REVIEW

An Overview of the Derivation and Function of Multinucleated Giant Cells and Their Role in Pathologic Processes

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Accepted for publication
February 13, 2019.

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Monocyte lineage cells play important roles in health and disease. Their differentiation into macrophages is crucial for a broad array of immunologic processes that regulate inflammation, neoplasia, and infection. In certain pathologic conditions, such as foreign body reactions and peripheral inflammatory lesions, monocytes fuse to form large, multinucleated giant cells (MGCs). Currently, our knowledge of the fusion mechanisms of monocytes and the regulation of MGC formation and function in discrete pathologies is limited. Herein, we consider the types and function of MGCs in disease and assess the mechanisms by which monocyte fusion contributes to the formation of MGCs. An improved understanding of the cellular origins and metabolic functions of MGCs will facilitate their identification and ultimately the treatment of diseases and disorders that involve MGCs. (*Am J Pathol* 2019, 189: 1145–1158; <https://doi.org/10.1016/j.ajpath.2019.02.006>)

Certain healthy tissues, including skeletal muscle, placenta, and bone, contain populations of multinucleated cells.¹ In contrast, the presence of multinucleated cells in other tissues is considered pathologic. Multinucleated giant cells (MGCs), which are polykaryons of monocytic origin, are often spatially associated with foreign bodies (ie, introduced exogenous materials) or comprise part of a tissue response to infection. MGCs also appear in autoimmune, neoplastic, and genetic disorders. Histologically, MGCs are morphologically similar to one another, despite their appearance in various discrete pathologies. Currently, the characterization of the various types of MGCs by histology alone is of limited value as the molecular determinants that specify the formation and function of these cells are not defined.

Although multinucleated cells of other origins exist in some pathologies, only those of monocytic origin will be discussed herein. Under the influence of specific cytokines and signaling molecules, monocytes can differentiate into discrete subtypes, macrophages and preosteoclasts. These more specialized cells can then undergo multiple fusion events to form multinucleated cells. Despite advances in the understanding of monocyte differentiation, the relationship

between various monocyte and macrophage subtypes and the different MGCs that they form is not defined. We focus herein on discrete, MGC-containing lesions and consider the fusion mechanisms and molecules involved in MGC formation. An in-depth understanding of the monocyte and macrophage subtypes that contribute to MGC formation in various pathologic conditions and the expression of molecules that enable cell fusion could lead to better diagnostic approaches through application of disease-specific MGC markers and new molecular targets for MGC-focused therapies.

Multinucleated Giant Cells in Pathology

MGCs exhibit similar morphologies under light microscopy but arise in pathologic lesions of widely varying etiology

Supported by a Queen Elizabeth II Graduate Scholarship in Science and Technology (P.J.B.), the Princess Margaret Cancer Centre Research Foundation (M.G.), and the Canada Research Chair in Matrix Dynamics and Canadian Institutes of Health Research operating grant MOP36332 (C.A.M.).

Disclosures: None declared.

(Figure 1). The diagnostic workup of giant cell–rich lesions can be challenging despite a priori information about patient demographics, anatomic location, and radiographic interpretation.² Currently, there is little understanding of MGC subtypes, which complicates the differential diagnosis of giant cell–rich lesions. Herein, we describe MGC-rich lesions based on their molecular expression profiles and proposed cell fusion mechanisms (Table 1).^{3–17} We focus on lesions of high prevalence that are associated with complex diagnoses and uncertainty of the pathogenic mechanisms that lead to their development.

Lesions Containing OCLGCs

The osteoclast-like giant cell (OCLGC) is a large, multinucleated cell with dispersed nuclei in a pale cytoplasm. These cells exhibit similar phenotypic markers as osteoclasts,¹⁸ but they are not always found on bone surfaces. Lesions that contain large numbers of OCLGCs but without other defining histologic characteristics are assigned to this diagnostic group.

Giant Cell Tumor, Aneurysmal Bone Cyst, and Osteosarcoma

The giant cell tumor of bone and the aneurysmal bone cyst are MGC-rich lesions of neoplastic origin. Giant cell tumors of bone display locally aggressive behavior and are composed of large numbers of OCLGCs, spindle-shaped stromal cells, and epithelioid-shaped mononuclear cells. In contrast, aneurysmal bone cysts contain blood-filled cavities that are lined by fibrous septa with more scarce and scattered MGCs.¹⁸ A mutation in the *H3F3A* gene (found in 92% of giant cell tumors²) induces the neoplastic spindle cells to drive reactive formation of OCLGCs. This process results from spindle cell secretion of monocyte chemoattractant protein and macrophage colony-stimulating factor, which promote monocytic cell recruitment and

differentiation into preosteoclasts. Spindle cells also express receptor activator of NF- κ B ligand (RANKL), which acts on RANK-expressing preosteoclasts to promote cell fusion. Cytogenetic abnormalities have been identified in all primary aneurysmal bone cysts. These abnormalities involve the ubiquitin-specific protease 6 and osteoblast cadherin 11, along with others.¹⁹ Expression of these molecules attenuates osteoblastic maturation, which is associated with increased osteoclastogenesis arising from the expression of RANKL by osteoblasts.

