Histol Histopathol (1996) 11: 821-830

Histology and Histopathology

Invited Review

Osteoclast differentiation antigen

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Summary. Osteoclasts are the primary cells which perform bone resorption. The origin of these multinucleated giant cells is the haematopoietic stem cells. The differentiation pathway of the osteoclasts has so far been well studied and the cell-lineage of these bone resorbing cells is considered to be close but not identical to the monocytes/macrophages. Owing to the development of *in vitro* culture systems for evaluating osteoclast differentiation, it has been elucidated that various cytokines are involved in the differentiation of the osteoclasts. However, there is still ambiguity concerning the molecular mechanism of the differentiation of the osteoclasts. One approach for clarifying the molecular mechanism is to find unique antigen molecules involved in the process of osteoclast differentiation. In this review article, we introduce such immunological studies concerning osteoclast differentiation. We also refer to our recent establishment of a panel of monoclonal antibodies recognizing rat osteoclasts. One of the monoclonal antibodies recognizes cell surface antigen (Kat1-antigen) expressed on cells in osteoclast-lineage and not on monocytes/macrophages. Cross-linking of the cell surface antigen using this monoclonal antibody showed that the Kat1-antigen is the unique cell surface molecule involved in the regulation of the affinity of the calcitonin receptor and also involved in the modulation of the bone resorption. In this review article, we overview, the current issues which should be elucidated for understanding the differentiation and activation of the osteoclasts. We further emphasize the utility of the immunological approach for solving these current target issues.

Key words: Cell surface antigen, Monoclonal antibody, Osteoclast formation, Osteoclast activation

Background

Osteoclasts are multinucleated giant cells which resorb bone. In bone tissues, functional osteoclasts express a high polarized morphological feature. At the apical side facing the bone, osteoclasts have the typical morphologically identified structure called «ruffled border» bearing numerous ruffled villi. This prominent structure plays a central role in bone resorption. Surrounding the area of the ruffled border, there exists a specified region called «clear zone», in which no cytoplasmic organelle is observed but which has abundant cytoskeletal components involving actin filaments. In addition, these cells have a large number of mitochondria. These characteristic morphological structures are thought to be essential for the bone resorbing function of the osteoclasts. The origin of the osteoclast is the hematopoietic stem cell, and mononuclear osteoclast precursors committed to differentiate only to osteoclasts are formed under the control of the bone microenvironment (Burger and Nijweide, 1991; Chambers, 1992; Suda et al., 1992). These mononuclear precursors fuse to each other to form multinucleated osteoclasts. Formed multinucleated cells are further activated by an unknown mechanism to form activated osteoclasts having the ruffled border and the clear zone. Studies in osteopetrotic mutant mice have provided important findings in osteoclastogenesis. Extensive studies using homologous mutant mice lacking protooncogene c-*src* demonstrated defects in the formation of the ruffled border (Soriano et al., 1991; Boyce et al., 1992), a defect in «the activation» of the osteoclasts. Homologous mutant mice lacking the protoncogene *c-fos* showed defects in the formation of multinucleated osteoclasts (Grigoriadis et al., 1994). In contrast to these osteopetrotic mice, in which the cause of the osteopetrosis lies on defects in cells in the osteoclast lineage, osteopetrotic *op/op* mice have defects in the bone microenvironments required for osteoclastogenesis. These mice have the point mutation in the open reading frame of gene of macrophage-colony stimulating factor (M-CSF) resulting in a failure of producing active M-CSF in mutant mice (Yoshida et al., 1990). Felix et al. (1990a) and Kodama et al. (1991a) cured these osteopetrotic *op/op* mice by injection of recombinant M-CSF, confirming the essential role of this cytokine in *in vivo* osteoclastogenesis. Furthermore, Kodama et al. (1991b) and Hofstetter et al. (1992) showed evidences

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indicating the expression of protooncogene *c-fms*, receptors for M-CSF, on murine osteoclasts.

In this review, we focused on the «Osteoclast Differentiation Antigen» identified by the monoclonal antibodies involved in he process of the osteoclast differentiation. Before describing «Osteoclast Differentiation Antigen» we would like to present an overview concerning the current target issues in cell biology of osteoclast differentiation.

