CHAPTER 10
OSTEOCLAST PRECURSOR CELLS

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1. ORIGIN OF THE OSTEOCLAST

The hematopoietic origin of the osteoclast is now clear. Walker (Walker 1975b, Walker 1975a, Walker 1975c) first demonstrated that the precursor cells of osteoclasts were hematopoietic. These studies showed that the transplant of spleen cells from osteopetrotic mice, which have dysfunctional osteoclasts, into irradiated normal animals caused the normal animals to become osteopetrotic within four weeks. Conversely, it was shown that transplant of normal spleen cells into irradiated osteopetrotic mice caused the osteopetrotic mice to develop normal bone remodeling within the same time period. More recently, bone marrow transplants into humans with osteopetrosis have, in selected cases, led to reversal of the condition (Coccia, Krivit, Cervenka, et al. 1980).

Testa et al. first illustrated that feline bone marrow cultures could be induced to form multinucleated osteoclast-like cells (OCL) (Testa, Allen, Lajtha, et al. 1981, Allen, Testa, Suda, et al. 1981). Subsequently, Ibbotson et al characterized this system and demonstrated that stimulators of bone resorption enhanced the rate of formation of OCL (Ibbotson, Roodman, McManus, et al. 1984). Culture systems that use human or mouse marrow have been extensively studied and these demonstrate that the OCL that form have many characteristics of true osteoclasts. These include the abundant production of tartrate resistant acid phosphatase (TRAP) and calcitonin receptors (CTR) as well as the ability to form resorption lacunae when the cells are cultured on dentin or bone slices. Udagawa et al showed that spleen cells from mice could be cultured with stromal cell lines or primary osteoblastic cells and induced to form OCL (Udagawa, Takahashi, Akatsu, et al. 1989). The cells formed numerous resorption lacunae and had other characteristics of true osteoclasts. These authors also demonstrated that cell contact between hematopoietic osteoclast precursors in the spleen cell population and stromal or osteoblastic precursors was essential for osteoclast formation.

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2. CHARACTERIZATION OF OSTEOCLAST PRECURSOR CELLS

The osteoclast precursor has been studied to characterize its lineage. Initial work demonstrated that osteoclasts share many characteristics of macrophages (Athanasou, Heryet, Quinn, et al. 1986). Although, osteoclasts and macrophages appear to express some common antigens (Sminia and Dijkstra 1986), there are clear differences in the expression of surface antigens, which separate these two cell types (Kukita and Roodman 1989, Tsurukai, Takahashi, Jimi, et al. 1998). Mononuclear cells that can differentiate into OCL in a variety of in vitro culture systems are present in the bone marrow and the peripheral blood (Quinn, Sabokbar and Athanasou 1996, Fujikawa, Quinn, Sabokbar, et al. 1996).

2.1. Relationship of Osteoclast Precursor Cells to Macrophages

The availability of multiple antibodies recognizing cell surface molecules, which are expressed on hematopoietic cells (Spangrude, Heimfeld and Weissman, et al. 1988, Akashi, Traver, Miyamoto, et al. 2000, Kondo, Weissman, Akashi, et al. 1997, Uchida and Weissman 1992), has allowed the identification of bone marrow and spleen cell populations that can form osteoclast-like cells (OCL) in vitro. Studies from several laboratories have identified several candidate populations with the ability to form OCL in coculture with stromal cells or when cultured alone in liquid media or methylcellulose. In experiments performed before the identification of RANKL, investigators relied on cocultures of various fractions of bone marrow cells with stromal or osteoblastic cells (either primary or cell lines), which were activated to induce osteoclastogenesis by treatment with a stimulator of resorption like 1,25 OH_2 vitamin D. The majority of these early studies focused on monocyte-macrophage lineage cells. Initial studies of cells isolated from bone marrow or spleens showed that cells expressing mature macrophage markers gave rise to OCL when they were cocultured with marrow stromal cells (Cecchini, Hofstetter, Halasy, et al. 1997). Muguruma and Lee identified an osteoclast progenitor population in bone marrow that was negative for mature markers of B-lymphocytes (B220), granulocytes (Gr-1), macrophages (CD11b/Mac-1), and erythroid cells (Ter-119). This population did not express Sca-1, which is a marker that is found on hematopoietic stem cells (HSC) but was positive for the progenitor marker c-kit/CD117 (Muguruma and Lee 1998). These cells could progress to TRAP-expressing mononuclear cells when they were cultured in semi-solid media and OCL cells when they were cultured with vitamin D-treated-ST2 stromal cells. However, the cells in this fraction were considerably multipotential, since they were also able to differentiate into granulocytes, macrophages and erythroid cells. Interestingly, when the c-kit low population was separated, it also could generate osteoclasts, but in a more restricted fashion. Tsurukai et al. isolated cells from coculture of hematopoietic cells and osteoblastic cells by separation through a Sephadex column and found the population of osteoclast precursors to express monocytic markers but not markers of B or T-lymphocytes (Tsurukai, Takahashi, Jimi, et al. 1998). Using a coculture assay with ST2 cells, Hayashi et al found, that osteoclast precursors were in the c-Kit positive fraction and furthermore, that expression of c-fms, the m-CSF receptor, inhibited the efficiency that c-Kit positive cells formed OCL in culture (Hayashi, Miyamoto, Yamane, et al. 1997).

