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Dendritic Cell-Mediated In Vivo Bone Resorption

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Osteoclasts are resident cells of the bone that are primarily involved in the physiological and pathological remodeling of this tissue. Mature osteoclasts are multinucleated giant cells that are generated from the fusion of circulating precursors originating from the monocyte/macrophage lineage. During inflammatory bone conditions in vivo, de novo osteoclastogenesis is observed but it is currently unknown whether, besides increased osteoclast differentiation from undifferentiated precursors, other cell types can generate a multinucleated giant cell phenotype with bone resorbing activity. In this study, an animal model of calvaria-induced aseptic osteolysis was used to analyze possible bone resorption capabilities of dendritic cells (DCs). We determined by FACS analysis and confocal microscopy that injected GFP-labeled immature DCs were readily recruited to the site of osteolysis. Upon recruitment, the cathepsin K-positive multinucleated osteoclasts were observed in bone-resorbing pits. Additionally, chromosomal painting identified nuclei from female DCs, previously injected into a male recipient, among the nuclei of giant cells at sites of osteolysis. Finally, osteolysis was also observed upon recruitment of CD11c-GFP conventional DCs in Csf1r−/− mice, which exhibit a severe depletion of resident osteoclasts and tissue macrophages. Altogether, our analysis indicates that DCs may have an important role in bone resorption associated with various inflammatory diseases. The Journal of Immunology, 2010, 185: 1485–1491.

The osteoclast is a member of the monocyte/macrophage family for which the terminal differentiation is controlled by two critical cytokines, receptor activator of NF-κB ligand (RANK-L) and CSF-1 (1–4). Upon differentiation, precursor cells fuse into tartrate-resistant acid phosphatase (TRAP)-positive and cathepsin K-positive multinucleated osteoclasts (5). At sites of active bone resorption, the osteoclasts form a specialized cell membrane, the ruffled border, following organization of the actin cytoskeleton in conjunction with αvβ3 integrin-mediated matrix recognition. On attachment to bone, matrix-derived signals polarize intracellular secretory vesicles that deliver H⁺ATPase to the plasma membrane and release proteolytic enzymes into the resorptive microenvironment. Degradation of the bone Ca⁺⁺/phosphate inorganic matrix is achieved through ATPase-mediated active secretion of protons, which create an acidic environment in the resorbing lacunae (6). In contrast, the organic matrix, mostly composed of collagen and elastin, is proteolysed by secreted cystein and aspartic proteases, among which cathepsin K has a major role (7).

At steady state, osteoclasts are thought to arise from osteoclast progenitors present in the bone marrow as well as in the peripheral circulation (7). Two mouse mutants, CSF-1–deficient osteopetrotic (Csf1r−/−) and CSF-1R knockout (Csf1r−/−) mice, indicate that compromising myeloid differentiation into osteoclasts induces defective bone resorption with increased bone density and osteopetrosis (8, 9). In an osteopetrous mouse (Csf1r−/−), a recessive mutation in the CSF1 gene compromises the development of monocyte-derived osteoclasts and tissue macrophages in several organs (8). Similarly, Csf1r−/− mice have a profound reduction in the number of tissue macrophages and bone osteoclasts (9). However, in the Csf1r−/− mice, the development and total number of conventional dendritic cells (DCs) and Langerhans cells is not compromised (8), whereas in Csf1r−/− mice, Langerhans cells are absent but the conventional DC population is not affected (9, 10).

During conditions of acute and chronic inflammation, such as occur in osteoarthritides, rheumatoid arthritis, aseptic osteolysis, more inflammatory infiltrates are observed not only at the bone surface, but also in the soft tissue surrounding the bone. However, it is still uncertain whether the inflammatory infiltrate only participates in bone resorption by favoring osteoclastogenesis through RANK-L and M-CSF production or whether the inflammatory infiltrates may play a more active primary role in bone resorption. In principle, because a CD11b⁺/GR1low/c-fms−/− common myeloid progenitor gives rise to macrophages, DCs, and osteoclasts, it is possible that DCs attracted to the site of inflammation could potentially be induced to express osteoclast activity (11–13). The presence of the CSF-1R on conventional DC precursors (11), as well as expression of cathepsin K, TRAP, and RANK-L in immature DCs, supports this possibility (12). Indeed, several recent in vitro experiments indicate that both human (monocyte-derived) and mouse (bone marrow-derived) DCs can function as osteoclasts in the presence of CSF-1, RANK-L, and bone-like matrix (13–15). However, whether this occurs in vivo is not known.

