($HMGB1^{-/-}$) die shortly after birth but the $HMGB1^{-/-}$ embryos reveal that long bone development is significantly compromised whereas the calvaria of these mice appear relatively unaffected [Taniguchi et al., 2007]. Immunohistochemical and in vitro culture revealed that HMGB1 moves to the cytosol of hypertrophic chondrocytes at the growth plate to be released into the immediate environment. The released HMGB1 into the cartilage functions as a chemoattractant for invading osteoclasts, osteoblasts, and endothelial cells [Taniguchi et al., 2007].

The observation describing distinct roles of HMGB1 in endochondral and intramembraneous ossification [Taniguchi et al., 2007] is consistent with our recent report characterizing differences in the response of marrow- and calvarial-derived osteoblasts to recombinant HMGB1 (rHMGB1) [Yang et al., 2007]. Bone marrow stromal cell cultures (BMSC) maintained under osteoblastogenic conditions respond to rHMGB1 by significantly increasing the RANKL/OPG steady state mRNA ratio in these cells, and releasing pronounced amounts of

both IL6 and TNFα into the culture medium. Conversely, challenge of the calvarial-derived MC3T3-E1 cells with rHMGB1 had no affect on these parameters. Additionally, rHMGB1 had differential effects on both the activation of signal transduction pathways and on proliferation in these two types of bone cells. These results suggest that extracellular HMGB1 acts as a potent bone resorption signal within the confines of the long bone marrow microenvironment but it may have a distinct role within the confines of the diploë of the flat bones [Yang et al., 2007].

HMGB1 appears to be constitutively released by primary osteoclasts and osteoblasts in culture, as well as some osteoblast-like cell lines [Charoonpatrapong et al., 2006] however, in osteoblasts, this basal release is diminished as the cell acquires the mature phenotype [Yang et al., 2007]. Whether this release is mediated via apoptosis (see above) or some other pathway is not known but thus far, immunocytochemistry has shown HMGB1 moving out of only apoptotic bone cells and not healthy, living cells [Charoonpatrapong et al., 2006; Yang et al., 2007].

Bone cells express the HMGB1 receptors, RAGE, TLR2, and TLR4 [see Charoonpatrapong et al., 2006 and references therein] and RAGE knockout mice ($RAGE^{-/-}$) exhibit a significant bone phenotype, but whether this is related to HMGB1/RAGE signaling is not presently known [Ding et al., 2006; Zhou et al., 2006]. Nevertheless, $RAGE^{-/-}$ mice have a significantly increased bone mass, enhanced bone biomechanical strength, and a decreased number of osteoclasts compared to wild-type mice [Ding et al., 2006]. The $RAGE^{-/-}$ mice do not lose bone mass following ovariectomy in contrast to wild-type mice [Ding et al., 2006]. Osteoclasts from $RAGE^{-/-}$ mice exhibit a disrupted actin ring and sealing zone structures, impaired differentiation, and attenuated bone resorption activity [Zhou et al., 2006].

A PROPOSED ROLE FOR HMGB1 AS AN OSTEOCYTE ALARMIN IN INFLAMMATORY BONE LOSS

Pathogenic bone erosion is often associated with inflammation and a key discovery from the emerging field of osteoimmunology is that shared cytokine-signaling pathways at the immune-bone interface likely provide the molecular basis by which the abnormal and/or prolonged activation of the immune system leads to bone loss [Takayanagi, 2007 and references therein]. For example, the signaling axis comprised of receptor activator of nuclear factor kappa B (RANK), osteoprotegerin (OPG), and their ligand RANK ligand (RANKL) is expressed by both immune and bone cells and plays a central role in coupling inflammation with bone erosion [Takayanagi, 2007 and references therein]. Similar to HMGB1, RANKL is a survival factor for DCs and is chemotactic to monocytes [Baud'huin et al., 2007 and references therein]. Additionally, RANKL null mice exhibit deficits in lymph node organogenesis, splenic, and Peyer s patches organization, and thymocyte development [reviewed in Baud'huin et al., 2007]. RANKL is also a primary mediator of osteoclastogenesis and mature osteoclast activity [Baud'huin et al., 2007 and references therein]. Whereas, OPG acts as a decoy receptor for RANKL and modulates its activity on osteoclastogenesis, this molecule also has a role in B cell development [Baud'huin et al., 2007 and references therein]. Recent work has shown that this RANKL-mediated crosstalk between blood and bone plays a significant role in the etiology of rheumatoid arthritis [Sato et al., 2006]. Briefly, within the synovium the infiltrating TH17 cells not only secrete RANKL but also secrete the pro-inflammatory cytokine IL-17 that, in turn induces the release of RANKL from the synovial fibroblasts thus activating osteoclastogenesis and bone erosion [Sato et al., 2006]. T cell-derived RANKL also appears to play a significant role in bone loss associated with periodontitis [Theill et al., 2002 and references therein] and multiple myeloma cells induce the release of RANKL by BMSCs, thus mediating the bone loss accompanying this B-cell malignancy [Yeh and Berenson, 2006]. Clinical trails characterizing the efficacy of a RANKL-specific antibody for treating postmenopausal osteoporosis, arthritis, and bone metastasis are promising [Takayanagi, 2007 and references therein].