Current issues in osteoclast differentiation (Figs. 1 and 2)

i) Identification of the transcription factor involved in the commitment of cells in osteoclast-lineage

Studies in mutant mice lacking the c-*fos* allele demonstrated the importance of this transcription factor in osteoclastogenesis (Johnson et al., 1992; Grigoriadis et al., 1994). As the differentiation of bone marrow macrophages is not suppressed in these osteopetrotic mutant mice, c-*fos* expression is considered to be required in the commitment of osteoclast progenitors, the common differentiation unit of osteoclasts with monocytes/macrophages, to osteoclasts. Osteopetrotic mutant mice, *mi/mi* mice, have been revealed to have a defect in the helix-loop-helix type transcription factor (Hodgkinson et al., 1993). The differentiation of osteoclasts and mast cells is abnormal in *mi/mi* mutant mice. Hoyland et al. (1994) showed that the nuclear transcription factor NF-IL6 is expressed in human normal and pagetic osteoclasts. In their report, NF-IL6 and IL6 receptors are expressed both in normal and pagetic osteoclasts, but IL6 is only expressed in the pagetic osteoclasts, suggesting the link of the possible paramixovirus in the regulation of IL-6 expression, which is considered to be involved in the incidence of the disease. No other transcription factor has been identified to be involved in he determination of the osteoclast-differentiation.

ii) Identification of the cell surface molecules expressed on preosteoclasts, involved in the interaction with osteoblasts

The vascular cell adhesion molecule (VCAM-1) expressed on osteoblast is involved in the interaction between osteoblasts and cells in osteoclast lineage (Duong et al., 1994) in the process of the differentiation of the osteoclasts. Kurachi et al. (1993) reported the involvement of the intercellular adhesion molecule (ICAM-1) in the process of the osteoclast formation. There are few other reports concerning these molecules in osteoclastogenesis.

iii) Identification of the soluble factor produced by osteoblasts which induces osteoclast differentiation

Numerous studies have been reported concerning this issue. M-CSF is essential in the terminal differentiation of the osteoclasts (Felix et al., 1990a,b; Takahashi et al., 1991; Kodama et al., 1991a,b, 1993). IL6 produced from osteoblasts has been demonstrated to have key roles in osteoclastogenesis (Lowik et al., 1989; Ishimi et al., 1990; Littlewood et al., 1991; Jilka et al., 1992; Udagawa et al., 1995). Some research groups have shown the importance of GM-CSF for the differentiation of the osteoclasts (MacDonald et al., 1986; Kurihara et al., 1989). In addition to known cytokines, many researchers believe that there should be unknown factors which induce osteoclastogenesis.

iv) Identification of the cell surface molecules expressed on osteoclast-precursors which participate in the specific recognition and specific cell fusion of these mononuclear osteoclast precursors

Kumegawa et al. (1987) studied the dynamic fusion process of the mononuclear precursors of the osteoclasts using time-lapse cinematography. Although this interesting dynamic process attracts many researchers, little is known about the molecular

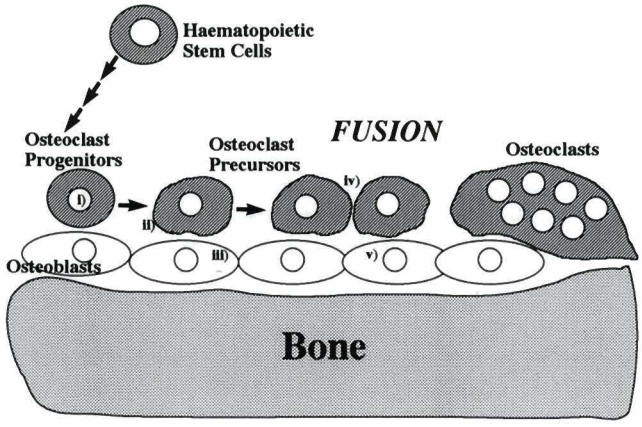

Fig. 1. Diagrammatic illustration of the osteoclast formation. Numbers in Roman style show the respective «current issues» in this review.