OCLGCs are also found in malignancies of bone. Indeed, one-quarter of osteosarcomas (commonly the telangiectatic and giant cell–rich variants) contain MGCs.²⁰ Osteoclastogenesis in these lesions involves aberrant RANKL expression by malignant osteoblasts. All three of these lesions contain MGCs that express the osteoclast markers tartrate-resistant acid phosphatase, vacuolar ATPases, carbonic anhydrase II, cathepsin K, and matrix metalloproteinase-9 (Table 1). As these proteins are involved in bone demineralization and collagen degradation,^{3,20} and in view of their location on bone surfaces, MGCs in these lesions are probably involved in bone resorption. Accordingly, OCLGCs likely contribute to the growth and expansion of the lesions by invasion through bone. Denosumab, an antibody that neutralizes RANKL, inhibits the formation and activity of MGCs and is approved for treatment of inoperable giant cell tumors of bone and of sarcomas to decrease tumor-induced osteolysis.^{5,21}

Cherubism and Brown Tumor of Hyperparathyroidism

Herein, we consider the MGCs of cherubism and brown tumor of hyperparathyroidism together as they are manifestations of systemic conditions, and patients may present with multiple lesions. Cherubism is a mainly autosomal dominant disorder, characterized by bilateral, centralized giant cell lesions of the maxillae that produce substantial

Figure 1 Multinucleated giant cells in pathologic lesions (hematoxylin and eosin staining). **A:** Chronic tuberculosis, buccal mucosa. **B:** Central giant cell granuloma, posterior mandible. **C:** Foreign body giant cell reaction, gingival tissue. **D:** Brown tumor of hyperparathyroidism, mandible. **E:** Cherubism, reparative stage, posterior mandible. **F:** Aneurysmal bone cyst, mandibular condylar head. Scale bars = 100 μ m (A–F). Original magnification, $\times 20$ (A–F).

Table 1 MGCs in Pathologic Entities

Disease process	Proposed cell type	Phenotypic markers	Mechanism of formation
Cherubism ³	Osteoclast-like giant cells	TRAP ⁺ , CD68 ⁺ , V-ATPase ⁺ , cathepsin K ⁺ , and carbon anhydrase II ⁺	Src homology 3 domain—binding protein 2 (SH3BP2) mutation up-regulates NFAT signaling, which promotes osteoclastogenesis
Giant cell tumor of bone ^{3,4}	Osteoclast-like giant cells	CD45 ⁺ , CD68 ⁺ , TRAP ⁺ , RANK ⁺ , V-ATPase ⁺ , and carbonic anhydrase II ⁺	Nonclonal and clonal chromosomal alternations lead to increased M-CSF and RANKL expression, which promotes osteoclastogenesis
Central giant cell granuloma ³	Osteoclast-like giant cells	CD68 ⁺ , TRAP ⁺ , RANK ⁺ , V-ATPase ⁺ , and carbonic anhydrase II ⁺	Unknown, considered reactive in nature with RANKL expression by spindle cells promoting osteoclastogenesis
Osteosarcoma ⁵	Osteoclast-like giant cells	TRAP ⁺ , RANK ⁺ , and carbonic anhydrase II ⁺	Malignant osteoblasts express RANKL—promoting osteoclastogenesis
Aneurysmal bone cyst ³	Osteoclast-like giant cells	TRAP ⁺ , RANK ⁺ , and Vacuolar-ATPase ⁺	Ubiquitin carboxyl—terminal hydrolase 6 (USP6) translocations result in the up-regulation of osteoblastic differentiation promoting osteoclastogenesis
Giant cell arteritis ^{6,7}	Classically activated macrophage (M1) and Langhans giant cells	CD68 ⁺ , iNOS ⁺ , and Langhans-type S100 ⁺	Th1-secreted IFN-γ drives classic polarization, proposed toll-like receptor 2—driven fusion events
Acute tuberculosis ^{8–10}	Classically activated macrophage (M1) and Langhans giant cells	CD68 ⁺ , iNOS ⁺ , CD40 ⁺ , and Langhans-type DC-STAMP ⁺	CD40-CD40L monocyte—Th1 interactions with Th1-secreted IFN-γ drives classic polarization with CD40-CD40L interactions, leading to DC-STAMP—driven fusion
Chronic tuberculosis ^{8,9,11}	Alternatively activated macrophages (M2b) and Langhans giant cells	CD68 ⁺ , iNOS ⁺ , myeloid differentiation primary response 88 (MyD88) ⁺ , and Langhans-type DC-STAMP ⁺	IL-1—driven fusion events, through TLR2 and TLR4 and associated MyD88 cell fusion
Sarcoidosis ^{12,13}	Alternatively activated macrophages (M2a)	Arginase ⁺ , CD206 ⁺ , and CD44 ⁺	Th2-cell—secreted IL-4/IL-13 drives alternative polarization, proposed CD44-mediated fusion events
Foreign body giant cell ^{13–15}	Alternatively activated macrophages (M2a)	CD68 ⁺ , DC-STAMP ⁺ , and E-cadherin ⁺	IL-4/IL-13 promotes DC-STAMP— and E-cadherin—associated fusion events
Xanthogranulomas ^{16,17}	Touton giant cells (M2d?)	CD68 ⁺ and lysozyme ⁺	IFN-γ and IL-6 formed cells with possible TLR-activated fusion, fusion mechanism unknown

CD40L, CD40 ligand; DC-STAMP, dendritic cell—specific transmembrane protein; IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; M-CSF, macrophage colony-stimulating factor; MGC, multinucleated giant cell; NFAT, nuclear factor of activated T cells; RANK, receptor activator of NF-κB; RANKL, RANK ligand; Th1, type 1 helper T cell; TLR, toll-like receptor; TRAP, tartrate-resistant alkaline phosphatase.

swelling of the faces of young children.²² The characteristic mutation of Src homology 3 domain-binding protein 2, which is an activator of nuclear factor of activated T cells (NFAT), leads to gain of function to promote NFAT signaling, thereby enhancing osteoclastogenesis.²³ The brown tumor of hyperparathyroidism can mimic the giant cell tumor and arises secondary to hyperparathyroidism from parathyroid neoplasms or end-stage renal disease. Parathyroid hormone secretion leads to increased recruitment of monocytic precursors and RANKL-induced osteoclastogenesis.²⁴ For clinical management, endocrine abnormalities should be ruled out in individuals with OCLGC-rich lesions by analysis of calcium, phosphate, and parathyroid hormone levels.²⁵ Histologically, both lesions contain OCLGCs and can resemble the giant cell tumor of

bone and the central giant cell granuloma (CGCG; *Central Giant Cell Granuloma*). The MGCs of cherubism and brown tumor express osteoclastic markers, suggesting that these cells are involved in bone resorption.

