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ORIGINAL PAPER

Dendritic Cell-Associated Osteoclastogenesis and Bone Loss

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Abstract The present osteoimmunology paradigm whereby inflammation in the periosseous tissue is significantly associated with an increase in osteoclasts (OC) frequency, activity and bone destruction (i.e., inflammation-induced osteoclastogenesis and bone loss) has now been fully implemented. We and others have studied the role(s) of dendritic cells (DC) during this process and thereafter proposed that, in addition to innate effector functions and their critical role as antigen-presenting cells (APC) involved in triggering and orchestrating the adaptive immune responses, they could be directly implicated as osteoclast precursors (OCp) during inflammation at the osteo–immune interface [called; DC-derived OC or DDOC]. Further understanding of the role(s) of DC in the inflammation-induced osteoclastogenesis and bone loss will benefit future treatment approaches; especially, when targeting DC as a therapeutic vehicle is translated into clinical strategies to ameliorate both tissue inflammation and bone destruction, associated with pathogenic progression in the inflammatory bone diseases such as arthritis and periodontitis. Herein, the author summarizes, discusses and presents a synoptic review of recent evidence, with the focus on the role(s) of DC at the osteo–immune cross-road as the alternative osteoclastogenesis pathway/mechanism(s) underlying inflammation-induced bone loss.

Keywords CD11c^{+}dendritic cells · Myeloid dendritic cells · Osteoclasts · Inflammation · Osteoclastogenesis and bone loss · RANKL-RANK/OPG signaling · Osteo–immune interface · Innate versus adaptive immunity

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Introduction

Infection and associated immune responses often cause damage to the surrounding tissues as a result of inflammation, and the bone is no exception. Both acute and chronic inflammations in the periosseous tissues are associated with perturbed bone remodeling, leading to alveolar bone and tooth loss in periodontitis, increased risk for bone/joint damage, loss of function and eventually disability and morbidity in devastating arthritic conditions [1, 2]. Inflammation enhances the development, activity and survival of the "bone-eating cells", osteoclast (OC), therefore overriding bone formation mediated by osteoblasts and disturbing the balance of bone remodeling [3]. To date, it is well known that during inflammation OC activated by receptor activator of NFκB ligand (RANKL), via its receptor (named: RANK) signaling and other critical osteotropic cytokines, are the prime culprits in inflammation-induced bone loss [4, 5]. For example, activated CD4^{+}T-cells triggered by environmental stimuli (i.e., invading pathogens or autoimmunity) constitute a major source for RANKL production, which directly promote inflammation-induced osteoclastogenesis and bone loss as observed in the experimental models of rheumatoid arthritis (RA) and periodontitis in vivo [6, 7]. Importantly, blocking RANKL/RANK signaling by osteopetrogerin

(OPG: the natural decoy receptor of RANKL) administration was found to almost completely inhibit (i.e., >80–100%) the subsequent osteoclastogenesis and bone loss in several inflammatory bone diseases including periodontitis, osteoporosis, RA and cancer-induced bone metastasis in vivo [6–12]. This has become a central paradigm in the osteoimmunology field, which has provided fundamental research and conceptual platform [8, 13], and consequently promoted the design of a new generation of novel molecules aimed to enhance the efficacy of anti-resorptive clinical therapy for inflammatory bone disorders [8, 13–15].

DC are professional antigen-presenting cells (APC) that function outside the realm of bone remodeling under the steady-state conditions, as DC deficient animals have no detectable skeletal defects or bone abnormalities [16]. However, they are found in the bone adjacent areas of synovial and periodontal tissues in human and experimental RA and periodontitis models, respectively [17–20], where they have been estimated to constitute ~5% of total inflammatory infiltrates [21]. The exact nature and roles of various DC subsets, and their contributions to inflammation-induced osteoclastogenesis and bone loss in vivo are currently unclear. Moreover, (1) although DC have been shown to interact directly with T-cells to form aggregates at the inflammatory foci, thus playing a critical role in driving the immunopathology of synovitis and periodontitis [22–24] and (2) despite their suggested common progenitors shared with OC [25], the intriguing question as to whether DC can directly contribute to inflammation-induced bone loss by acting as OC or osteoclast precursors (OCp) or not remained unaddressed. Recently, using in vitro and in vivo models, our group and others have examined the direct contribution of DC, as OCp, to inflammation-induced osteoclastogensis and bone loss. The osteoclastogenic potential of murine and human DC were assessed, and the phenotype and function of $CD11c^+$DC-derived OC (called: DDOC) were carefully characterized [26–28]. In this review, we update and highlight the novel role of DC at the osteo–immune interface during inflammation and discuss their possible direct involvement as OCp during inflammation-induced osteoclastogenesis and its implications in bone loss (e.g., arthritis and periodontitis).

Bone Remodeling and Osteoclastogenesis in Health and Disease

Bone remodeling is a tightly controlled process involving the opposing and coupled activities of osteoblasts (OB) and OC [2–5, 13], through the resorption of the bony matrix by OC and deposition of newly synthesized bone by OB [29]. Normally, OC and OB activities are regulated by several growth factors and hormones, including parathyroid hormone (PTH), vitamin-D3, sex hormones (e.g., estrogens), calcitonin and bone morphogenetic proteins [BMPs; 29, 30]. Furthermore, various immune cytokines including TNF-α, interleukins (e.g., IL-1, IL-6, IL-11) and inflammatory mediators such as prostaglandins (e.g., PGE_2) are also involved in regulating bone remodeling under the stress, infection and/or inflammatory conditions [13, 31, 32].