In the current study, a model of aseptic bone inflammation was used to determine the osteoclastic potential of conventional DCs. Among the possible experimental models that we could select to...
study the osteolytic potential of DCs, we chose ultra-high m.w. poly-
ethylene (UHWMPE)-induced osteolysis for several reasons: 1) in this
model, the bone inflammatory response is induced by injecting
sterile alkane particles (UHWMPE) under the calvarial peristium
to generate an aseptic inflammatory reaction that is easier to control
compared with pathogen-induced osteolysis (16); 2) the resulting
inflammatory reaction is localized to the site of injection and there-
fore easier to quantify than a more systemic reaction; and 3) the
inflammatory infiltrates are mostly formed by local osteoclasts,
recruited macrophages, and DCs, with very few T and B cells
(16). Experimental mice were performed in Csf1r−/− mice, which are pro-
foundly deficient in osteoclasts and tissue macrophages, conclus-
ively demonstrated the in vivo osteolytic potential of CD11c+ con-
ventional DCs recruited to the sites of bone resorption.

Materials and Methods

UHWMPE-induced calvarial osteolysis

UHWMPE-induced calvarial osteolysis was performed according to pub-
lished protocols (16). Briefly, mice in each group (C57BL/6 female 8–12
wk old) were sedated with isoflurane gas and anesthetized with ketamine (20%
ketamine HCl, 15% xylazine, and 65% saline at 0.1 ml per 20 g body
weight). A 10-mm incision was made over the sagittal midline suture of the
calvarium and a 1.0 × 1.0 cm area of the periosteum exposed. In sham
control mice, the incision was closed without any further intervention,
whereas experimental mice received 20 μl of diluted UHWMPE particles with
an average size of 53–75 μm (Sigma-Aldrich, St. Louis, MO). The par-
ticles were distributed over the periosteum using a sterile sharp surgical
spatula and the incision sutured. Twelve days postoperation, a microcom-
puted tomography (micro-CT) scan was performed to confirm and verify
the development of particle-induced osteolysis. Micro-CT scans were per-
formed on the Aloka LaTheta laboratory CT scan machine (Aloka, System
Engineering Co., Tokyo, Japan). The x-ray voltage was set at high. For
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In vitro bone marrow cell differentiation

Bone marrow cells were purified using the lineage-negative magnetic bead kit, which depletes cells expressing the following lineage Ags: CD5, B220,
Lin−, CD11b, Gr-1, CD3, and Ter-119 (Miltenyi Biotec, Auburn, CA). Lineage
negative (Lin−) precursors were then cultured in DMEM (Life Technolo-
gies, Rockville, MD), supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM l-glutamine, 0.1 mM nonessential amino acids, 1 mM
pyruvate, and 10 mM HEPES containing either GM-CSF (10 ng/ml) for 8
Cell culture

Intravenous injection of GFP+ DCs and FACS analysis of cells
recovered at sites of osteolysis

In some experiments, 2 wk postoperation, mice (8–12-wk-old female C57BL/
6) were injected i.v. with 104 bone marrow-derived GFP+CD11c+ DCs
prepared from C57BL/6-transgenic (TG) CAG-GFP) mice (The Jackson
Laboratory, Bar Harbor, ME). The CD11c+ cells were purified from bone marrow cells that had been cultured in GM-CSF for 7 to 8 d, using
CD11c+ Ab conjugated magnetic beads (Miltenyi Biotec). Cell pu-
rity following magnetic bead separation was assessed by FACS. Only
preparations with a purity ≥99% were used. In some experiments, 96 h
after DC injection, calvaria were isolated from sham and UHWMPE-
implanted mice and directly observed as a whole mount using differential
contrast imaging and fluorescence microscopy. In other experiments, 96 h
following GFP+DC injection, cells were retrieved from the calvaria by
extensively washing the bone in DMEM and gently scraping the bone
surface. Retrieved cells were either analyzed directly by FACS (to quantify
the percentage of GFP+ cells) or stained for cathepsin K. For staining, cells
were fixed for 10 min in 2% paraformaldehyde, washed in 10 mM glycine,
and permeabilized for 30 min in 0.05% saponin. Cells were incubated on
ice for 30 min with 10 μg/ml Ab to mouse cathepsin K (Santa Cruz Bio-
technology, Santa Cruz, CA) followed by an anti-mouse IgG conjugated to
Alexa Fluor 568. Cells were analyzed on an FACSScan flow cytometer (BD
Biosciences, San Jose, CA).