We propose that the HMGB1/RAGE/TLR2/TLR4 cytokine signaling axis, like the RANK/RANKL/OPG triad, couples immunity with bone homeostasis and that the release of HMGB1 by apoptotic, as well as necrotic osteocytes acts as an alarmin in both normal bone remodeling and inflammatory bone loss. Glucocorticoid excess, microdamage, and estrogen loss all typically

result in osteocyte apoptosis [Noble, 2003; Kogianni and Noble, 2007 and references therein] and osteocyte death is likely a significant aspect of glucocorticoid-induced osteoporosis (GIOP) [O'Brien et al., 2004], arthritis [Noble et al., 1997], and periodontitis [Yamamoto et al., 1999].

Our hypothesis maintains that the osteocyte release of HMGB1 into the bone microenvironment triggers the expression and release of the potent bone resorption agents RANKL, TNFα, and IL6 from BMSCs, osteoblasts, and macrophages upon binding to the receptors RAGE, TLR2, and/or TLR4. Furthermore, this released HMGB1 is chemotactic to the alerted osteoclasts [Taniguchi et al., 2007] thus targeting their action (see Fig. 2). Finally, HMGB1 may induce the release of pro-inflammatory cytokines, including HMGB1 itself from the osteoclast precursors and mature osteoclasts themselves, as it does with macrophages and monoctyes [Andersson et al., 2000; Park et al., 2003].

We propose that in normal bone remodeling, the release of modest amounts of HMGB1 brings the appropriate number of osteoclasts and osteoblasts to the breach in the osteocyte network caused by microdamage during normal turnover. In the pathological setting of an overwhelming loss of osteocytes and osteoblasts due to glucocorticoid excess, bacterial insult, or arthritic erosion, the massive one-time release of this alarmin will mobilize a disproportionate number of osteoclasts but since the bone forming cells have been decimated the reparative arm of remodeling response will be compromised. This is consistent with the observed initial dramatic loss of bone followed by a slower bone turnover with a disproportionate reduction of bone formation over an increased bone resorption in GIOP patients [reviewed in Pennisi et al., 2006].

Our proposed model for HMGB1-mediated inflammatory bone erosion initiated by osteocyte death provides a number of potential therapeutic targets for attenuating pathological bone resorption and the predicted consequence of neutralizing these targets is consistent with recent clinical and laboratory observations. TNFα blockers have an established role in the prevention of inflammatory bone loss in rheumatoid arthritis [reviewed in Romas and Gillespie, 2006] and show promise in attenuating bone resorption in postmenopausal women [Charatcharoenwitthaya et al., 2007]. In rodent