OB are mesenchymal stem cell-derived and can be phenotypically defined by the expressions of osterix, type-I collagen and osteocalcin [33]. During OB development, the receptors for PTH, prostaglandin, IL-11, insulin growth factor-1 and TGF-β are found to be up-regulated [33]. In contrast, OC are derived from the myeloid precursors of monocytes/macrophages (Mo/MQ) lineage [34–38], where their precursor frequency in mice has been estimated to range from ~0.5 to 5% in the bone marrow (BM) and the circulating peripheral blood [39, 40]. Several molecules including CD11b, F4/80, Ly-6C, c-Fms (M-CSFR), c-kit (CD117) and RANK have been used to aid the identification of mouse OCp [37, 39–43], while CD14 and CD16 are used to study Mo-derived human OCp [44, 45]. Despite that Mizoguchi et al. [46] recently described the presence of "quiescent" OCp at the site of osteoclastogenesis maintained by local OB, OCp populations are still not very well characterized, and their behavior and activities are not totally understood. As the development of Mo/MQ lineage and their myeloid subsets are highly heterogeneous, the characterization of OCp populations remains rather challenging, and the results are often very controversial [40].

Active OC are multinucleated cells that express tartrate resistant acid phosphatase (TRAP), calcitonin receptor (CT-R), cathepsin-k, integrins $\alpha_v\beta_3$ and are capable of demineralising and resorbing bone matrix [2–4], and thereby play an essential role in both bone remodeling and tooth eruption [2, 3, 13, 29, 47]. Additionally, it has been recently shown that OC are capable of degrading the endosteum, thereby promoting selective egress of immature haematopoietic progenitors residing in BM to the circulation under inflammatory condition [e.g., effects of lipopolysaccharides (LPS); 48]. While the molecular mechanisms underlying this process remain to be further explored, it is suggested that via some particular chemokine signals (i.e., CXCR10), the egressed OCp in the circulation are selectively recruited to the inflamed bone tissues where they can fuse and develop into functional/terminal polykaryonic OC, often with ≥10 nuclei [3, 4]. This report provided the first direct evidence linking OC, bone resorption and the release and recruitment of haematopoietic precursors or progenitors in response to the inflammatory stimuli.

RANKL/RANK/OPG in Bone Homeostasis and Beyond

The homotrimeric TNF family member, RANKL (also called TRANCE, OPGL or ODF), RANK and OPG have been shown to be the key regulators of bone remodeling and are directly involved in controlling OC differentiation, activation and survival [49–52] in the presence of macrophage-colony stimulating factor [M-CSF; 53]. OPG acts as "decoy receptor" by binding to RANKL, thus preventing the activation of RANK signaling pathways. Genetic mutations of RANKL and RANK molecules result in similar phenotypes with defective OC development and severe osteopetrosis [13, 49, 54, 55]. On the other hand, OPG transgenic mice are osteopetrotic; whereas OPG deficient mice are severely osteoporotic [9, 13, 51]. Additional knockout studies in mice have identified other molecules, down-stream of RANKL/RANK signaling, involved in OC development and functions, including adapter signaling proteins such as TNF receptor associated factor 6 (TRAF6), p38/MAP Kinase, Syk & c-Src and the transcription factors such as NFATc1 (NFAT2), NF-κB (p50/p52), c-fos/AP-1 complex and PU.1 [56–65]. Importantly, recent studies have also demonstrated that mice lacking immuno-receptor tyrosine-based activation motif (ITAM)-harboring adaptors, including Fc receptor common γ-chain subunit (FcR-γ) and DNAX-activating protein (DAP12), exhibit similar severe osteopetrotic phenotype, indicating that ITAM-dependent "co-stimulatory signals" activated via multiple immune-receptors are involved in the osteoclastogenesis [66] and, thus, ITAM-mediated signals also play critical roles in OC function or/and activity.

RANKL is expressed as both membrane-bound and soluble forms [13, 67] and produced by different cell types, including OB, BM stromal cells, chondrocytes, mammary gland epithelial cells, inflamed intestinal epithelia, synovial fibroblasts, keratinocytes, mast cells, endothelial cells, platelets and, importantly, activated T- & B-lymphocytes [50, 68–73] (see Table 1). Interestingly, beside its involvement in bone homeostasis, RANKL is known to be a part of other cellular activities and interactions. For instance, it has been shown to mediate Mo recruitment to inflammation sites by up-regulating chemokines such as CCL22 & MCP-1 [74, 75]. In experimental atherosclerosis, vascular endothelia have been shown to increase RANKL expression leading to OC formation, in association with Mo adhesion and transendothelial migration [76]. Its signaling can regulate Mo as well as DC survival, lymph node formation and organogenesis, intrathymic self-tolerance, cancer cell migration and associated bone metastasis and specific DC/T-cell interactions [54, 70, 71, 77, 79–81]. The observation that 2/3 of the OPG deficient mice develop a late medial calcification in the renal and aortic arteries, where abundant endogenous OPG expression normally occurs in otherwise healthy animals [76, 82, 83], suggests that OPG also plays a critical and protective role in the vascular system, modulating the osteo–immune interactions associated with atherosclerosis [76]. Collectively, these findings implicate RANKL/RANK/OPG triad in the molecular networks of host physiology spanning bone homeostasis and remodeling, immunological development, functions and inflammatory responses, and recently vascular patho-physiology and cancer metastasis [12, 76, 80, 84–86].