Western blot analysis

Cultured cells (DC or osteoclasts) were collected, washed in PBS, and lysed
in 150 mM Tris-HCl (pH 8), 150 mM NaCl, 5 mM EDTA, and 1% Nonidet P-40
supplemented with a mixture of protease inhibitors (Roche, Basel,
Switzerland). Eighty micrograms protein was run on a 12% SDS-
PAGE. After blotting, membranes were probed with the following primary
Abs: cathepsin F, K, L, S, and TRAP (Santa Cruz Biotechnology). Sec-
ondary Abs were HRP-labeled, and detection was performed using the
Super Signal West Pico Chemiluminescent substrate (Pierce, Rockford, IL).

Confocal microscopy

The calvaria from sham and UHWMPE-implanted mice were decalcific-
ted, as described above, and embedded in either OCT-compound
(Tissue-Tek, Sakura, Torrance, CA) for frozen sections or in paraffin accord-
ing to standard procedures. Slides were stained for 30 min at room temper-
ature with 10 μg/ml Ab to mouse cathepsin K (Santa Cruz Biotechnology)
followed by Alexa Fluor 568-conjugated goat anti-mouse secondary Ab.
Stained slides were examined using a scanning confocal microscope (Leica
AOBS system, Leica Microsystems, Deerfield, IL). All images were col-
clected using identical fluorescence settings.

Fluorescence in situ hybridization

Fluorescence in situ hybridization was performed using a painting probe
specific for the murine Y chromosome. DNA for the Y chromosome (M. A.
Ferguson-Smith, University of Cambridge, Cambridge, U.K.) was labeled
by degrading oligonucleotide-primed PCR with Spectrum Orange-dUTP
(Vydyne, Robst Molecular Technol. U.K.). Fluorescence in situ hybridiza-
tion (FISH) paraffin embedded tissue sections were baked overnight at 56˚C, dipped
twice in xylene for 5 min at room temperature, and rehydrated in ethanol
(100–90–70%). Slides were then washed in 4× SSC/0.5% Tween 20
at room temperature for 30 min in a rotating shaker. Pretreatment to digest
cytosplasm was performed with pepsin (final concentration 0.4 mg/ml)
at 37˚C for 30 min. Slides were denatured in formamide for 1 min and 45 s,
and then a probe was applied and incubated overnight at 37˚C in a humid-
ified chamber. After washing, nuclei were counterstained with DAPI for 10
min. Interphase cells were imaged with an Olympus BX61 microscope
equipped with a Cooke SensiscanQE camera with IPLab for image acqui-
sition (Olympus, Melville, NY). An IPLab script was generated to acquire
images of interphase cells for the Spectrum Orange dye and for a differen-
tial contrast image. Multiple focal planes (13) were acquired as a whole
mount using differential contrast imaging. Signal detection on different focal planes was included. Images were analyzed with tools available through ImageJ (National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/).

Generation and subcloning of the CD11c promoter

Two hundred nanograms genomic DNA from a 129 mouse was amplified
using the following primers: forward primer 5′-CATGCTCAGAGA-
TCCAGAGCTGGCTGCTG-3′ (designed with XhoI and BamHI
restriction sites at the 5′) ; reverse primer 5′-CATGACCGGTGACAGAG-
GGCTCTGAGCA-3′ (designed with the AgeI restriction site at the 3′).
The amplified 2013-bp CD11c promoter corresponds to bases 3790–5802
of the published 1211c promoter sequence (Gene Bank DQ658851.1).
Following PCR amplification, the promoter was digested with XhoI and
AgeI restriction enzymes and cloned into the pCCL.PPT.GFP.PGF.PRE
lentivector upon removal of the PGK promoter (17).