Central Giant Cell Granuloma

The CGCG is an MGC-rich lesion with a poorly understood etiology that is found predominantly in the maxillae of young individuals. The CGCG was previously thought to be a reparative granuloma of jawbones due to its less aggressive behavior from giant cell tumors.³ Generally, CGCGs are histologically indistinguishable from giant cell tumors and peripheral CGCG variants; they are considered inflammatory lesions and commonly occur on the gingiva.²⁶ Some CGCGs exhibit rapid growth and recurrence,

suggesting a potential neoplastic origin. In CGCGs, spindle-shaped mononuclear cells can secrete RANKL, which promotes the formation of MGCs with an osteoclast phenotype (Table 1).³

CGCGs were previously considered reparative or reactive in response to intrabony hemorrhage or inflammation; however, new molecular phenotyping suggests a neoplastic origin.²⁷ Comprehensive genomic analysis has revealed mutations in *BRAF*, *KRAS*, *HRAS*, guanine nucleotide-binding protein G(S) subunit (*GNAS*), microphthalmia-associated transcription factor (*MITF*), and platelet-derived growth factor receptor B in CGCGs. The first five of these genes are known drivers in human neoplasms. Although it is not known whether these mutations result in the formation of MGCs, mutations of *BRAF* and *KRAS* are associated with Noonan syndrome, which manifests as bilateral CGCG-like lesions.²⁸ Mutations of *HRAS* are associated with recurrent malignant giant cell tumors of bone. Mutations of *MITF* are associated with dysfunction in osteoclastogenesis through the RANKL-signaling pathway.^{29,30} As these cells exhibit an osteoclast phenotype, the MGCs of CGCGs likely resorb bone and promote lesion progression because even the peripheral variant of CGCGs can exhibit pathologic bone resorption.

Lesions Containing Classically Activated Giant Cells

Macrophages differentiate into two main subtypes (classically and alternatively activated) that are characterized by discrete metabolic activities and phenotypic markers. In a general sense, classically activated macrophages are associated with acute inflammatory responses, whereas alternatively activated macrophages are associated with resolving inflammatory lesions. In the context of the lesions described in *Acute Tuberculosis*, classically activated MGCs secrete several effector molecules, including nitric oxide (generated by inducible nitric oxide synthase), which promotes the inflammatory process.³¹

Acute Tuberculosis

The classic example of giant cell formation in response to microbial challenges is the granuloma caused by *Mycobacterium tuberculosis*, which is similar in appearance to the lesions of aspergillosis and leprosy. Dendritic cells signal T-helper-1 cells to promote secretion of interferon- γ (IFN- γ) in response to antigen presentation.³¹ IFN- γ then promotes activation and recruitment of macrophages to enable these avidly phagocytic cells to engulf and destroy invading organisms. The hallmark feature of tuberculosis is the presence of granulomas, which are collections of mononuclear epithelioid histiocytes organized into a ball-like structure surrounded by a ring of T-helper lymphocytes. Secretion of granulocyte-macrophage colony-stimulating factor, IFN- γ , and IL-3 by helper T cells recruits macrophages and promotes epithelioid changes.³² T-cell-induced secretion of IFN- γ leads to enhanced

expression of dendritic cell-specific transmembrane protein (DC-STAMP), which promotes the fusion of macrophages and the formation of Langhans giant cells.¹⁰ Furthermore, macrophages infected with *Mycobacterium tuberculosis* exhibit increased CD40 and CD57 expression.³³ Inhibition of either CD40 or CD57 abrogates Langhans giant cell formation, which underlines the significance in MGC formation in tuberculosis.¹⁰

An important protein expressed by MGCs during the formation of granulomas is E-cadherin, an intercellular adhesion molecule that is associated with mesenchymal-to-epithelial transition in granulomatous inflammation in tuberculosis.⁹ Increased expression of E-cadherin promotes the adhesion of adjacent macrophages, whereas inhibition of E-cadherin function blocks granuloma formation. Currently, it is not known whether the intercellular adhesion function of E-cadherin is directly linked to its apparent central role in the development of granulomas. In tuberculosis, Langhans giant cells are thought to restrict the spread of mycobacteria along with granulomas.⁹ The formation of granulomas, however, does not eliminate the causative organism, a situation that enables the development of chronic infections in which MGCs are abundant.

Giant Cell Arteritis

Giant cell arteritis, an ischemic condition in which intimal hyperplasia and luminal obstruction occur along with MGCs, also appears to be an acute inflammatory response, like some cases of tuberculosis (Table 1).⁶ In these lesions, MGCs may be a response to a microbial antigen or auto-antigen, and these cells are associated with expression of matrix metalloproteinase-9, which can promote the vascular destruction seen in this disease.⁷

Lesions Containing Alternatively Activated Type Giant Cells

On the basis of their activation and protein expression profiles, the MGCs described in *Chronic Tuberculosis and Sarcoidosis* and *Foreign Body Granulomas and Xanthomas* are thought to be derived from alternatively activated macrophages. The MGCs formed from anti-inflammatory macrophage precursors are avidly phagocytic, suggesting that these cells are associated with immune-mediated removal of exogenous materials or organisms.³⁴ If these materials are not phagocytosed or degraded, MGCs expand and persist within affected tissues. Notably, the functional role(s) of MGCs in these lesions has not been definitively demonstrated.

