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COMMENTARY

Identification and characterization of osteoclast precursor cells

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Commentary on: Charles JF, Hsu LY, Niemi EC, Weiss A, Aliprantis AO, Nakamura MC. Inflammatory arthritis increases mouse osteoclast precursors with myeloid suppressor function. J Clin Invest 2012;**122**:4592–4605
Jacome-Galarza CE, Lee SK, Lorenzo JA, Aguila HL. Identification, characterization and isolation of a common progenitor for osteoclasts, macrophages and dendritic cells from murine bone marrow and periphery. J Bone Miner Res 2013; **28**:1203–1213

Osteoclasts are unique bone-resorbing cells, and thus, the loss of osteoclast differentiation or function results in osteopetrosis, a skeletal disorder. In contrast, accelerated osteoclastogenesis promotes osteoporosis, bone erosion and bone destruction. Therefore, the regulation of oteoclasts is crucial to maintain bone homeostasis. Recently, an osteoclast precursor (OCP) population was identified and characterized by two different groups.[1,2] Charles *et al.*[1] identified $CD3^-B220^-Ter119^-CD11b^{-/low}Ly6C^{hi}CX3CR1^+CD115^+CD135^{low}CD117^+CD11c^-$ cells as an OCP population in the bone marrow (BM) of SKG mice, which are arthritis model mice. This population was also detected in wild-type BM, but the frequency was high in arthritic mice.[1] Meanwhile, Jacome-Galarza *et al.*[2] identified $B220^-CD3^-CD11b^{-/low}CD115^+CD117^+CX3CR1^+$ cells as common precursor (CP) cells of osteoclasts, macrophages and dendritic cells (DCs) in BM. They also identified $B220^-CD3^-NK1.1CD11b^+Ly6C^{hi}CD115^+Ly6G^-CD117^{intermediate}$ cells as CP cells of osteoclasts, macrophages and DCs in the spleen and peripheral blood.

OCPs and Arthritis

Rheumatoid arthritis is characterized by chronic inflammation and destruction of multiple joints.[3] Osteoclasts are strongly activated in subchondral bones of erosive joints.[4] Charles *et al.*[1] found that the frequency of $CD3^-B220^-Ter119^-CD11b^{-/low}Ly6C^{hi}$ cells increased in the BM of SKG arthritis model mice, and the frequency was three times higher in arthritic mice than normal control mice. The authors phenotypically characterized this population and found that these cells were $CX3CR1^+CD115^+CD135^{low}CD117^+CD11c^-$.[1] These cells had a high potency to differentiate into osteoclasts *in vitro* and *in vivo*, and thus, these cells were predicted to have a crucial role in joint erosion in SKG mice as OCPs. However, unexpectedly, the transplantation of OCPs into arthritis model mice strongly inhibited joint inflammation.[1] The OCPs strongly suppressed T-cell proliferation *in vitro*, and therefore, OCPs are considered immune-suppressive cells.[1] To inhibit T-cell proliferation, interferon gamma (IFNγ) expressed in T cells and the IFNγ receptor and Nos2 expressed in OCPs were required.[1] As OCPs in wild-type mice also inhibited T-cell proliferation, the increased OCPs in arthritic mice likely perform a protective reaction to control arthritis.

Identification and Characterization of Osteoclast, Macrophage and DC CP Cells

Osteoclasts, macrophages and DCs are all derived from hematopoietic stem cells and monocyte/macrophage lineage cells.[5] These cells were considered to develop from common progenitor cells.[6,7] Jacome-Galarza *et al.*[2] identified $B220^-CD3^-CD11b^{-/low}CD115^+CD117^+$ cells as a CP of osteoclasts, macrophages and DCs at a single-cell clonal level in mouse BM. The CPs formed functional bone-resorbing osteoclasts, phagocytic macrophages and antigen-presenting DCs.[2] They also identified $B220^-CD3^-NK1.1^-CD11b^+Ly6C^{hi}CD115^+Ly6G^-CD117^{intermediate}$ cells as CPs in the mouse spleen and peripheral blood. This group previously identified CPs as osteoclast progenitor cells that exhibited a high potency to differentiate into osteoclasts.[8] These cells were phenotypically similar to the clonogenic population of macrophages and DCs described by Fogg *et al.*[9] and thus, the authors tried to analyze the differentiation capacity of these cells into macrophages and DCs. They found that these cells also differentiated into macrophages and DCs.[2]