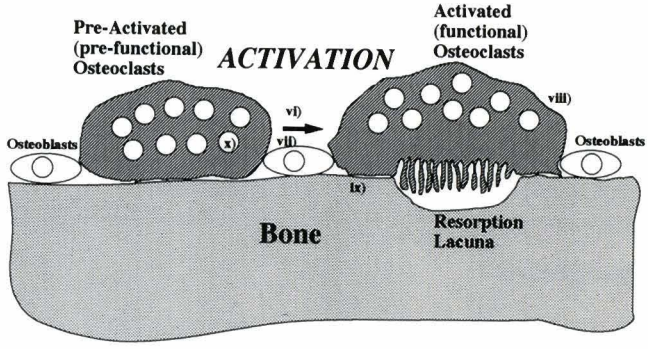

Fig. 2. Diagrammatic illustration of the osteoclast activation (or the terminal differentiation). Numbers in Roman style show the respective «current issues» in this review.

mechanism of the fusion process. Mbalaviele et al. (1995) recently showed an involvement of E-cadherin in this fusion process. Several research groups have tried to solve this issue, but little is known about the molecule involved in the specific interaction and specific fusion between osteoclast precursors. Key cell-surface molecules involved in the specific fusion events on cell surface of the osteoclast precursors may exist.

v) Identification of the cell surface molecule expressed on osteoblasts, and involved in the induction of the fusion process of the preosteoclasts

It has been emphasized from several research gorups that the unknown molecule expressed on the cell surface of the osteoblasts is essential for osteoclastogenesis (Burger and Nijweide, 1991; Chambers, 1992; Suda et al., 1992). The involvement of osteoblasts, bone forming cells, in the differentiation of the osteoclasts has been shown in several culture systems for osteoclast differentiation (Takahashi et al., 1988; Chambers et al., 1993; Kukita et al., 1993a,b; Matsumoto et al., 1995). Although the molecular entity of this molecule has not been identified, this cell surface molecule expressed on the osteoblasts having the potential ability of osteoclastogenesis is considered to be important.

vi) Elucidation of the mechanism underlying osteoclast activation; induction of the ruffled border formation

Mutant mice having disrupted proto-oncogene c-*src* provided quite important evidence that this proto-oncogene has essential roles in the activation of the osteoclasts. Soriano et al. (1991) reported that targeted disruption of the c-*src* gene leads to osteopetrosis in mice. Boyce et al. (1992) demonstrated that multinucleated osteoclasts are formed in these animals, but that these osteoclasts have no ruffled borders, suggesting that these mutant mice have defects in the activation of the osteoclasts. C-*src* has the tyrosine kinase activity and this tyrosine phosphorylation is involved in some tumorigenesis (Eiseman and Bolen, 1990). Substrate proteins of the c-src tyrosine kinase are considered to be the key molecules in the formation of the ruffled border. Tanaka et al. (1995) showed that 120 KDa protein is the specific substrate for c-*src* kinase in osteoclasts. There would be a cascade of tyrosine phosphorylation of several substrate proteins in the downstream of c-*src*. Identification of each component of this cascade seems to be essential for understanding the molecular mechanism of the ruffled border formation. Recently, Thomas et al. (1995) reported that phosphorylated cortactin is found in the cell-matrix adhesion of normal osteoclasts but is deficient in c-src$^-$ osteoclasts. These proteins associated with the cytoskeleton may be key molecules for osteoclast activation.

vii) Identification of the regulatory molecules secreted by osteoblasts and involved in the activation of the osteoclasts. Also, identification of the receptor molecule expressed on activated osteoclasts

Based on the hypothesis of Rodan and Martin (1981), Mcsheehy and Chambers (1986) presented the first experimental evidence suggesting the presence of osteoclast activating factors in the conditioned medium of osteoblasts. In spite of the efforts to identify such molecules, they have not yet to be identified.

viii) Elucidation of the apoptotic mechanism of the osteoclasts

In normal bone tissues, the number of osteoclasts and also that of the osteoclast precursors are defined by an unknown mechanism. The life span of an osteoclast is supposed to be defined by the normal apoptosis of the cell. Fuller et al. (1993) showed that M-CSF inhibits apoptosis of the osteoclast *in vitro*. The regulatory mechanism of osteoclast apoptosis may be clarified in the future.

ix) Identification of the bone matrix protein involved in the activation of the osteoclasts