Lentiviral vector production and concentration

Lentiviral particles expressing either the non–tissue-specific PGK promoter-
GFP construct or the tissue-specific mCD11c promoter-GFP construct were
generated by calcium phosphate-mediated cotransfection of 293T cells withour plasmids: 1) a CMV promoter-driven packaging construct expressing
the gag and pol genes; 2) a Rous sarcoma virus promoter-driven construct
to express rev; 3) a CMV promoter-driven construct expressing the VSV-g
evelope; and 4) the previously generated 2013-bp CD11c promoter con-
trolling GFP expression. Thirty hours following transfection, the culture
supernatant containing the packaged viral particles was collected and con-
centrated by ultracentrifugation. Collected viral particles were titrated on
Bone marrow transplant experiments

Six to 8-wk-old male mice (Csf1r<sup>−/−</sup> on an outbred C57BL/6J, C3Heb/FcJ-a/a, 129SvJ background) were killed with CO<sub>2</sub> and the bone marrow collected by flushing femurs and tibia. Lineage negative (Lin<sup>−</sup>) cells were purified as described above (Miltenyi Biotec MACS Lin<sup>−</sup> cells). Cells were transduced with either the PGK-prom-GFP lentiviral vector or the mCD11c prom-GFP lentiviral vector at a concentration of 10<sup>5</sup> TU/ml for 10<sup>⁶</sup> cells. Transduced cells were cultured for 24 h in StemSpan media (StemCell Technologies, Vancouver, British Columbia, Canada) supplemented with IL-3, IL-6, stem cell factor, and Flt-3 ligand cytokines. For bone-marrow transfer experiments, transduced cells were injected into the tail vein (10<sup>⁵</sup> cells/mouse) of 8-wk-old mice (C57BL/6 or Csf1r<sup>−/−</sup>) that had been previously lethally irradiated (2 × 6 Gy). Full engraftment of the transplanted hematopoietic stem cells was observed 2 mo following bone marrow transplantation.

Characterization of GFP<sup>+</sup> DCs in blood, bone marrow, and spleen of the transplanted mice

Spleen, blood, and bone marrow samples were harvested from each mouse 2 mo following bone marrow transplantation. Single-cell suspensions were prepared from each organ and RBCs removed by lysis. For immunostaining, 10<sup>⁵</sup> cells were incubated in staining buffer (PBS, 2% FBS) for 30 min at 4°C with 1 to 2 µg/ml of the following PE-conjugated primary Abs: CD19, CD11b, CD11c, Gr-1, CD3, and NK1.1 (BD Pharmingen, San Diego, CA). Following incubation, cells were washed three times in staining buffer and analyzed on an FACSscan flow cytometer (BD Biosciences).

**Results**

**UHMWPE calvarial model**

The mouse model of osteolysis was established by implanting microgram amounts of UHMWPE particles adjacent to bone in mouse calvaria as described previously (16). In sham-operated mice, the calvaria were surgically uncovered and the periosteum incised with no further intervention, whereas in the UHMWPE group, particles were deposited directly between the bone and the periosteum. Two weeks postsurgery, a strong cellular infiltrate was observed in the UHMWPE-implanted mice, and small pit-like erosions were present on the calvarial surfaces (Fig. 1a). Cellular fusion around the particles was also evident from the presence of multinucleated giant cells (mgcs) exhibiting 6–12 nuclei (Fig. 1b). To quantify the extent of calvarial osteolysis, mice were analyzed with micro-CT (Fig. 1c). In mice receiving UHMWPE particle implants, a statistically
significant decrease in cortical and total bone mass density was observed in the postsurgery specimens. In contrast, no significant differences were observed in the sham controls pre- and postsurgery (Fig. 1d). We therefore sought to adapt this model to gain further insight into how DCs might be involved in the bone-resorptive process.

**DCs are recruited to the site of osteolysis**

In the next set of experiments, we addressed the question of whether peripheral myeloid DCs were recruited to the tissue surrounding the area of osteolysis. Ten million CD11c+ immature bone marrow-derived DCs were prepared from C57BL/6-Tg (CAG-EGFP) mice, which express the enhanced GFP (EGFP) under the control of the β-actin promoter. Cells were injected into the tail veins of UHMWPE-implanted and sham-operated mice. Ninety-six hours later, the whole calvarium was observed using differential contrast imaging and fluorescent microscopy (Fig. 2a, 2b). Green fluorescent areas were observed in the frontal area of the calvaria in UHMWPE-implanted mice but not in the sham control (Fig. 2b). The calvarium area adjoining the site of surgery was then excised, decalcified, and 5-μm frozen sections generated for further fluorescent microscopic analysis. Extensive cellular infiltrates were observed under light microscopy in UHMWPE-implanted mice but not in the sham controls (Fig. 2d). Among the infiltrates, many fluorescent cells could be observed in the UHMWPE-implanted mouse (Fig. 2e, right panel, merged image of 2d and 2e shown in 2f). FACS quantification of the GFP-injected DCs recruited to the calvaria indicated that in the mice implanted with UHMWPE, 13% of the cells retrieved at the site of osteolysis were GFP+ (Fig. 2c). These results demonstrate an active recruitment of DCs to the site of osteolysis.

