seemingly more intact maternal innate immune system.

Together, using three distinct methods, these studies show that ZIKV readily infects placental trophoblasts and neurons *in vivo* and that murine fetuses with infected brains exhibit decreased brain/head size, similar to human microcephaly. As with any important scientific advance, these reports raise numerous questions. First, are all ZIKV strains – including those of the African lineage – equally capable of infecting a fetus and inducing neurological birth defects? What role do interferons play in preventing placental infection, particularly during different stages of pregnancy? Is ZIKV-induced neuronal damage a direct result of infection or does placental insufficiency along with an ensuing immune response contribute to pathogenesis?

Regardless of the answers, these new murine models are poised to answer several crucial questions regarding protective immunity to ZIKV infection and transmission to the unborn. For instance, what level of replication is needed to infect/breach the placenta and result in fetal growth restriction? Beyond the potential utility of mouse models in preclinical testing of vaccine candidates, the identification of a viral set point for vertical transmission in these models may inform human vaccine evaluation studies with the ultimate goal of preventing fetal infections. Furthermore, the systematic evaluation of these models will enable fundamental and translational research opportunities that shape strategies to blunt the global impact of this emerging pathogen.

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Special Issue: Aging and Rejuvenation

Forum

Osteoporosis: The Result of an 'Aged' Bone Microenvironment

Bo Yu[1] and Cun-Yu Wang[1,2,*]

Osteoporosis is an age-related progressive bone disease. Recent advances in epigenetics, cell biology, osteoimmunology, and genetic epidemiology have unraveled new mechanisms and players underlying the pathology of osteoporosis, supporting a model of age-related dysregulation and crosstalk in the bone microenvironment.

Osteoporosis is a 'silent bone disorder' characterized by low bone mass and bone fragility, contributing to an increased public health and economic burden for our aging population (Box 1). A significant number of osteoporotic cases go undiagnosed until the first bone fracture. Current treatment options, mostly antiresorptive agents (estrogen, bisphosphonates, and denosumab) are limited in their ability to restore bone loss once it is diagnosed. Newer and more effective treatment modalities for osteoporosis hinge on our evolving understanding of the players and mechanisms underlying this progressive bone loss.

The Forces at Stake: Osteoporosis Is Driven by Age-Related Mechanisms

The orchestrated balance between bone resorption by osteoclasts, and bone formation by osteoblasts, maintains a relatively stable bone mass in adulthood. In osteoporosis, accelerated osteoclastic resorption overwhelms compensatory bone formation, leading to net bone loss. Until the past decade the predominant cause of osteoporosis was thought to be estrogen deficiency. However, this estrogen-centric view has been challenged and revised in the recent decade, reflecting enhanced understanding of the skeletal aging process [1]. In both genders, trabecular bone loss occurs despite sex steroid sufficiency, suggesting that intrinsic aging-related mechanisms are at play.

Chronic, low-grade inflammation is a hallmark of aging. With advancing age, accumulating cytokines such as IL-6, TNF-α, and IL-1 render the bone marrow (BM) increasingly proinflammatory [2]. The connection between inflammation and osteoporosis has long been established *in vitro* and in animal research. For instance, the transcription factor nuclear factor κB (NF-κB) is activated in most inflammatory

Box 1. The Burden and Disease Etiology of Osteoporosis

Afflicting over 200 million worldwide, osteoporosis is by far the most common bone disease, leading to over 9 million fractures annually [11]. With one in three women and one in five men over 50 years old at risk, osteoporosis causes significant mortality (20–30% associated with first hip fracture) and morbidity in elderly individuals. Given the aging population, by 2025 the annual healthcare cost of osteoporotic fractures is predicted to reach $25.3 billion in the USA alone. The primary causes of osteoporosis are related to intrinsic age-related changes in bone metabolism, and have been historically associated with post-menopausal estrogen deficiency in women and with slowing production of testosterone in men. A growing number of underlying diseases (e.g., congenital connective tissue defects, metabolic and hematologic disorders, hypogonadal states, inflammatory diseases) nutritional deficiencies (e.g., vitamin D and malabsorption), and drugs (e.g., corticosteroids and thyroid replacement) are recognized as secondary causes of osteoporosis, and may be key etiological factors in premenopausal women and men. A better understanding of the molecular mechanisms driving this multifactorial bone loss is evolving away from an estrogen-centric paradigm to one focusing on age-related changes within the bone microenvironment.