Chronic Tuberculosis and Sarcoidosis

When the initial immune response to *M. tuberculosis* infection is not resolved, IL-1 β expression is enhanced, which drives IL-10 expression by tuberculosis-infected macrophages.³⁵ This process promotes the formation of macrophages with an alternatively activated expression

repertoire. These cells may contribute to the formation of MGCs in tuberculosis lesions, but this mechanism has not been rigorously defined. Furthermore, IL-10 stimulation decreases maturation of the phagosome, blocking phagosome-lysosomal binding and enabling survival and growth of the mycobacteria inside macrophages, which contributes to the chronicity of tuberculosis infections. Notably, toll-like receptors (TLRs) 2 and 4, expressed by macrophages, are associated with the formation of tubercloid MGCs.³⁶

Similar to chronic tuberculosis infections, sarcoidosis lesions contain granulomas populated by MGCs with enhanced arginase activity.¹² In granulomas of sarcoidosis, T-helper-2 cells secrete IL-4 and IL-13, which, in turn, drive alternative differentiation of macrophages in response to poorly defined antigens or autoantigens.¹³ The formation of MGCs in these lesions is possibly related to E-cadherin, which is expressed by mononucleated and multinucleated macrophages and is associated with their epithelioid transition into granulomas. CD44 and CCR5 are also involved in homotypic fusion events in the formation of the MGCs of sarcoidosis. Patients who are affected by sarcoidosis have monocytes and macrophages with higher levels of purinergic receptor P2X7; this receptor is a pharmacologic target for preventing the formation of MGCs and is used in the clinical management of sarcoidosis.³⁷ More in-depth insight into global, receptor-specific expression in MGC precursor cells could suggest new targets for therapeutic development.

Foreign Body Granulomas and Xanthogranulomas

Foreign body giant cells (FBGCs) form when the immune system is exposed to foreign material or to organisms that it cannot phagocytose or degrade. The surface chemistry and topology of foreign materials contribute to activation of cell adhesion molecules, especially β -integrins. This process leads to the secretion of IL-4 and IL-13, similar to sarcoidosis.³⁸ IL-4-induced fusion of macrophages into MGCs depends on $\beta 1$ integrins for cell spreading and intercellular adhesion, whereas $\beta 2$ integrins are required for initial cell attachment to the foreign body. IL-4 promotes the fusion of inflammation-resolving macrophages to form large FBGCs. In turn, FBGCs express CD68 and the major histocompatibility complex class II molecule, human leukocyte antigen D, which enables antigen presentation to T-helper cells and promotes the involvement of adaptive immune recognition systems.²⁰ E-cadherin, a prominent intercellular adhesion molecule, is also up-regulated in the foreign body response, similar to sarcoidosis. The expression of E-cadherin may explain why poorly developed granulomas are observed in close proximity to foreign materials.¹³ Similarly to osteoclasts and Langhans giant cells, FBGCs also express DC-STAMP, which complicates cell identification despite the different pathogenesis of these lesions.

Xanthogranulomas contain Touton-type giant cells, a variation of the FBGC, that arise from the fusion of foam cells.¹⁷ The formation of foam cells, which are macrophages

with lipid-filled cytoplasm, is associated with IFN- γ and lipopolysaccharide (LPS), suggesting these cells are a form of a proinflammatory macrophage. However, IL-6, which is associated with the formation of inflammation-resolving macrophages, is also linked to foam cell formation.³⁹ LPS may promote foam cell formation through activation of TLRs. Although LPS promotes proinflammatory activation of macrophages, TLR-associated cell fusion mainly involves anti-inflammatory macrophages. Therefore, although they exhibit some features of proinflammatory macrophages, it now seems that Touton-type giant cells of xanthogranulomas arise from the fusion of inflammation-resolving macrophages. This notion is rationalized by their reparative behavior in the removal of extracellular lipids and activation by IL-6, but the detailed mechanisms are not fully defined.⁴⁰

Multinucleated Giant Cell Subtypes and Their Formation

There are several different types of MGCs; the most intensively studied of these cells is the osteoclast, a cell type that provides considerable insight into the mechanisms that regulate fusion of precursor cells into MGCs.

Osteoclasts

Osteoclasts are MGCs specialized in the removal of bone matrix and mineral. These cells exhibit apically located nuclei and lysosome fusion with the basal cell membrane and ruffled border during periods of active bone resorption.⁴¹ Osteoclasts and macrophages express CD45 (the common leukocyte marker) and the pan-macrophage markers CD68 and CD14.⁴² During osteoclast differentiation, committed osteoclasts may cease expression of CD14, a coreceptor for bacterial LPSs.²⁰ The vitronectin receptor (CD51 or $\alpha v \beta 3$ integrin), which is also a marker for OCLGCs, enables cell attachment to bone matrix proteins. Tartrate-resistant alkaline phosphatase (TRAP) catalyzes phosphate ester hydrolysis and is highly expressed by osteoclasts. Although TRAP is a well-recognized osteoclast marker, the 5a TRAP isoform may also be expressed by macrophages, which may indicate the activation of these cells in response to inflammation and the production of reactive oxygen species.⁴³ In lesions, such as foreign body granulomas and tuberculosis, MGCs are TRAP positive, indicating that, unless specific isoforms are examined, TRAP is not a reliable marker for the differentiation of different MGC subtypes.⁴⁴

Other MGCs

Although the systems that regulate the fusion of osteoclasts have been examined in detail, other types of MGCs

(FBGCs, Langhans cells, and Touton giant cells) are formed through different mechanisms.