OCPs in BM

Two papers identified very similar or most likely the same population as OCP cells: $B220^-CD3^-CD11b^{-/low}CD115^+CD117^+CX3CR1^+$ cells from BM.[1,2] If we combine these two reports, these cells are considered phenotypically $Ly6C^{hi}CD135^{low}CD11c^-$ as well, have an immune-suppressive

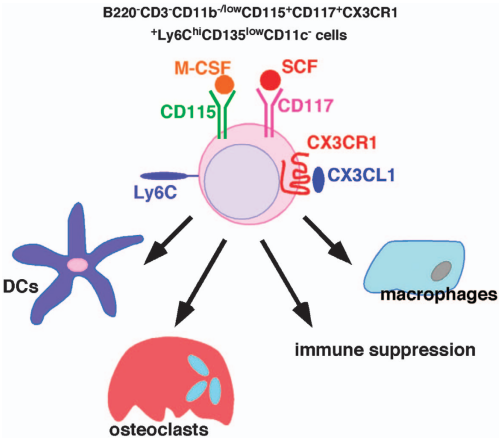

Figure 1 Characteristics of BM OCP cells. The cells identified in the two papers from mouse BM cells are likely the same cells, and the cell surface antigens are: $B220^-CD3^-CD11b^{-/low}CD115^+CD117^+CX3CR1^+Ly6C^{hi}CD135^{low}CD11c^-$. This population can differentiate into osteoclasts, macrophages and DCs, and show an immune-suppressive activity on T cells.

function, and differentiate into osteoclasts, macrophages and DCs (**Figure 1**).

A study to identify OCPs in BM was performed by Walker.[10,11] In this report, osteopetrotic animals were transplanted with BM cells and functional osteoclasts developed in osteopetrotic animals. Then, OCP cells were identified in hematopoietic cells.[12]

Previously, osteoclast differentiation was induced in coculture of OCP cells and stromal cells in the presence of osteotropic factors such as $1,25(OH)_2D_3$.[13] This system was suitable to induce osteoclastogenesis, but it was difficult to analyze the direct response to cytokines in OCP cells during differentiation due to the presence of stromal cells and the factors expressed by them. Identification of RANKL enabled us to analyze the direct response to cytokines in purified cells without stromal cells.[14,15]

The flowcytometric technique to isolate a purified population or clonogenic assay was widely used in the hematopoietic stem cell and immune research fields, and this technique is now frequently used in the bone cell biology field. Purified OCPs, $CD11b^{-/low}CD115^+CD117^+$ cells, were identified in BM.[16] The $CD115^+CD117^+RANK^-$ cells were demonstrated as common progenitors to differentiate into osteoclasts and DCs.[7] The $CD11b^-CD3^-CD19^-NK1.1^-Ia^{b-}CD11c^-B220^-TER\text{-}119^-Gr1^-CX3CR1^+CD117^+$ cells were described as a clonogenic BM progenitor specific for macrophages and DCs.[9] Two recent papers identified almost the same population, but added new information: immune-suppressive function and potency to differentiate into osteoclasts, macrophages and DCs (**Figure 1**).

M-CSF is required for osteoclastogenesis, and M-CSF stimulation before RANKL promotes effective osteoclast differentiation. RANK expression is induced in OCP cells by M-CSF and subsequent RANKL stimulation induces mature osteoclast formation,[16] and the characteristics of the precursor population are changed by M-CSF. Therefore, it is important to consider the relationship between frequency of 'OCP cells' and changes of bone resorption *in vivo*.

Future Prospects

The lineage-negative ($CD11b^-CD3^-B220^-TER\text{-}119^-Gr1^-$), $CD117^+Sca1^+CD34^{low/-}$ cells (LSKs) were identified as hematopoietic stem cells.[17] The cells demonstrated as osteoclast or CP cells, $CD11b^{-/low}CD3^-B220^-TER\text{-}119^-CD117^+$ cells, likely have more multi-potency to differentiate into multiple lineage cells like hematopoietic stem cells. It would be interesting to analyze the expression of Sca1 and CD34, and perform a transplantation assay to analyze the self-renewal activity and capacity to differentiate into multi-lineage cells.

Conflict of Interest

The author declares no conflict of interest.

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