M-CSF/M-CSFR and Osteoclastogenesis

M-CSF (also called: Csf-1) is required for OCp development, proliferation and survival, up-stream of RANKL/RANK signaling [87]. Both M-CSF and M-CSFR deficient

Table 1 Various immune cells capable of contributing to bone loss "in vivo"

Cell type	Functions	
	Osteoclastogenic potential or RANKL-expressing ability (i.e., unclear, negative, week, moderate to strong)	Key immune functions
T-lymphocytes ($CD4^+$ or $CD8^+$)	High RANKL producer with strong osteoclastogenic capability	Cell-mediate host defense (Th & cytotoxic T-cells); cytokines production; immune memory
B-lymphocytes	High RANKL producer with strong osteoclastogenic capability	Antibody production; complement activation; killing or neutralize the pathogens
Monocytes/macrophages	Can express RANKL; but their contribution to osteoclastic activity in vivo remain unclear, or indirect	Antigen presentation and processing; killing the invading pathogens; or may differentiate into active osteoclasts
Dendritic cells (DC) or specific subsets	Indirect or/and direct (specific $CD11c^+$DC can act as OC precursors: see text for details)	Trigger innate immunity; antigen presentation, activate & fine-tune adaptive immunity
Other innate immune cells or/and granulocytes	Unclear (but PMN & platelets have been shown to express RANKL & RANK recently for bone loss)	PMN can trigger innate immunity thus bridging to activate adaptive immune responses

mice manifest severe osteopetrotic phenotype, thereby highlighting their essential role in osteoclastogenesis [88, 89]. M-CSF deficient mice, however, suffer less severe osteopetrosis compared to their M-CSFR deficient cohorts, possibly due to the existence of a second ligand for M-CSFR, namely IL-34 [90]. Moreover, unlike RANKL deficient mice, M-CSF deficiency results in osteopetrosis that resolves with age [91]. M-CSF acts via its tyrosine kinase receptor M-CSFR encoded by the proto-oncogene c-Fms, activating ERK, GRB2 and AKT pathways via PI-3K signaling [92] and can also activate MITF, inducing anti-apoptotic programs such as the expression of Bcl-2 and Bcl-X_L for cell survival [93]. For instance, transgenic Bcl-2 expression was shown to partially rescue the osteopetrosis seen in M-CSF deficient mice, suggesting the role of M-CSF as a critical survival factor for OCp in the Mo/MQ lineage [94].

In other studies, M-CSF was reported to regulate the cytoskeletal organization via PI-3K and c-Src signaling associated with the migration of MQ and OC [95, 96]. In this process, the guanine nucleotide factor Vav3 becomes hyper-phosphorylated, leading to Rac-stimulated motility in OCp [97, 98]. Furthermore, it has been suggested that M-CSF and $\alpha_v\beta_3$ integrins collaborate in regulating OC activity. For example, M-CSF organizes the cytoskeleton via $\alpha_v\beta_3$-mediated matrix adhesion [91], where the retarded differentiation and cytoskeletal function observed in $\beta_3^{-/-}$OC can be rescued by adding exogenous M-CSF [99]. Further, M-CSF can enhance the osteoclastogenic potential of OCp, thereby promoting OC differentiation via the stimulation and up-regulation of RANK expression [53], which in turn increases their responsiveness to RANKL signaling. Interestingly, the requirement for M-CSF/M-CSFR signaling during DC development has recently been reported [100, 101], therefore establishing M-CSF as a common developmental and/or differentiation factor for Mo, MQ and DC in vivo.

Cytokines and Inflammation-Induced Osteoclastogenesis and Bone Loss

Alveolar bone loss associated with periodontal inflammation is one of the most clinically common osteopenia occurring in man [102], involving a vast array of commensal and invading biofilms [103, 104] accompanied by protective as well as destructive host immunity [105]. For example, *Aggrregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have been strongly associated with aggressive (AgP) and chronic periodontitis (CP), respectively [102, 105, 106], where it has been estimated that while AgP merely affects a small percentage (<1–1.5%) of the teenagers and young adults <20 years old, CP affects up to 80% of the worldwide population in adults [105].

Inflammation-induced osteoclastogenesis and bone loss are also part of the immunopathology for RA. As an autoimmune inflammatory bone disorder, RA is characterized by polyarthritis with cartilage and bone destruction [107, 108], and the presence of auto-reactive lymphocytes in the joint lining (synovitis), synovial hyperplasia (i.e., pannus), angiogenesis, and remarkable infiltration of PMNs (neutrophils), NK cells, Mo/MQ, T- & B-cells, and DC to the affected tissues [19, 109, 110]. The presence of rheumatoid nodules can be detected in ~20% of the arthritic cases, consisting of T-, B-cells & DC aggregates, and resemble the tertiary lymphoid tissues as seen in the periodontitis lesions [21, 111]. In the synovial and periodontal tissues, an influx of various leukocytes occurs at the disease site and includes neutrophils, Mo and MQ, DC as well as activated T- and B-lymphocytes [1–3, 13, 20–23, 32, 102], where activated T- & B-cells have been shown to provide a rich source of RANKL in vivo. Such complex cellular infiltrates provide ideal cytokine environment to modulate OC function and activity [3, 13, 87], thereby disrupting the balanced OB versus OC activities in response to inflammatory cytokines and resulting in inflammation-induced bone loss. For instance, T-cells, in addition to local fibroblasts and tissue resident MQ secrete an arsenal of active pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-7, IL-11 and IL-17 as well as PGE2, which can regulate OC development and activities via RANKL/RANK-OPG dependent and/or independent pathways [13, 22, 23, 112], thereby impacting bone remodeling often with pathological consequences.

TNF-α is a very potent pro-inflammatory cytokine with pleotropic effects on both the immune and skeletal systems. It is primarily produced by activated T-cells, synoviocytes and tissue MQ during inflammation [113, 114]. RANKL- and M-CSF-dependent TNF-α's contributions to tissue destruction, and bone loss has been clearly documented [115], in addition to its suggested RANKL-independent role [116, 117]. TNF-α was shown to promote the proliferation and differentiation of OCp by up-regulating c-Fms on OCp pool in a murine model for erosive arthritis [118, 119]. Meanwhile, when acting with IL-1, it has been shown to work both synergistically with, and independently of, RANKL signaling to modulate bone resorption in the arthritis and some other osteoporotic disorders [115, 117, 120].