**DCs recruited to sites of osteolysis express cathepsin K**

In the next series of experiments, we sought to determine whether conventional DCs that migrate to the site of osteolysis express cathepsin K. Among the possible markers associated with osteolytic activity, we chose cathepsin K because it is a well-characterized osteoclast marker, and its activity in bone resorption is indicated by the bone macromegalia observed in cathepsin K knockout mice (18). More importantly, CD11c+ conventional DCs express low levels of cathepsin K, which can be readily upregulated in vitro following culture of DCs with M-CSF and RANK-L, indicating, at least in vitro, the bone resorption potential of DCs (Fig. 3a).

To this goal, CD11c+ DCs were purified from the bone marrow of C57BL/6-Tg (CAG-EGFP) mice (Fig. 3b) and injected into syngenic non–GFP-recipient mice, which were either sham operated or implanted with UHMWPE. Confocal analysis determined that in the calvaria of sham-operated mice, essentially no recruited CD11c+ GFP+ cells could be observed (Fig. 3c), and the only cathepsin K+ cells (red) were the resident osteoclasts (Fig. 3c). In contrast, the calvaria of mice that had been implanted with UHMWPE particles exhibited a rich infiltrate of recruited GFP+ DCs (green), some of which stained positive for cathepsin K (Fig. 3d, 3e, red). In addition, some cells that were observed in contact with the bone (Fig. 3f, left panel) were positive for both GFP and cathepsin K (Fig. 3f, right panel, arrow), indicating the possibility that DCs may form bone-resorbing pits in the UHMWPE-implanted mice (Fig. 3f). To better quantify the number of CD11c+GFP+ cathepsin K+ cells recruited to the site of osteolysis, the calvaria of sham- or UHMWPE-implanted mice was removed. Cells retrieved from the site of osteolysis were stained for cathepsin K and analyzed by flow cytometry. UHMWPE-implanted mice showed an increased calvarial recruitment of cathepsin K+ GFP+ DCs as compared with sham controls (Fig. 3g).

Taken together, these data indicate that cathepsin K+ GFP+DCs are readily recruited to the site of osteolysis. **DCs recruited to the site of bone inflammatory osteolysis fuse into mcgs**

Active osteoclasts, the primary cells involved in bone erosion, are organized as mcgs that are closely connected to the erosion pits observed during inflammatory bone resorption. To determine whether DCs are recruited into the mcgs observed around the UHMWPE particles (Fig. 1b), bone marrow DCs were prepared from a C57BL/6-Tg (CAG-EGFP) female donor. These cells were then injected into male recipients that were previously surgically implanted with UHMWPE or had undergone a sham procedure. DC recruitment and their osteolytic potential were monitored by tracking GFP+ cathepsin K+ cells as described above (Fig. 4). In Fig. 4b, a GFP+ cell can be observed in the inflammatory infiltrates, which, in a serial section, did not stain for the Y chromosome (Fig. 4c, white arrow). Thus, this cell was clearly derived from the transplanted bone marrow DCs. A similar group of inflammatory cells adjacent to the bone are shown in Fig. 4d-f. Transplanted bone-marrow-derived recruitment into mcgs was demonstrated by detection of nuclei missing the Y chromosome in mcgs around the UHMWPE particles (Fig. 4g). These cells contained multiple nuclei, the majority of which were positive for the Y chromosome (recipient cells), but a small number were missing the Y chromosome, indicating that they were derived from the transplanted female donor DCs (Fig. 4g, white arrows, second and fourth panels). These data support the proposition that