Foreign Body Giant Cells

These cells are spatially associated with introduced foreign materials and may exhibit up to 200 nuclei dispersed throughout the cytoplasm. Their formation is mediated, in part, by IL-4 and IL-13.^{32,45} Arginase-1 is overexpressed by mouse monocytes treated with IL-4, consistent with the notion that inflammation-resolving macrophages may fuse to form FBGCs. Furthermore, IL-4 inhibits certain proinflammatory activators and increases expression of CD206 (a mannose receptor), which is important for fusion events.⁴⁶ E-cadherin may also contribute to cell fusion as inhibition of the function of E-cadherin prevents the formation of FBGCs.⁴⁷

Langhans Giant Cells

Langhans giant cells exhibit peripherally located nuclei (up to 20 nuclei per cell) that surround the Golgi apparatus in a characteristic, horseshoe shape. Ultrastructural studies of these cells show lysosomes that are evenly distributed throughout the cytoplasm. Langhans giant cells are associated with granulomas generated in response to microbial infections, and they are thought to be derived from proinflammatory macrophages as a result of stimulation by granulocyte-macrophage colony-stimulating factor, IFN- γ , IL-3, and tumor necrosis factor (TNF)- α .³² Inhibition of the intercellular adhesion receptor DC-STAMP decreases fusion events, whereas CD40 blockade completely stops the formation of these cells. Therefore, DC-STAMP and CD40 are likely crucial for Langhans giant cell formation, although this notion has not been directly tested.¹⁰

Touton Giant Cells

Touton giant cells exhibit multiple, clustered nuclei that are surrounded by a foamy cytoplasm composed of accumulated lipids. When stimulated by macrophage colony-stimulating factor, IFN- γ , or IL-6, monocytes fuse and form MGCs. Touton giant cells are thought to be derived from inflammation-resolving macrophages because of their reparative behavior and IL-6-mediated activation.⁴⁰ The fusion molecules associated with Touton giant cell formation are not well characterized but appear to be associated with activation of TLRs.³⁹

Monocyte, Osteoclast, and Macrophage Differentiation

As described in *Multinucleated Giant Cells in Pathology*, which discusses the more prevalent inflammatory lesions that contain MGCs, the formation of these cells involves the fusion of monocytic cells, which, in turn, require a priori priming into activated macrophages. An in-depth understanding of the differentiation of monocyte precursor cells

into MGCs is crucial for the development of diagnostic markers and for the identification of potential therapeutic targets.

Monocyte Differentiation

The granulocytic progeny of hematopoietic stem cells differentiate into monocytes and then into the macrophages of the mononuclear phagocyte system. These macrophages, as well as osteoclast precursors, fuse to form MGCs and bone-resorbing multinucleated osteoclasts. Circulating monocytes exist as two populations: patrolling and inflammatory monocytes.⁴⁸ Patrolling monocytes are identified as CX3 chemokine receptor 1 (CX3CR1)^{high}/CD14^{dim}/CD16⁺ in humans and CX3CR1^{high}/lymphocyte antigen 6 complex C (Ly6C)[−] in mice. Inflammatory monocytes exhibit CCR2^{high}/CD14^{high}/CD16[−] profiles in humans and CCR2^{high}/Ly6C⁺ in mice. Human CD16⁺/CD14⁺ cells are designated as intermediate inflammatory monocytes.⁴⁹ Patrolling monocytes survey the endothelium and can differentiate into dendritic cells. Inflammatory monocytes, which are the most likely precursor cells for MGCs, are recruited by chemokines that include chemokine (C-C motif) ligand 2, the ligand for CCR2.⁵⁰ On entry into peripheral tissues, monocytes differentiate into macrophages or preosteoclasts. The knowledge that circulating inflammatory monocytes are the most likely source of pathologic MGCs is important as insights associated with the differentiation repertoire of these cells may suggest new therapeutic approaches to target recurrent lesions that contain MGCs. Notably, the biology of MGC formation is complicated by the observation that embryonic resident tissue macrophages can also provide a source of precursor cells for macrophages and MGCs.⁵¹

Osteoclastogenesis

As discussed in *Osteoclasts*, osteoclasts provide useful models for definition of the mechanisms that regulate fusion of monocyte precursor cells to form MGCs. Osteoclasts form by the fusion of preosteoclasts, which differentiate from monocytes. The fusion events that lead to osteoclast formation are regulated by a large cadre of cytokines. Monocytes are activated by macrophage colony-stimulating factor-1, which stimulates expression of RANK on the cell surface. RANKL, which is expressed by osteoblasts, stromal cells, and T cells, promotes the fusion of preosteoclasts.⁵² Osteoprotegerin helps to regulate osteoclastogenesis by acting as a decoy receptor for RANKL. In turn, RANKL promotes osteoclast formation by activating c-Jun N-terminal kinase and NFAT.^{23,53} Although RANKL or RANK-deficient animals cannot generate osteoclasts, TNF- α and LPS (with peptidoglycans) can promote macrophage fusion and osteoclast formation.⁵⁴ These cytokines activate c-Jun N-terminal kinase and NF- κ B signaling pathways in the formation of RANKL-independent osteoclasts. The findings

that the same signaling pathways for osteoclastogenesis are activated by inflammatory mechanisms indicate why osteoclasts are associated with acute inflammation; however, in addition, IL-1 β , which was initially described as an osteoclast-activating factor, also promotes multinucleation in monocytes. As mentioned in *Osteoclasts*, osteoclasts express TRAP, cathepsin K, and DC-STAMP.⁵⁵ IL-1 β induces the fusion of monocytes, a process that involves the TNF receptor—associated factor 6, which, in turn, interacts with IL-1 receptor—associated kinase to promote osteoclast activation and to stimulate fusion involving cytoskeletal rearrangement.⁵⁶ Furthermore, IL-1 acts indirectly on osteoblasts to increase expression of prostaglandin E2 and RANKL, which increase osteoclast differentiation.⁵⁷ These well-known processes in osteoclasts have not yet been definitively elucidated in the MGCs that form in the lesions of macrophage origin.

Macrophage Differentiation

Macrophages, which are derived from monocytes, are heterogeneous populations of cells, as shown by their broad gene expression profiles and by the molecules that they phagocytose and secrete.⁵⁸ Similarly, MGCs, which are formed by the fusion of monocytes or macrophages, exhibit an array of singular features. Macrophage differentiation has been associated with a broad spectrum of activation states, which were initially classified on the basis of the notion of classically activated macrophages (M1 cells; considered as proinflammatory) and alternatively activated macrophages (M2 cells; considered as inflammation resolving).⁵⁹ More recent insights show that the M1/M2 classification system only partially represents the spectrum of macrophage phenotypes. Conceivably, as more subtypes are characterized, the protein expression repertoires and functional profiles of cells will motivate that development of a more comprehensive classification system. Nevertheless, macrophage populations that exhibit various proinflammatory and anti-inflammatory properties contribute to the formation of the MGCs that comprise non-OCLGC-containing lesions.