In contrast, the inflammatory cytokine IFN-γ has been reported to exert both stimulatory and inhibitory effects on RANKL-associated osteoclastogenesis, where its inhibitory effect has been shown to induce STAT1-dependent TRAF6 degradation in OCp [121]. Gao et al. [122] have recently shed some light on the possible reasons behind the dual

effect of IFN-γ. In three different osteopenic models, including ovariectomy, LPS-stimulation and suppressing TGF-β-mediated T-cell activation, where subsequent RANKL and TNF-α expressions are significantly increased, they reported data clearly demonstrating that, in vivo, pro-inflammatory cytokine IFN-γ induces pro-osteoclastogenic effects when acting indirectly on OCp, while its anti-osteoclastogenic effects are direct, resulting in a net outcome of higher osteoclastogenesis and bone loss. These findings are in concordance with the evidence that IFN-γ up-regulates the expressions of MHC-class II and other accessory molecules on APCs, other leukocytes and mesenchymal cells, which may further recruit critical signaling molecules and/or immune effectors associated with bone remodeling [120, 123, 124]. Together, these reports strongly suggest that the net effect of cytokine regulations (i.e., IFN-γ) lies between the interactive balances of promotion versus inhibition of osteoclastogenesis and the subsequent bone loss in vivo.

Similarly, TGF-β has also been shown to have complex interactions that induce either positive or negative influences on osteoclastogenesis, depending on whether it acts directly or indirectly on OC or OCp in the bone microenvironment [125, 126]. TGF-β is known as an anti-inflammatory cytokine that down-regulates the immune responses [127]. Due to its ubiquitous and abundant expression in bone tissues ($\sim$200 μg/kg of bone) and ability to induce coupling effect on bone resorption and bone formation, it has been considered a central player in bone turnover [128–130]. In addition, TGF-β is a potent osteoclastogenic cytokine associated with inflammation-induced bone loss [126]. For instance, depending on the cell type and culture system employed, the effects of TGF-β can be either stimulatory or inhibitory, as it is influenced by not only the cytokine concentration but also the duration and timing of its activity [125, 126, 131–133]. It is now believed that at least in vitro TGF-β can inhibit osteoclastogenesis and bone loss in the presence of OB and/or stromal cells, possibly through up-regulating OPG production, thereby inhibiting RANKL/RANK signaling in OC and OCp [134, 135]. In contrast, TGF-β can promote osteoclastogenesis in OB- and/or stromal cells-free cultures and in lymphocyte-rich microenvironment [125, 136]. Further evidence of TGF-β's involvement during OC differentiation came from studies where RANKL was unable to induce OC formation in the absence of TGF-β [125, 137, 138]. These findings collectively point to the existence of a rather complex cytokine interactive network driving and/or regulating RANKL-RANK/OPG-signaling pathways during osteoclastogenesis in vivo, including the recently described function of IL-17 during bone destruction phase of experimental RA [23, 130]. Future studies are essential to understand the exact molecular interactions and mechanisms underlying the effect of host immune cytokine responses on skeletal homeostasis.

Why DC, as Osteoclast Precursors, During the Osteo–Immune Interactions?

DC is a heterogeneous population of leukocytes with key innate immune functions including anti-bacterial and anti-viral properties [139, 140]. They were first discovered by Steinman [141] in 1975, as leukocytes with distinctive dendritic morphology. They are currently considered to be the most specialized professional APC critically involved in triggering adaptive immunity against invading pathogens, as well as inducing and maintaining immune tolerance for self antigens [142]. Steady-state DC subsets have been described in mouse and human, including typ-1 IFN-producing plasmacytoid DC (pDC) and conventional DC (cDC) [143]. pDC do not express T-, B- or myeloid-lineage markers but exhibit plasma-cell like morphology in steady-state. cDC are further classified according to their tissue localizations: such as skin DC including Langerhan's cells (LC) in the epidermis versus other DC subsets in the dermis, mucosal tissue-associated DC, lymphoid tissue-associated DC including splenic marginal zone DC, T-cell zone-associated interdigitating DC, germinal center DC (or follicular DC), thymic DC and interstitial tissue DC including liver versus lung DC. In contrast, DC that are not found in the steady-state but develop during or post-infection and inflammation include Mo-derived DC, TNF-producing and inducible nitric oxide synthase (NOS)-expressing DC (TipDC) and are referred to as inflammatory DC [144–148].

To date, our knowledge and understanding of DC biology have expanded significantly, which continues to be an active area of research. For a detailed summary of DC ontogeny, subtypes, origin, development and function, please refer to other reviews [146, 149–151]. In the following paragraphs, the author summarizes the recent findings supporting what is now a generally accepted notion that at least certain DC subsets are not functionally committed and/or fully differentiated post-lineage commitments [152] and manifest certain functional plasticity with uncharacterized potential to mount differential responses to various stimuli in the tissues microenvironment (see "Functional Plasticity of DC"). In addition, the author reviews and discusses the involvement of DC in periosseous inflammation and their mononuclear phagocytic link (see "DC and Inflammation in Bone" and "The Mononuclear Phagocytes Link", respectively). Collectively, these observations have been the impetus for our pursuit of studying the osteoclastogenic potential of DC

and the exploration of their candidacy as the precursors during inflammation-induced osteoclastogenesis.