**FIGURE 3.** Cathepsin K-positive DCs are recruited to the site of osteolysis. a, Western blot analysis for cathepsin S, L, F, and K and TRAP of bone marrow-derived immature DCs differentiated for 8 d in GM-CSF (DC) or further differentiated with RANK-L and M-CSF for an additional week (osteoclasts). b, FACS analysis of CD11c expression of purified GFP+ DCs prior to injection into GFP+ recipient mice (solid line) or isotype control (dotted line). c–f, Confocal light (left panel) and fluorescent (right panel) images of calvarial sections analyzed for expression of cathepsin K+ (red) and GFP+ (green) DCs recruited to the site of surgery in sham (c) or UHMWPE-implanted mice (d–f). c and d, Original magnification ×63; e and f, original magnification ×100. Arrow indicates cathepsin K+ GFP+ DCs recruited to areas of osteolysis. g, FACS profile of GFP−cathepsin K+ DCs retrieved from the calvaria of sham or UHMWPE-implanted mice. One of three experiments is shown. In each experiment, three mice for each experimental condition (sham or UHMWPE) were used.
recruited DCs can participate in the formation of giant cells found at inflammatory sites adjacent to the bone.

**DCs recruited to the inflammatory site possess bone resorption activity**

To determine in a more definitive manner whether DCs recruited to sites of bone inflammation possess osteolytic potential, we performed the UHMWPE calvarial model experiments in mice devoid of osteoclasts. To do this, we used Csf1r<sup>−/−</sup> mice, which are severely depleted of macrophages and osteoclasts and which phenotypically present with extensive osteopetrosis (9). Also, because these mice lack the CSF-1R, monocytes that might have been recruited to the inflammatory site cannot differentiate into osteoclasts. The Csf1r<sup>−/−</sup> mice are difficult to breed because the majority of newborn mice die within 1 mo. The few mice that survive are very frail, and several attempts to perform surgical experiments on these mice failed, and so chimeras were created.

To create an endogenous population of GFP<sup>+</sup> DCs, Csf1r<sup>−/−</sup> mice were lethally irradiated and transplanted with Csf1r<sup>−/−</sup> bone marrow cells that had been previously transduced with a lentiviral vector expressing GFP under the control of the CD11c promoter. The tissue specificity of the CD11c promoter was compared with the non–tissue-specific PGK promoter. As expected, transplantation of bone marrow cells with the non–tissue-specific PGK promoter controlling GFP expression gave rise to green fluorescent cells in both the lymphoid (T and B cells) and myeloid populations (macrophages, DCs, and granulocytes) (data for spleen shown in Fig. 5a). In contrast, in mice transplanted with total bone marrow cells transduced with the tissue-specific CD11c promoter, GFP expression was restricted to the myelomonocytic population in the spleen (Fig. 5a).

Two months after irradiation, chimeric (Csf1r<sup>−/−</sup> recipient and Csf1r<sup>−/−</sup> bone marrow donor) mice were either surgically implanted with UHMWPE on their calvaria or were sham operated. Two weeks following surgery, the calvaria were removed and analyzed by CT scan for bone erosion and resorption. One hundred sixty-two scan images were overlapped as a three-dimensional image (Fig. 5b). Sham-operated chimeras or control Csf1r<sup>−/−</sup> did not display any sign of osteolysis (Fig. 5b, first and third panels). In contrast, UHMWPE-injected mice exhibited extensive osteolysis (Fig. 5b, second and fourth panels). Mice competent to produce osteoclasts, macrophages, and DCs (Fig. 5b, fourth panel), as well as mice repopulated with DCs but lacking osteoclasts and macrophages (Fig. 5b, second panel), displayed bone surface erosion (Fig. 5b), indicating the in vivo bone resorption activity of recruited DCs.

**Discussion**

Under physiological conditions, maintenance of bone mass is achieved through a delicate balance between the activity of bone-forming osteoblasts and bone-resorbing osteoclasts (19). This constant bone remodeling is necessary to support calcium homeostasis and to meet the structural needs of the musculoskeletal system. However, in certain inflammatory diseases, such as rheumatoid arthritis, this balance is lost, and osteolytic activity predominates, resulting in diffuse periarticular osteopenia and localized bone erosion (20–22). Understanding the mechanisms that determine the relative activity of osteoblasts and osteoclasts is important for the development of strategies for minimizing skeletal injury in these inflammatory diseases.