Classically Activated Macrophages

M1 macrophages were originally considered to be generated from monocytic precursor cells after exposure to IFN- γ , TNF, and damage- and pathogen-associated molecular patterns.⁶⁰ Damage-associated molecular patterns are produced by the host, and their pathogen-derived counterparts, pathogen-associated molecular patterns, can include bacterial products, such as LPSs, that promote a proinflammatory activation. These cytokines include those expressed in giant cell arteritis and acute tuberculosis.⁷ M1s are highly phagocytic cells that remove pathogens and debris and exhibit a shift from arginine-1 metabolism to nitric oxide synthase, which facilitates killing of pathogens. Their phenotype is characterized by an inducible

nitric oxide synthase—positive/CXCL11⁺/IL-12^{high}/IL-23^{high}/IL-10^{low} profile.⁶¹ Furthermore, M1 cells secrete other cytokines and chemokines (eg, TNF- α , IL-1 β , and IL-6) that activate other macrophages and perpetuate the proinflammatory immune response. In the context of MGC formation, there are rich opportunities for definition of MGC subtypes through examination of the expression repertoire of molecules associated with the so-called M1 macrophages.

Alternatively Activated Macrophages

Alternatively activated M2s, the reparatory macrophages, produce ornithines through the arginase pathway, which are associated with enhanced cell proliferation, healing, and suppressed T-cell responses.⁶² M2 macrophages are also avidly phagocytic and produce extracellular matrix molecules, chemotactic factors, and angiogenic factors. Alternatively activated macrophages are subdivided into four types (a to d) based on their activation stimuli; however, evolution of the classification system may result in new subdivisions based on metabolic profiling. M2a macrophages are generated by exposure to IL-4 or IL-13, as are the MGCs seen in foreign body reactions. These cells are known to be found in inflammatory zone 1 (FIZZ1)⁺/arginase 1 (Arg1)⁺/IL-12^{low}/IL-23^{low} and secrete IL-10 on Fc receptor- γ activation.⁶¹ M2a macrophages attenuate M1-associated inflammation; contribute to extracellular matrix synthesis, encapsulation of foreign bodies, and fibrosis; and inhibit T cells.⁶³ M2b macrophages (type 2) are generated after exposure to IL-1; are activators of TLRs and immune complexes, such as those seen in chronic tuberculosis; and show CD80^{high}/CD14^{high}/human leukocyte antigen-DR^{low}/IL-12^{low}/IL-23^{low} expression.^{8,11} M2b macrophages up-regulate antigen presentation and promote T-helper-2 responses. M2c cells arise in response to IL-10, transforming growth factor- β , or glucocorticoids; they are referred to as deactivated macrophages because of their expression of an M2 profile after activation in culture.⁶⁴ These cells induce T-regulatory cells to aid in their anti-inflammatory activities. M2d cells are formed in response to IL-6 and adenosine, and they are associated with angiogenesis and tissue repair.⁶⁵ The determination of the proportion and relative abundance of M1 and M2 markers of MGCs that populate MGC-containing lesions would seem to be a reasonable starting point for advancing our understanding of their ontogeny.

Cell Fusion in MGC Formation

Identification of the molecules involved in the formation of MGCs from various monocyte- and macrophage-derived precursors could help to define the role of these cells in MGC-associated diseases, elucidate their pathogenesis, and serve as potential pharmacologic targets. Indeed, the molecules and processes involved in the formation of MGCs are just now being defined. It is generally recognized that for all

Figure 2 **A** and **B**: Formation of multinucleated giant cells (MGCs) begins with monocyte precursor cells (**A**) that obtain fusion competence through the presence of profusogenic cytokines and then extend actin-rich filopodial projections (**B**). **C**: The cells undergo migration toward one another, and adhesion occurs via filopodial projections or fusopods; at opposing sites, fusogens enable approximation of plasma membranes. **D**: Removal of the intervening membranes allows for cytoplasmic sharing and the formation of a binucleated cell. **E** and **F**: Cells migrate and adhere again (**E**), followed by cytoplasmic sharing to form an MGC (**F**).

cell types that undergo fusion (eg, myoblasts, trophoblasts, and monocytes), three discrete stages of fusion are involved: the development of fusion competency; migration; followed by intercellular adhesion and cytoplasmic sharing (Figure 2).⁶⁶ Fusion competency indicates that the cells express the appropriate proteins and exhibit the required function to undergo fusion. This process occurs when potentially fusogenic cells migrate toward one another, approximate their plasma membranes, and then lose the intervening membrane bilayers to enable cytoplasmic sharing. In this section, we discuss in detail the first two stages of MGC fusion as they relate to the formation of MGCs in pathologic lesions.

Fusion Competence

For fusion to occur, cells must become fusion competent, which indicates that they must express the adhesion molecules (fusogens) that will enable cell membrane approximation and the expression of the machinery required for fusion. Fusogens lower the energy barrier between lipid bilayers, which allows cells to be in close proximity despite charge repulsion. Fusogens are delivered via membrane lipid rafts to the approximating plasma membranes. The membranes also recruit other adhesion molecules to the adhesion site because of the assembly of subcortical actin filaments and filament flow in the plan of the cortex.⁶⁷ The fusion molecules important in the formation of each type of MGC are depicted in Figure 3. The intercellular adhesion machinery in the fusion process is linked to the generation of signals that bring opposing membranes toward one another. Here, we consider some

of the best-described intercellular adhesion molecules that are involved in fusion.