Functional Plasticity of DC

Though most DC subsets share common features, it is evident that these various subsets have distinct developmental origins, life-spans and functions [146, 150, 151]. Recently, certain DC subsets have been shown to be involved in immune surveillance for tumors [153], in addition to the previously characterized and essential roles of other subsets during follicle development in lymph nodes [154]. DC are widely distributed in the body to rapidly access and sample the environment for the foreign antigens (Ags) and the danger signals. Upon responding to stimuli such as bacterial products and/or inflammatory cytokines, activated DC reach maturity quickly and then migrate to the draining lymph nodes, where they interact with naïve T-cells to activate adaptive immune responses [139, 140, 146]. Mature DC are subject to the influences of extracellular signals; however, immature DC are more susceptible to external cues in the surrounding microenvironment [146, 148, 153, 155], suggesting they may be functionally flexible or adaptive. Splenic $CD11c^{bright}$ DC, which can be categorized into at least three subsets based on CD4 and CD8α expressions, are the best characterized DC in the murine system [156], with differential abilities to prime T-helper/Th-cell responses [157]. For instance, it was previously shown that splenic $CD8\alpha^+$ and $CD8\alpha^-$DC subsets can preferentially prime Th_1 versus Th_2 responses, respectively [158–160]. More recently, studies have been further pursued to address whether distinct DC subsets have evolved to serve distinct functions such as the TLR expression profiles mentioned earlier [160–163]. Collectively, these findings provided supportive evidence that various DC subsets can indeed act both independently and in cooperation to regulate Th responses.

Meanwhile, there has been compelling evidence in support of DC plasticity based on recent studies that explored the functions of different steady-state versus inflammatory DC subsets [164, 165]. For instance, it was shown that pDC down-regulate their expression of TLR-7 and TLR-8 and the ability to produce IFN-α, while up-regulating TLR-4 expression associated with cDC-like phenotype [166]. Further, it has been shown that under inflammatory conditions, DC may respond differently depending on the nature of the surrounding milieu [i.e., cytokines; 146], therefore suggesting that certain DC subsets may not be terminally differentiated cells, despite their lineage commitments [152]. For example *L. monocytogenes* infection stimulates the development of MQ-like DC, known as TipDC [148]. Moreover, immune responses against certain tumors have been shown to trigger the generation of cytotoxic cells called: IFN-producing killer DC (IKDC), with the tumoricidal activity through secreting high levels of IFN-γ and TRAIL-dependent lysis of tumor cells [153, 167]. Although the exact nature of IKDC remains controversial (i.e., DC versus NK subsets), recent evidence suggests that IKDC are indeed a cell type distinct from NK cells [168, 169]. Nonetheless, these studies further support the idea of DC's functional plasticity, adding yet another level of complexity to the DC biology.

DC and Inflammation in Bone

The detection of various DC subsets in the rheumatoid nodules as well as periodontal lesions, led to a series of studies exploring the role and contribution of DC to inflammation-induced bone loss [138, 155]. However, the patho-physiological contributions and the molecular mechanisms of individual DC subset(s) to the resulting bone loss under these conditions remained unclear. In the synovial biopsies of RA, Page et al. [19] detected immature DC in the lining and sub-lining of perivascular synovial tissues, whereas mature DC were found in the periphery of peri-vascular synovium and lymphoid aggregates. Meanwhile, other reports indicated that (1) synovial fluid in human and experimental arthritis is rich in immature DC [i.e., $MHC\text{-}II^{low\ or-}$; 18], which quickly up-regulate MHC-II and the co-stimulatory molecules' expressions upon stimulation by growth factors such as GM-CSF and IL-4 [170] and (2) the abundant TGF-β in bone can act to modulate the DC maturity and function [18, 127].

Additionally, recent studies have demonstrated that (1) $RANKL^+$ cells are localized to the synovial lining and lymphocytic infiltrates and (2) $RANK^+$ cells are more restricted to the peri-vascular infiltrates, suggesting the involvement of RANKL/RANK signaling in DC/T-cells interactions during arthritic inflammation [19, 20]. Thus, with their APC function and their ability to modulate T-cell activity, DC have been suggested to be indirectly involved in inflammation-induced osteoclastogenesis and bone loss in RA [155], and likely so in periodontitis [20, 171]. In an ovariectomy-induced osteoporosis rat model, Grassi et al. [172] have shown that activated T-cells drive the subsequent bone loss via enhanced DC activity in BM, suggesting an indirect role for DC subsets during pathological bone loss. Taken together, it is evident that DC in the synovial fluid likely represents a part of the critical DC/T-cell interactions in the periosseous tissues (e.g., synovium & peridontium) during disease pathogenesis; yet, our understanding of their exact roles and physiological functions in such processes remains very limited.

The Mononuclear Phagocytes Link

DC, Mo and MQ are found throughout most tissues and are known to form a network of phagocytic cells, with M-CSFR dependent developmental programs [101, 173]. These cells are often referred to as the mononuclear phagocyte system and are known to play major roles in the development, scavenging, inflammation and immune responses to invading pathogens [142, 174]. The DC pool contains multiple heterogeneous subpopulations; for example, the $CD11b^{+}RANK^{+}$ subset, share the indicated expression pattern with many Mo and MQ subsets, including OCp, thus making the phenotypic distinction between DC and Mo/MQ rather challenging and difficult [175, 176]. In mice, although CD11c expression is currently considered a bona fide marker for DC, it appears neither exclusive nor required for DC development, as $CD11c^{+}$ Mo subsets have been detected in vivo [147, 176]. Therefore, the assessment of allogenic immune response via mixed lymphocyte reaction (MLR) is essential and generally accepted to distinguish DC from Mo/MQ at the functional level [177], particularly when studying cDC and the inflammatory DC subsets.