responses. While activation of NF-κB signaling is a key step required in osteoclast differentiation, it can also potently inhibit osteoblastic bone formation [3]. Three recent large-cohort human epidemiological studies confirmed this immunological link, wherein a 1.5–3-fold increase in osteoporotic fracture risk was associated with a higher level of inflammatory 'markers' [4]. Consistently, estrogen withdrawal promotes T cell activation and immune cytokine production in both rodents and humans. Furthermore, oxidative stress (OS) increases during aging, with the accumulation of excess intracellular reactive oxygen species. Mounting *in vivo* evidence in rodents suggests that age-induced OS may contribute to osteoporotic bone loss [1]. Both estrogen deficiency via ovariectomy (OVX) and aging-related bone loss result in increased OS markers. The build-up of OS also leads to the activation of NF-κB in various aging tissues. Hence, age-related chronic inflammation of the bone microenvironment could be a unitary driving force in the pathogenesis of osteoporosis. However, it remains unclear how intrinsic changes in aged BM niches might lead to chronic inflammation, and whether osteoporosis and altered bone metabolism in turn exacerbate the inflammatory states of an aged BM.

The Players: Crosstalk between Skeletal Systems, Immune Systems, and Beyond

As noted above, the onset of osteoporotic bone loss involves aberrant activation of the adaptive immune system. One of the most intense areas of research focuses on delineating the osteoimmunological interactions between various cell types residing in the bone microenvironment.

Osteoblasts and osteoclasts are long known to be coupled to the physiological maintenance of bone mass. Insulin-like growth factor I (IGF-1) and transforming growth factor β are classical matrix-derived coupling agents released by osteoclastic resorption to stimulate bone formation. A series of osteoclast-derived clastokines, including PDGF-BB, have also been recently shown to promote bone formation [5]. In contrast to current osteoclast-targeting antiresorptive agents, odanacatib, a small-molecule inhibitor of cathepsin-K, suppresses resorption without affecting osteoclast survival. In fact, odanacatib exploits the coupling between osteoclasts and osteoclasts by increasing the number of osteoclast precursors, and thereby promoting the secretion of osteogenic PDGF-BB [5].

Since the turn of this millennium, T and B lymphocytes have been recognized to play an indispensable role in the onset of osteoporosis by regulating bone cell functions. Estrogen depletion reportedly stimulates T/B cell expansion and the production of osteoclastogenic cytokines TNF-∝ and RANKL. T cells, normally associated with osteoclast activation, have recently been shown to reciprocally suppress osteoclasts both *in vitro* and *in vivo* via CTLA-4 [6]. Tightly regulated interactions between the immune and skeletal systems have reaffirmed that aberrant immune responses have a strong potential to drive the disequilibrium of bone metabolism in osteoporosis. However, these findings also raise intriguing questions: (i) How does aging disrupt osteoimmune feedback, thereby leading to osteoporosis? (ii) Could age-related weakening of the immune system prime the aging bone for osteoporotic bone loss?

Other important constituents of the bone microenvironment are adipocytes; these derive from the same mesenchymal stem cell (MSC) progenitor pools as osteoblasts. The lineage commitment towards osteoblasts and adipocytes is considered to be mutually exclusive. In osteoporosis or skeletal aging, aberrant lineage allocation of MSCs leads to overwhelming marrow adipose tissue (MAT) accumulation at the expense of bone formation. The hormone leptin is secreted by adipocytes, but its peripheral/local effects on bone metabolism remain controversial [7]. Intriguing findings from a recent Prx1Cre–Lepr$^{fl/fl}$ mouse model showed that local leptin signaling in limb bone marrow MSCs promoted adipogenesis while inhibiting osteogenesis [7], suggesting that aberrant increases in MAT could influence MSC lineage decisions. In addition, as the bone responds to various environmental cues during aging, epigenetic regulation of MSC lineage specification may also play a role in osteoporosis. Histone demethylases KDM4B and KDM6B favor osteogenesis over adipogenesis from human MSCs by removing gene-silencing trimethylated histone H3 lysine 9 (H3K9me3) and H3K27me3 chromatin marks on the promoters of osteogenic master regulator genes [8]. Indeed, KDM6B knockout mice exhibit impaired osteoblastogenesis [9]. Furthermore, H3K9me3 and H3K27me3 expression is elevated in BM MSCs of aged and OVX mice [8]. Concordantly, EZH2, an H3K27-specific methyltransferase, is upregulated in BM MSCs from OVX