Arai et al. used both coculture with ST2 stromal cells and direct stimulation with RANKL and M-CSF as well as antibodies against the M-CSF receptor, c-fms, and the monocytic marker CD11b/Mac-1, to demonstrate that bone marrow cell populations expressing c-kit formed OCL in culture (Arai, Miyamoto, Ohneda, et al. 1999). These authors concluded that a population of murine bone marrow cells with the phenotype c-kit$^+$ c-fms$^+$ CD11b^{lo}, contained a multipotential progenitor cell population that gave rise to osteoclasts with high frequency. This population did not express RANK when it was isolated from bone marrow. However,

when it was cultured with M-CSF it produced a population of cells that expressed RANK. Interestingly, these precursors were not completely restricted to osteoclastogenesis since in methylcellulose cultures they generated macrophages and mononuclear TRAP positive cells. Microglia, which are specialized phagocytic cells in the central nervous system, appear to also arise from a precursor cell that can give rise to osteoclasts (Servet-Delprat, Arnaud, Jurdic, et al. 2002).

2.2. Relationship of Osteoclast Precursor Cells to Dendritic Cells

The relationship of osteoclasts to dendritic cells, which present antigen to T-lymphocytes as part of the adaptive immune response (Reis e Sousa 2006), is now also established. Cells expressing early markers of myeloid dendritic cell lineage commitment can differentiate into osteoclasts in culture (Servet-Delprat, Arnaud, Jurdic, et al. 2002, Rivollier, Mazzorana, Tebib, et al. 2004, Miyamoto, Ohneda, Arai, et al. 2001). In addition, it appears that relatively mature dendritic cells that can present antigen to T-lymphocytes retain their ability to form osteoclasts, at least, in vitro (Alnaeeli, Penninger, and Teng 2006). The ability of a common progenitor cell to differentiate into macrophages, osteoclasts and myeloid dendritic cells has been proposed for some time (Miyamoto, Ohneda, Arai, et al. 2001, Rivollier, Mazzorana, Tebib, et al. 2004). However, only recently has the identity of a cell that has the capacity to differentiate into macrophages and dendritic cells been demonstrated (Fogg, Sibon, Miled, et al. 2006). It is currently unknown, but highly likely, that the macrophage/myeloid dendritic cell precursor can also differentiate into an OCL.

Expression of the myeloid specific antigen CD11b (Mac-1) has been used by a number of investigators to identify a circulating osteoclast precursor cell (De, Carpentier, Lories, et al. 2004, Yao, Li, Zhang, et al. 2006, Li, Schwarz, O'Keefe, et al. 2004a, Li, Schwarz, O'Keefe, et al. 2004b). The number of these cells in circulation is regulated by the inflammatory state of the organism and in particular tumor necrosis factor. Most recently Yao et al demonstrated that expression of CD11b and Gr1 could be used to identify the levels of this osteoclast precursor in the circulation and the periphery (Yao, Li, Zhang, et al. 2006). We have found that the osteoclast precursor cell in bone marrow is initially negative for expression of CD11b but becomes positive after stimulation with M-CSF and that mature OCL are negative for this antigen (Jacquin, Gran, Lee, et al. 2006). The more important question, which is currently unanswered, is what is the role of the circulating osteoclast precursor cell in normal and pathologic bone turnover? It is currently unknown if this cell homes to bone to form osteoclasts in all conditions where resorption is occurring or if its role is restricted to the development of bone resorption at sites in bone that are not adjacent to marrow. The extramedullary maturation of B and T-lymphocytes is well established (Picker and Siegelman 1999). However, whether similar events occur during osteoclast development is unknown.