DC-STAMP

DC-STAMP is a transmembrane protein that is critical for the formation of multinucleated osteoclasts. When DC-STAMP is deleted in mice, multinucleation is blocked.¹⁴ Although the ligand of DC-STAMP is not known, it may be a chemokine, based on the similarity of the structure of DC-STAMP with other chemokine receptors. RANK promotes binding of c-Fos and NFATc1 to the *DC-STAMP* promoter.⁶⁸ Accordingly, in IL-4–induced FBGC formation, c-Fos, NFATc1, PU.1, and NF- κ B regulate DC-STAMP expression. Activation of DC-STAMP leads to NFAT and calcium signaling, which are important for osteoclastogenesis.⁶⁹

DC-STAMP contains an immunoreceptor tyrosine-based inhibition motif in its cytosolic domains that is essential for NFAT nuclear translocation and cell fusion.⁶⁹ Immunoreceptor tyrosine-based inhibition motifs recruit tyrosine phosphatases (eg, Src homology 2 domain tyrosine phosphatase), which regulate signaling of immunoreceptor-activating motifs to activate downstream signaling. Although DC-STAMP is important in RANKL-mediated osteoclastogenesis, it is not required for the formation of FBGCs involving the fusion of M2a cells.⁶⁸

Cadherins

Cadherins are transmembrane adhesion receptors, one of which (E-cadherin) is important for homotypic fusion in cells of monocytic origin.⁴⁷ IL-4 and IL-13 increase surface expression of E-cadherin through a STAT6-dependent signaling pathway.⁷⁰ Although the E-cadherin promoter

Figure 3 Formation of multinucleated giant cells (MGCs) from various types of monocyte differentiation. Activation of monocytes by various cytokines leads to the differentiation of macrophages. Actin-rich cellular extension processes, filopodia, approximate cells with one another; and the proposed fusogens expressed by these cells enable fusion events to form the various MGCs. DC-STAMP, dendritic cell—specific transmembrane protein; IFN- γ , interferon- γ ; LPS, lipopolysaccharide; MFR, macrophage fusion receptor; MR, mannose receptor; MyD88, myeloid differentiation primary response 88; RANKL, receptor activator of NF- κ B ligand; TGF- β , transforming growth factor- β ; TLR, toll-like receptor; TLRL, TLR ligand; TNF, tumor necrosis factor.

does not contain a STAT6 binding site, polyamines produced in M2a macrophages, in response to IL-4 up-regulation of arginase metabolism, are crucial cofactors for the induction of E-cadherin expression.⁷⁰ Although the recruitment of E-cadherins (along with β -catenin and p120-catenin) enables homotypic adhesive interactions, reduced expression of E-cadherin does not inhibit MGC formation entirely.⁷⁰ IL-4-naïve cells are able to undergo fusion events with IL-4-treated cells, indicating that donor and acceptor cell adhesion events occur independently of homotypic binding involving E-cadherin.

In cultured cells, E-cadherin is a ligand for killer cell lectin-like receptor subfamily G member 1.⁷¹ The killer cell lectin-like receptor subfamily G member 1 also contains an immunoreceptor tyrosine-based inhibition motif cytosolic component that is similar in structure to DC-STAMP. However, binding of E-cadherin to monocytes leads to reduction of secretion of inflammatory cytokines, indicating a modulatory role of this adhesion receptor. Osteoclasts also use E-cadherin in fusion processes: blockade of E-cadherin in RANKL-treated cells diminishes multinucleation in osteoclast formation.⁷² Inhibition of E-cadherin also retards

the expression of TRAP, cathepsin K, DC-STAMP, and NFATc1, but this effect is evidently not crucial for osteoclastogenesis. Accordingly, E-cadherin may be associated with the induction of genes in osteoclasts that promote cell motility and fusion competency.

Mannose Receptor

A critical regulator of adhesion in osteoclasts and FBGCs is CD206 (a mannose receptor).⁷³ CD206 is a lectin receptor expressed on cells that detects microorganisms expressing mannose oligosaccharides. The mechanism by which the mannose receptor promotes cell fusion in MGCs is unknown but may involve collagen clearance and enhanced cell migration through the extracellular matrix to increase the likelihood of cell approximation.⁷⁴

The expression of CD206 is enhanced by IL-4 and IL-13, whereas inhibition of CD206 decreases IL-4–induced MGC formation.⁷⁵ However, the expression of CD206 is not required for MGC formation, indicating that other fusogens may play more central roles in critical fusion events. Osteoclasts and their precursors also express CD206, but inhibition of CD206 function only partially attenuates the formation of TRAP-positive osteoclasts. In the context of the formation of MGCs, CD206 can recognize several pathogens, notably *M. tuberculosis*. In these lesions, CD206 mediates macrophage adhesion and MGC formation.⁷⁵ The development of CD206-directed therapeutics is now underway for treatment of tuberculosis through improved uptake of drugs and the targeting of medication-resistant granulomas that form.⁷⁶ A potential shortcoming of this approach is that CD206 is expressed by MGCs in several different pathologic processes, including osteoclasts, which may inadvertently impact normal physiological processes.

MFR and CD47

The macrophage fusion receptor (MFR; alias signal-regulatory protein α) belongs to the Ig superfamily of proteins and interacts both homotypically and heterotypically with other members of the Ig superfamily, integrins and cadherins.⁷⁷ MFR is a transmembrane adhesion protein expressed on the plasma membrane of macrophages and dendritic cells.⁷⁸ Its adhesion partner, CD47, is a member of the Ig superfamily. CD47 is expressed ubiquitously and was originally associated with the $\alpha v \beta 3$ integrin.⁷⁹ Inhibition of the MFR-CD47 interaction reduces osteoclast number *in vivo*,⁸⁰ in part by affecting cell fusion. The MFR-CD47 interaction recruits SHP-1 and SHP-2 to phosphorylated immunoreceptor tyrosine-based inhibition motifs, a process that promotes fusion of preosteoclasts through activation of nonmuscle myosin.⁸¹ CD47 also participates in MGC formation, which has been demonstrated in experiments using CD47 antibody blockade to inhibit macrophage fusion.⁸² One report has indicated that expression of a mutated MFR did not affect osteoclast formation in mice, although the osteoclasts in these animals exhibited increased activity, indicating a possible regulatory role of MFR in cell fusion and

osteoclastogenesis.⁸³ The MFR-CD47 interaction may facilitate cell-cell fusion through initial approximation and subsequent adhesion of adjacent plasma membranes; a smaller MFR isoform decreases the distance between fusing cell membranes. CD47 also interacts with integrins to encourage chemotaxis and migration. Accordingly, increased CD47 availability in cells with mutant MFR could lead to increased activity of these cells.