Osteoclastogenesis depends upon progenitor cells of the monocytic hematopoietic lineage. These cells differentiate into osteoclasts under the influence of M-CSF and RANK-L, derived at least in part from bone stromal cells (23, 24). DC-specific transmembrane protein and αvβ3 integrin are also part of the osteoclast differentiation pathway. DC-specific transmembrane protein controls cell fusion, which leads to mgs, whereas αvβ3 integrin is important for providing giant cells with the ability to adhere to the bone surface and to form an acidified extracellular microenvironment. Within this latter space, cathepsin K and TRAP are released to promote...
A still-unresolved question is the source of osteoclast precursors. At the present time, we do not know how many cells are derived from the bone pool of physiological preosteoclasts versus the recruitment of peripheral circulating precursors. This question is particularly important for understanding bone resorption in inflammatory conditions. Monocytes and circulating DCs are normally recruited to inflamed tissue (24, 26, 27), where monocytes may directly differentiate into osteoclasts (26). However, DCs could play either a direct or indirect role in osteoclastogenesis (28, 29). An indirect role would involve the ability of these cells to produce cytokines, such as IL-1, IL-6, and TNF-α, that augment expression and release of TRAP and cathepsin K by resident osteoclasts (30). DCs could also promote osteoclastogenesis indirectly by stimulating T cells to express RANK-L, a major differentiation factor for osteoclast precursors (31). In contrast, DCs could potentially directly participate in bone resorption following cathepsin K release. Indeed, several in vitro studies support the notion that immature DCs can differentiate into osteoclast-like cells (14, 32, 33). Culture of DCs with naïve T cells and human periodontal pathogens indicated the in vitro ability of DCs to fuse into multinucleated cells with bone resorptive activity (14). In the current study, we addressed the osteolytic potential of DCs using an in vivo model that permits us to track recruitment of DCs to sites of bone inflammation, as well as testing knockout mice that lack macrophages and osteoclasts (9). The results demonstrated that exogenous myeloid DCs migrate to an inflammatory site of osteolysis, where they are active participants in bone resorption.

A still-open question is whether the same growth factors that control physiological osteoclastogenesis are also involved in de novo inflammatory-induced osteoclastogenesis. In vitro analysis indicates that, similarly to bona fide osteoclast precursors, immature DCs are also dependent on RANK-L and M-CSF for differentiation into mature osteoclasts (14). These data support the notion that, at least in vitro, a similar growth factor-mediated differentiation pathway is used by both precursors. However, it is also possible that additional cytokines or growth factors play a role during the in vivo process of DCs’ differentiation into osteoclasts. In fact, Langerhans cells have been shown to fuse into mononuclear cells with bone-resorptive properties (34). Thus, it is possible that conventional DC fusion into multinucleated cells with bone-resorption properties is also dependent on other cytokines and growth factors. It would be of great interest to determine whether the biological function of mature osteoclasts derived from bona fide osteoclast precursors or derived from immature DCs differs. This point is of extreme interest because the fusion of DCs and their role in bone resorption is primarily observed during inflammatory

**FIGURE 5.** DCs induce inflammatory osteolysis in absence of endogenous macrophages and osteoclasts. a, FACS profile of T cells (CD3), B cells (CD19), granulocytes (Gr-1), macrophages (CD11b), DCs (CD11c), and NKT cells (NK1.1) from the spleen of mice previously transduced with lentiviral vector expressing GFP under the control of a general promotor (PGK) or a tissue-specific promotor (CD11c). Mice were analyzed 2 mo after receiving the bone marrow transduced with the lentiviral vectors. Ten days prior to the FACS analysis, mice were injected with the B16-GM-CSF cells that produce endogenous GM-CSF, thus increasing the number of splenic DCs and facilitating the analysis of the CD11c promotor tissue specificity. b, Csf1r+/- recipient mice were lethally irradiated and transplanted with the bone marrow of either Csf1r+/- or Csf1r-/- mice. The procedure generates chimeras that only express CD11c+ DCs (first and second panels) or DCs, macrophages, and osteoclasts (third and fourth panels), respectively. CT scan of the calvaria of chimeras mice implanted with UHMWPE or sham controls. One of two experiments is shown (in each experiment, three mice for each experimental condition [sham or UHMWPE] were used).
conditions. Intervening in the recruitment of DCs to the bone matrix or their fusion into giant cells could be part of a therapeutic approach for limiting joint injury in diseases, such as osteoarthritis and rheumatoid arthritis (36). This model could be a useful tool for the development and testing of such agents.

Disclosures

The authors have no financial conflicts of interest.

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The authors have no financial conflicts of interest.