Langerhan's cells have been shown to be derived from circulating Mo precursors during inflammatory conditions [100]. Moreover, several cloning and adoptive transfer studies in the mouse have shown that many MQ subsets, most of the cDC in the secondary lymphoid organs and at least a fraction of DC subsets in the thymus, probably originate from the myeloid progenitors [178–181]. Thus, the inclusion of DC in the mononuclear phagocyte system becomes even more justified, as one closely examines the studies by Geissmann et al. [182, 183] exploring the lineage relationship(s) between MQ and DC in vivo. To date, precursors with common MQ/DC potentials called MQ-DC precursor (MDP) have been identified and are shown to give rise to cDC, pDC and Mo, including $Gr\text{-}1^{+}$ inflammatory Mo that differentiate into TipDC during infection [183]. In contrast, common DC precursors (CDP), which like MDP are present in the $M\text{-}CSFR^{+}$ Lin^{-} fraction of BM progenitors, are shown to give rise to pDC and cDC but not Mo or MQ [184–186]. These intriguing observations suggested that MDP have broader differentiation potential than CDP, and thus may represent an earlier precursor population [183]. Thereafter, Liu et al. [187] recently reported findings showing that DC development progresses from the MDP to CDP, which in turn give rise to pDC, splenic cDC but not Mo and finally to the committed precursors of cDC (pre-cDC), after which pre-cDC enter lymph nodes through and migrate along high endothelial venules and later disperse and integrate into the local DC network. Collectively, we predict that the developmental history and/or programs of certain DC subsets shared with certain Mo and MQ sub-populations (e.g., specific M-CSF/M-CSFR signaling requirements) likely hold the key toward a better understanding of what we described recently regarding DC's behavior/function, as OCp, during inflammation and subsequent bone destruction or loss.

DC During Osteoclastogenesis: The Silent Offenders at the Osteo–Immune Interface

Several immunohistochemical studies have detected both mature and immature DC in the "rheumatoid nodules" [188, 189] and "oral lymphoid foci" in RA and periodontitis, respectively [19, 20, 171, 190]. Such unique DC subsets form aggregates with T-cells in and around the inflammatory infiltrates [18, 20, 171, 190] and involve RANKL-RANK and other cytokines interactions associated with osteoclastogenesis and bone loss during different phases of disease progression [138, 155]. Based on studies of RA and periodontal disease models, there is ample evidence supporting that DC are situated at the crossroad of osteo–immune interface where they play a critical role in driving the immuno-pathological pathway(s) to tissue inflammation in concert with other leukocytes and immune effector molecules [24, 191]. For instance, DC can respond directly to danger stimuli including some endogenous antigens such as nuclear component high mobility group box chromosomal protein-1 (HMGB-1; a potent trigger of experimental arthritis) and crystalline uric acid, both of which are released during tissue damage, and capable of triggering DC maturation [192, 193]. Leung et al. [194] showed that type-II collagen-pulsed mature DC can induce arthritis in DBA/1 mice 10-days after adoptive transfer. Interestingly, the inflammatory environment in RA promotes myeloid DC differentiation, which preferentially activates Th_1 responses [195] and exhibits prolonged life span with enhanced resistance to the suppressive effects of IL-10 ex vivo [196]. DC in the joints of RA has been shown to express some modest levels of MHC and co-stimulatory molecules [197, 198]. In addition, a single injection of BM-derived DC engineered to express IL-4 reduces the incidence and severity of collagen induced arthritis (CIA) and suppresses Th_1 cells activity [199]. Similarly, significant suppression of CII-reactive T-cells or CIA was demonstrated in vivo by systemic administration of BM-derived DC genetically engineered to express Fas-L and TRAIL molecules [200, 201], thereby supporting DC's roles in disease pathogenesis as the important immune effectors and regulators of the body defenses during local and systemic inflammatory responses.

The concept of possible DC involvement during inflammation-induced bone destruction, through their

development into functional OC has only been recently and independently proposed by Delprat's group and us [26, 27, 202]. We discovered that, immature murine $CD11c^+$DC can develop into functional OC (i.e., DDOC) during immune interactions with $CD4^+$T-cells, in response to microbial sonicates or protein Ags and the essential RANKL-RANK signaling in the bone environment in vitro and post-adoptive transfer in vivo [26, 202, 203]. In parallel, Rivollier et al. [27, 28] showed that human blood Mo-derived and murine BM-derived immature DC can "transdifferentiate" into functional OC in the presence of M-CSF and RANKL. Moreover, it has been recently shown that pDC subset does not appear to manifest the capability or plasticity of developing into OC in response to M-CSF and RANKL [28], despite that $CD11c^-$ $B220^+$DC (i.e., pDC) are also affected in Csf-$1^{-/-}$op/op mice [101, 204]. However, whether or not pDC can become OC in response to Ag stimulation in the presence of RANKL is of some interest and remains to be further investigated. In our studies, DDOC manifested distinctive phenotype and behavior with certain key differences compared to those reported by Rivollier et al. [e.g., derived from immature $CD11c^+$DC being non-proliferative and carrying $CD11c^+$MHC-II^+ phenotype with dendrites; 202, 203]. Interestingly, DDOC appear to be smaller polykaryons than the classical OC [Teng et al. unpublished observations]. It remains unclear, though, whether other developmental and/or functional differences between the classical OC and the DDOC do exist [202], where further studies are currently underway to systematically compare the two subsets.