Cell Approximation

In cultured monocytes undergoing fusion, morphologic studies have shown that a larger founder cell seeks out a smaller follower cell for fusion to occur.⁸⁴ In addition to membrane fusion events, these processes centrally involve the assembly of subcortical actin filaments at the cell fusion sites.

Cytoskeletal Regulation of Fusion

The importance of actin filaments in fusion processes is readily apparent in cells in which inhibition of Ras-related C3 botulinum toxin substrate 1 (Rac1), a small GTPase that regulates the actin cytoskeleton, blocks RANKL-mediated fusion.⁸⁵ Inhibition of Rac1 does not affect the phagocytic functions of MGCs.⁸⁶ Rho GTPases, such as Rac, control osteoclast formation by regulating the assembly of filopodia, lamellipodia, and podosomes.⁸⁷ Filopodia, which are cell extensions composed of short bundles of actin filaments, are at the forefront of fusion events.⁸⁵ These cell extensions are associated with chemotactic sensing, the direction of cell migration, and cell attachment to substrates. The filopodia that enable approximation and membrane fusion are termed fusopods. During the formation of FBGC, filopodia contribute to the sharing of cytoplasm between fusing cells.⁸⁸ Other cytoskeletal molecules that affect fusion processes include Rac2, a GTPase that is distinct from Rac1; and filamin A, an actin filament cross-linking protein.^{89,90}

Phospholipid Membrane Regulation of Fusion

Regulation of fusion events is modulated, in part, by molecules embedded in the phospholipid bilayer of fusing cell membranes. Immobilization of phosphatidylethanolamine, a small-headed phospholipid, prevents the formation of filopodia and, as a result, osteoclastogenesis is inhibited.⁹¹ Modification of the lipids in the leaflets of the plasma membranes is critical in the early stages of membrane fusion.⁹² Phosphatidylserine is a negatively charged phospholipid located on the cytosolic side of the phospholipid membrane, but it translocates to the outer membrane before fusion. Phosphatidylserine depletes cholesterol, which increases membrane fluidity and promotes the movement of the lipid raft. When phosphatidylserine is masked, IL-4–induced fusion of macrophages is inhibited.⁹²

Intercellular Adhesion and Cytoplasmic Sharing

Before cytoplasmic sharing can occur, the fusogens that endow fusion-competence need to be activated by their

fusion partner. In this context, once intercellular adhesion has occurred, the purinergic receptor P2X7 is thought to control phospholipid exposure, which is then recognized by CD36, the scavenger receptor.⁹³ P2X7 is an ATP-gated ion channel that enhances ion permeation during MGC formation as a result of plasma membrane permeabilization.⁹⁴ Partial silencing of P2X7 in Raw264.7 cells leads to decreased gene expression of NFATc1 and cathepsin K, along with reduction of the size and bone-resorbing function of osteoclasts.⁹⁵ Inhibition of P2X7 reduces the probability of cell fusion events and is thought to be associated with the final stage of cell fusion events, cytoplasmic sharing.

Fusion Models

On the basis of our lack of knowledge of the molecular determinants of MGC formation in a wide variety of lesions that contain these cells, an important future goal will be to develop robust models that will enable the characterization of the critical molecules that enable fusion to occur. Notably, knockout mouse models of DC-STAMP, OC-STAMP (a variant of DC-STAMP), P2X7 receptor, and CD47 have been used to determine the roles of these proteins in cell fusion in intact animals.^{14,79,96,97} Measurement of the ability of cells to form MGCs in experimental lesions or under experimental conditions in which these potential fusogens are deleted could give insight into how these molecules regulate the formation of specific types of MGCs. In cultured cells, the functional roles of potential fusogenic proteins in the formation of actin-rich filopodial projections has been examined using three-dimensional imaging, which enables high-resolution analysis of fusopod-driven events.⁸⁵ In these approaches, cells are cultured on membranes containing small pores that allow the monitoring of filopodia as they extend through the pores, allowing for the study of the assembly of actin filaments and the impact of specific actin regulatory proteins on cell extension formation.⁹⁸ These methods will help to uncover the mechanistic details of MGC formation.

Applications and Conclusions

Evidently, there are important gaps in our understanding of MGC formation, including the molecular determinants of cell fusion. Therefore, it is critical to obtain an in-depth understanding of the molecules that promote formation of MGCs and thereby to link specific fusion molecules with discrete, MGC-associated diseases. The identification of these MGC type-specific molecules could significantly advance diagnosis of these diseases and lead to the discovery of new drug targets. Currently, our ability to discriminate MGC variants on histologic criteria alone is challenging and is a major hurdle for further progress. Notably, the discovery of a specific fusion molecule involved in the formation of MGCs led to the discovery of

the role of the mannose receptor in the formation of tubercloid granulomas.^{76,99} This knowledge has been applied to the generation of mannose receptor-targeted therapies, which can penetrate medication-resistant granulomas in the treatment of tuberculosis. Presumably, if these differentially expressed fusogens could be identified, the diagnosis of MGC-containing lesions would be expedited. The application of broad and unbiased proteomic approaches could be used to detect novel fusogenic molecules that are differentially expressed in discrete MGCs. Identification of molecules with membrane surface expression could provide new targets for diagnostics and molecule-targeted therapies.

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