3. WHY DO OSTEOCLASTS ONLY FORM IN BONE?

One interesting aspect of osteoclastogenesis is that cells with a cell surface phenotype that is similar to that of osteoclast precursor cells in bone marrow can be identified in the spleen, an organ that also has sources of M-CSF and RANKL. However, osteoclastogenesis does not occur in the spleen under any known condition. One possible explanation for this paradox is that the population of cells found in the spleen, despite having a similar phenotypes to cells found in the bone marrow, are missing crucial elements, which prevent them from forming osteoclasts in splenic tissues. However, this hypothesis seems improbable since multiple investigators have established the in vitro osteoclastogenic potential of splenocytes. Another possibility is that the microenvironment

in the spleen does not allow the production of osteoclasts either because it lacks critical signaling molecules or because it produces inhibitory signals. Miyamoto et al. proposed that in order to complete osteoclastogenesis an adherent condition, which is defined by the expression of specific molecules on osteoclast progenitors, is required (Miyamoto, Arai, Ohneda, et al. 2000). This would ensure the correct interactions between osteoclast progenitors and supporting cells that express the correspondent ligands. Osteoblastic lineage cells in the bone marrow might produce these signals. The absence of these signals in the spleen and other peripheral tissues would explain the inability of these tissues to support osteoclastogenesis.

The latter hypothesis is supported by the recent findings that late osteoclast differentiation and activation require a novel combination of co-stimulatory molecules, which act in concert with M-CSF and RANKL to complete osteoclastogenesis (Colonna 2003). These molecules involve proteins containing immunoreceptor tyrosine-based activation motif (ITAM) domains, which are found in adapter molecules like DAP12 and the Fc receptorγ (FcRγ). The search for receptors associated with these ITAM adaptors in myeloid cells have identified at least two candidates that associate with FcRγ (OSCAR or osteoclast associated receptor, and PIR-A) and two that associate to DAP12 (the triggering receptor expressed by myeloid cells-2, TREM-2), and the signal regulatory protein β1 (SIRP β1) (Koga, Inui, Inoue, et al. 2004). The ligands for these receptors are currently unknown.

4. CAN B-LYMPHOCYTES LINEAGE CELLS FORM OSTEOCLASTS?

Although the myeloid origin of osteoclast is well established, it has been proposed that cells of the B-lymphoid lineage can also give rise to osteoclast progenitors. Several groups have proposed the existence of bipotential progenitors for B lymphocytes and macrophages in bone marrow, which have the ability to differentiate into osteoclasts (Manabe, Kawaguchi, Chikuda, et al. 2001, Blin-Wakkach, Wakkach, Rochet, et al. 2004, Sato, Shibata, Ikeda, et al. 2001). We have also found that Pax-5−/− mice, which have a block in B lymphocyte development at the pro-B cell stage, have an increased number of osteoclasts in their bones and decreased bone mass (Horowitz, Xi, Pflugh, et al. 2004). However, the osteoclast population in these mice appears to be myeloid in origin. In previous work (Katavic, Grcevic, Lee, et al. 2003), we found that OCL formed in cultures of murine bone marrow cells that express the B-lymphocyte marker CD45R/B220. These studies relied on populations of CD45R positive murine bone marrow cells that were separated by fluorescence activated cell sorting (FACS) to a purity of 98–99%. However, in more recent work (Jacquin, Gran, Lee, et al. 2006), we determined that purification of the CD45R/B220 positive murine bone marrow population by a second round of FACS to a purity >99.9% essentially eliminated the ability of the purified CD45R population to form OCL in vitro. Hence, it appears that the OCL, which form in these cultures, require the presence of a contaminating non-CD45R -expressing population of cells to form OCL. We are aware of no other studies of the osteoclastic potential of CD45R -expressing murine bone marrow cells that purified their populations to the degree that we have now done. However, we suspect that additional rounds of purification of other CD45R positive populations, which have been proposed to contain osteoclast precursors, will likely also demonstrate them to be contaminated with small amounts of non-CD45R -expressing osteoclast precursors, which are critical for OCL formation in these cultures.

5. OSTEOCLAST PRECURSOR CELLS IN MURINE BONE MARROW

We have found that osteoclasts precursor cells are enriched in a population of murine bone marrow cells that do not express the antigens CD45R or CD3 and were low for expression CD11b (Jacquin, Gran, Lee, et al. 2006). Culture of these cells with M-CSF and RANKL stimulates

formation of multinucleated TRAP positive osteoclast-like cells (OCL) with high efficiency in as little as 3 days. Interestingly, we also found that murine bone marrow cells, expressing high levels of CD11b, formed osteoclasts with less efficiency than did murine bone marrow cells, which did not express this antigen. OCL in both the CD11b negative/low, (CD45R negative and CD3 negative) and the CD11b high, (CD45R negative and CD3 negative) populations have a number of characteristics of authentic osteoclasts, including the ability to form resorption pits when cultured on bovine cortical bone and high level expression of calcitonin receptors. The highest efficiency of OCL generation was found in the population of murine bone marrow cells that were C-kit and C-fms positive, CD11b negative/low as well as CD45R and CD3 negative (Jacquin, Gran, Lee, et al. 2006).

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