DC in the oral buccal mucosa have been shown to capture Ags and migrate to the regional draining lymph nodes [205]. For instance, upon responding to infection, LC in the oral mucosa increased their numbers during the development of gingivitis and periodontitis [17, 171]. Cutler et al. [171, 191] have suggested that both mature and immature DC drive the formation of "oral lymphoid foci" that localize around the inflamed periodontium at least in part through TLR signaling on LC and/or their progenitors. Our in vitro analyses suggest that TLR-4 signaling likely triggers an overall inhibitory effect on inflammation-induced DDOC development and its associated bone loss [203; Teng et al. unpublished data], suggesting a negative role for TLR signaling in this process. However, additional studies are required to ascertain the effects of the ligation of other specific TLRs on DDOC development and subsequent functions for modulating bone loss in vivo.

Our recent observations using in situ quantitative immunohistochemical analysis of experimental RA in mice revealed the presence of multinucleated $CD11c^+TRAP^+$ OC-like cells (possibly DDOC) located on the eroded bone surface of the arthritic joints (DBA mice immunized and boosted by chicken type-II collagen) but not in the healthy controls [206]. At present, whether or not the detected $CD11c^+TRAP^+$ OC-like cells are derived from local and/or infiltrating DC in this model remains to be further clarified. Interestingly, these $CD11c^+$OC-like cells appear to constitute a significantly higher proportion of TRA-P^+OC cells in the inflamed synovial and arthritic lesions compared to the $CD11c^-$ population [206; Teng et al. unpublished data]. The study of Da Costa et al. [207] where OC-like multinucleated giant cells expressing CD11c and HLA-DR were found in the local LC lesions of patients with histiocytosis, provided further echoing evidence supporting the idea that DDOC may indeed develop as a result of inflammatory disease-associated pathology in vivo. Using our NOD/SCID calvarial model for local tissue inflammation in a proof-of-principle experimental system, we found that a significant number of fluorescently labeled immature $CD11c^+$DC become $TRAP^+$ multinucleated OC-like cells within 7 days post-injection onto the calvarias surfaces [Teng et al. manuscript in preparation]. Although suggestive of bona fide osteoclastogenic potential for $CD11c^+$DC in vivo, these findings provide only inconclusive evidence in support of physiological significance regarding the DDOC development during inflammation, thereby requiring further investigations.

To date, the direct evidence for DC's involvement as OCp during inflammation-induced osteoclastogenesis and bone loss (e.g., DC depletion in vivo) remains lacking. By using diphtheria toxin receptor transgenic mouse model under the control of CD11c promoter, where depletion of $CD11c^+$ cells, presumably DC, can be achieved in vivo [208], Wakkach et al. [209] have recently suggested the direct involvement of DC in bone homeostasis as OCp and reported the rescue of the osteopetrotic phenotype in oc/oc mice after adoptive transfer of WT but not transgenic $CD11c^+$DC. However, the interpretations provided in these studies are compromised or complexed by the presence of OC and OCp in the oc/oc mouse model used. Because it has been previously shown that the defect of bone-resorbing activity in oc/oc mice is primarily due to the subcellular localization of V-ATPase essential for ruffled border formation and OC function, but neither to its expression level nor activity [210]. Moreover, because it remains unclear whether osteoclastogenesis can occur through the fusion of DC with OC precursors of Mo/MQ lineage in vivo; thereby a partial rescue of the osteopetrotic phenotype observed upon the injection of $CD11c^+$DC may be due to their fusion with endogenous OC or OCp, as a source for membrane V-ATPase.

At the junction of cytokine interactions during the osteo–immune cross-talks, Speziani et al. [28] have recently described the effects of TNF-α, IFN-α, IFN-γ, IL-1β, IL-2, IL-4 and IL-10 on OC development from $Flt3^+$ BM precursors-derived DC. The authors showed that,

similar to classical osteoclastogenesis, the studied cytokines promoted RANKL-dependent DDOC development with the exception of IL-2, IFNs and IL-4 [28]. These findings suggest a positive role for TNF-α, IL-1β and IL-10 during DDOC development, consistent with the results of our recent studies where cytokine depletion was achieved and tested using in vitro DC co-cultures. Interestingly, our latest studies have also indicated and concluded that TGF-β plays a very important role in promoting DDOC development. However, in contrast to Speziani et al., we found IFN-γ to promote rather than inhibit DDOC development at least in vitro (Teng et al. unpublished observations), the exact reasons behind this disagreement remain unclear but it could be due to differences in the culture conditions employed. The complex roles of immune cytokines in regulating DDOC development are compellingly evident in