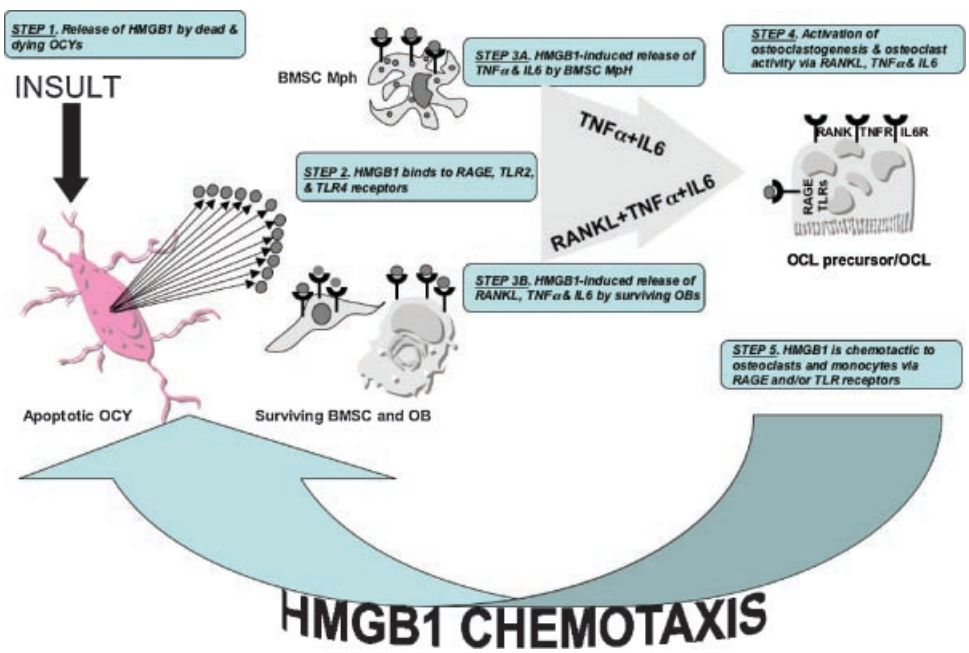

Fig. 2. Proposed role of HMGB1 as an osteocyte alarmin. HMGB1 is released by dead and dying osteocytes (OCYs). As a cytokine in the bone microenvironment HMGB1 triggers the release of RANKL, TNFα, and IL6 from marrow cells including, bone marrow stromal cells (BMSCs), osteoblasts (OBs), and macrophages (MpH). These agents of bone resorption enhance osteoclastogenesis and the activity of mature osteoclasts (OCLs). Ultimately, HMGB1 is chemotactic to these osteoclasts (and OBs) via its interaction with RAGE and/or TLR2 and TLR4 and target their activity to the breach in the osteocyte network.

models of arthritis, treatment with neutralizing antibodies to HMGB1 [Kokkola et al., 2003] or soluble RAGE [Hofmann et al., 2002] moderate tissue destruction including bone loss. Therefore, HMGB1 may be a promising target for ameliorating bone loss in GIOP and/or periodontitis.

Studies are now required using in vivo model systems to test the hypothesis that HMGB1 is an osteocyte alarmin. Mouse models present an attractive experimental preparation due to the myriad of available genetic approaches but there are a number of potential confounding issues that must be addressed. Any experimental approach must disentangle the dual functions of HMGB1 as a transcription factor and cytokine. For example, over-expressing HMGB1 in osteocytes using the dentin matrix protein-1 promoter may have unforeseen consequences on chromatin organization and gene expression in these cells while not significantly impacting the amount of HMGB1 released into the bone microenvironment upon cell death. Another complicating factor is that posttranslational modifications of HMGB1, specifically acetylation, may have considerable impact on this cytokine's function at the immune-bone interface. A third consideration is that HMGB1 is a ligand for numerous receptors, therefore, *RAGE*$^{-/-}$, *TLR2*$^{-/-}$, and *TLR4*$^{-/-}$ mice are appropriate for exploring the action of HMGB1 in the bone microenvironment. The use of HMGB1 neutralizing antibodies circumvents some and perhaps all of these issues and this approach as been useful in recent studies using rodent models (see above). Measuring serum HMGB1 in mouse models of inflammatory bone loss and similarly in patients, although correlative, will determine whether appreciable amounts of this protein are released preceding bone erosion. Indeed, HMGB1 serum levels are elevated in rheumatoid arthritis patients [see Lotze and Tracey, 2005 and references therein].

SUMMARY

Numerous studies support the premise that osteocyte apoptosis is key to normal bone turnover and makes a significant contribution to pathological bone loss. It is presumed that these dying cells release a death signal that alerts osteoclasts to the rent in the osteocyte network, but the identity of this putative alarm is not known. HMGB1 fits the profile of this presumptive cue for bone repair. In vitro studies show that it is released by apoptotic osteocyte-like cells as well as osteoblasts, is highly pro-inflammatory in the bone marrow triggering the release of bone resorption agents, and is chemotactic to both osteoclasts and osteoblasts. Like RANKL, HMGB1 also has significant roles in immunity thus likely coupling the chronic activation of the immune system to inflammatory bone loss. Furthermore, the proposed one-time massive release of HMGB1 by dying osteocytes could explain the initial dramatic loss of bone in GIOP patients. HMGB1 neutralizing antibodies have been efficacious in ameliorating a variety of sequelae in various rodent disease models and thus may be useful in attenuating inflammatory bone erosion in patients. In vivo studies are now required to further test the hypothesis that HMGB1 is an osteocyte alarmin active in both normal bone remodeling and pathological bone loss.

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