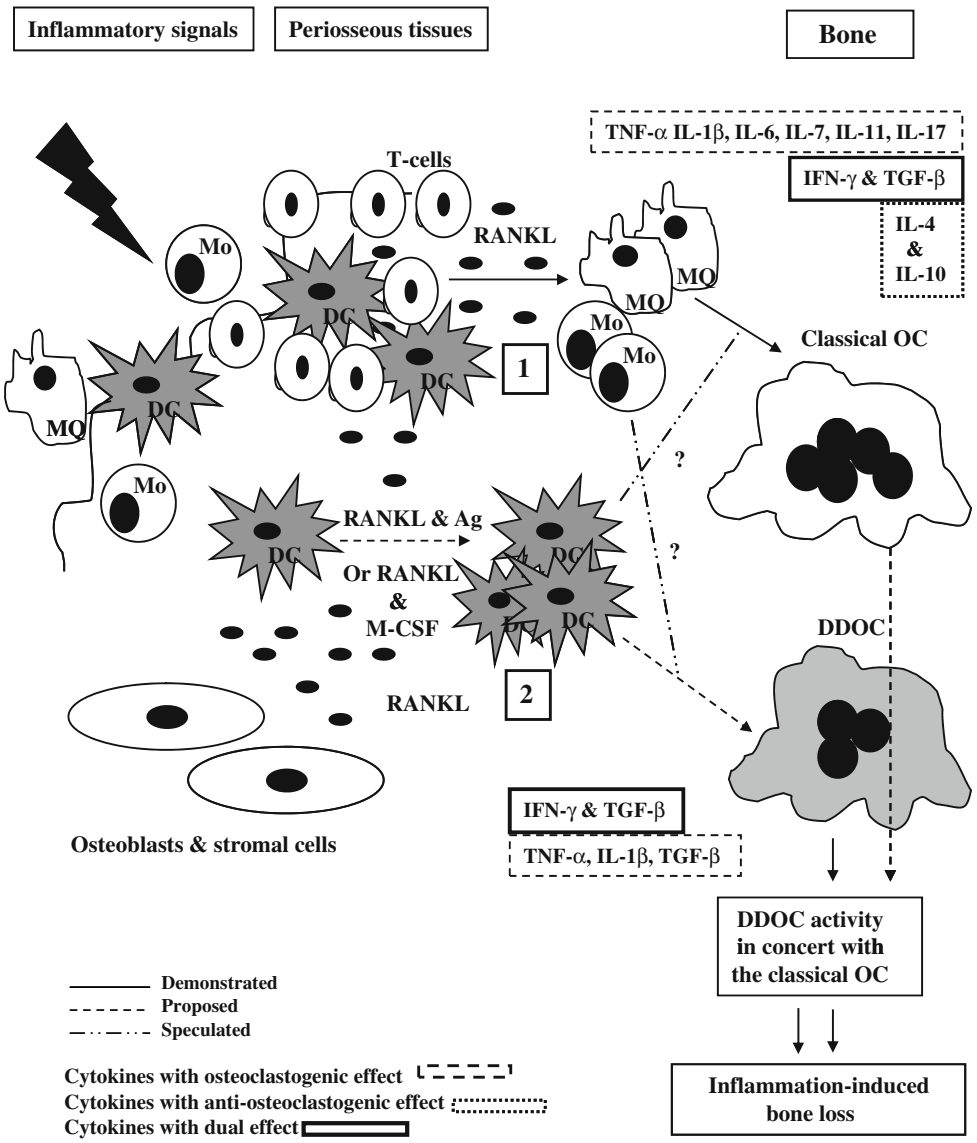

Fig. 1 Illustration of the possible mechanisms underlying the inflammation-induced osteoclastogenesis and bone loss. Inflammation or infection in the tissues adjacent to bone proper (i.e., periosseous or periodontal tissues) induces the infiltrations and activation of various subsets of leukocytes, including monocytes/macrophages, T- & B-cells and mesenchymal/residential versus stromal cells. The indirect contribution of DC as APC, which activate naïve T-cells, and lead to RANKL production, thereby triggering the classical OC development and activation is depicted in the pathway [as 1] (labeled classical OC; the *solid line*). In contrast, the direct contribution of $CD11c^{+}$DC as OC precursors (OCp) is depicted in the proposed pathway [as 2] (labeled DDOC; the *regular dashed line*). The release of several different (pro)-inflammatory and anti-inflammatory cytokines with osteoclastogenic and anti-osteoclastogenic effects supports the links to promote or inhibit the inflammation-induced osteoclastogenesis and bone loss, which ultimately control the balance versus dis-regulation of bone remodeling. Whether DC fuse with the classical OCp during the inflammation-induced osteoclastogenesis remains unclear and is speculated in the present model (the *irregular dashed line*)

our recent study using inflammation-induced advanced alveolar bone loss murine models, where the suppressor of cytokine signaling-3 (SOCS-3) was found to play a critical role in modulating cytokine signaling involved in RANKL-associated DC-mediated osteoclastogenesis during immune interactions with T-cells [211]. Collectively, based on our findings and other's, we propose that during disease pathogenesis of inflammatory bone disorders and in response to a complex network of signals modulated by locally released cytokines, DC develop functional OC phenotype and activities in a RANKL-dependent manner during their interactions with T-cells, stromal cells or OB; thus representing an alternative pathway of osteoclastogenesis and acting as the silent offenders directly involved in the inflammation-induced bone loss (see Fig. 1 for the proposed model). Moreover, in light of the reported overlap and/or continuity of DC and Mo/MQ subpopulations, a scenario whereby inflammation-induced osteoclastogenesis may result from DC fusion with OCp of the Mo/MQ lineage becomes conceivable but remains to be further evaluated (see Fig. 1 for the speculated model).

Summary

During the past decade, the critical roles played by DC in regulating T-cell-mediated immunity during inflammation-induced osteoclastogenesis and subsequent bone loss have been brought to light, thereby further expanding the current paradigm of osteoimmunology. Today, emerging evidence from our laboratory and others' independently suggests that specific DC subsets in mouse and human are likely involved in inflammatory bone diseases where they not only can act as potent APC for immune functions, but also directly impact bone destruction or osteolysis [26, 202, 203, 205]. Based on our phenotypic and functional characterization studies, we suggest that DC can act as OCp that further develop into DDOC with distinctive phenotype and behavior under the inflammatory conditions (Fig. 1). If proven physiologically significant, a direct contribution of certain DC subsets to inflammation-induced bone loss may prove to be a promising therapeutic target for controlling inflammation and the subsequent amelioration of bone pathology in diseases.

In summary, despite the lack of rigorous approach for conclusive and final evidence, our recent studies and results strongly support a critical and direct role for DC as OCp at the osteo–immune interface during inflammation in the periosseous tissues. The next stage of investigation underway is to assess the physiological relevance and significance of DDOC development in experimental and human inflammatory bone disorders such as RA, osteomyelitis and periodontitis. Such studies will determine whether DC is indeed an important source of OCp during periosseous inflammation in vivo. If proven true, DC could become perhaps the most actively studied cell subset in the osteoimmunological research, with their active and significant roles at the immune and skeletal sides of the disease equation.

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