Osteopontin is a candidate molecule for modulating the activity of osteoclasts (Miyauchi et al., 1991; Ross et al., 1993). This bone matrix protein contains an RGD sequence and binds to the $\alpha_v\beta_3$ integrin (vitronectin receptor), highly expressed on osteoclasts. Reinholt et al. (1990) showed a localization of the vitronectin receptor at the area of osteoclast clear zone and localization of the bone matrix osteopontin at the corresponding area of the bone surface with the clear zone, but other research groups reported controversial results, in that the vitronectin receptor was not involved in the osteoclast clear zone (Lakkakorpi et al., 1991). Other unknown molecules causing osteoclast activation may be involved in the bone matrix.

x) The molecular mechanism concerning the induction of a series of gene expression required for bone resorption

In osteoclasts, carbonic anhydrase II (Gay et al., 1974; Vaananen and Parvinen, 1983; Väänänen, 1984) and vacuolar type proton ATPase (proton pump) (Baron, 1989; Vaananen et al., 1990) are expressed for acidifying the bone surface resulting in decalcification and facilitating the action of the proteolytic lysosomal enzymes during bone resorption. Furthermore, various proteolytic enzymes are required in bone resorption for digesting bone matrix proteins. Cathepsin B (Van Noorden et al., 1989), cathepsin C (Baron et al., 1988), cathepsin L (Rifkin et al., 1991), and cathepsin D (Goto et al., 1992) are shown to be expressed in osteoclasts. Osteoclasts also express gelatinase B (Wucherpfennig et al., 1994) and tartrate-resistant acid phosphatase

(Minkin, 1982). Furthermore, osteoclasts express abundant calcitonin receptors (Warshawsky et al., 1980; Nicholson et al., 1986) as the most important regulatory cell surface molecule. How these genes are activated during the process of osteoclast differentiation remains to be elucidated. Transcription of each gene is considered to be induced by their unique transcription factor(s) selectively expressed in osteoclasts and their mononuclear precursors.

Basic immunological approach for identifying molecules sharing the regulation of osteoclast differentiation

To identify key molecules involved in osteoclast differentiation, the immunological approach using B cell hybridoma technology provides marked advantages in the search for novel molecules. In this approach, a panel of monoclonal antibodies are raised against whole cells or homogenates of cells. Specific antibodies or selective antibodies recognizing osteoclasts are screened by several assays using immunohistochemical staining or immunocytochemical staining. After preparing osteoclast-selective or osteoclast-specific monoclonal antibodies, their biological activity is estimated in several bioassay systems for evaluating bone resorption and osteoclast differentiation. Any antigen molecule recognized with the monoclonal antibody having inhibitory or stimulatory effects obtained in these assay systems is considered to be the key molecule in the differentiation and activation of the osteoclasts. Once such a monoclonal antibody recognizing the unique protein is obtained, cDNA cloning of the antigen of interest can be done by the molecular biological techniques.

The B cell hybridoma technology has furthered our understanding of the molecular mechanism of lymphocyte homing to the specific region of the high endothelial cell wall (high endothelial venule) present in lymphoid tissues, from which peripheral blood lymphocytes are recirculated into the lymphoid tissue (Springer, 1994). The high endothelial venule is identified easily by histological observations under a microscope. In these studies for finding novel molecules involved in lymphocyte homing, the histological screening of the antibodies is a powerful assay system for evaluating monoclonal antibodies. In these bioassays, lymphocytes are incubated on the cryosection of the lymphoid tissues to bind specifically to the histologically identified high endothelial venule (Stamper-Woodruff assay) (Stamper and Woodruff, 1976). By use of this assay system, monoclonal antibodies having the ability to inhibit lymphocyte binding to the high endothelial venules have been obtained and antigen molecules of each monoclonal antibodies have been elucidated to be a series of novel molecules involved in lymphocyte homing, e.g.; L-selectin (Gallatin et al., 1983), MECA-79 carbohydrate antigens (Streeter et al., 1988a,b), GlyCAM-1 (Lasky, 1992), MAdCAM-1 (Streeter et al., 1988a,b), and CD34 (Baumhueter et al., 1993). As shown in these studies, histological techniques, which seem to be classical methods, are useful to find novel molecules involved in some biological processes.

Tartrate resistant acid phosphatase and the calcitonin receptor are available markers of identifying the osteoclasts, but more convenient immunological markers are required in distinguishing osteoclasts and their precursors from other cell lineages. Such immunological reagents enable cells in osteoclast lineage to be analyzed by fluorescence activated cell sorter, FACS. Detailed analysis of the cell population of the osteoclasts and their mononuclear precursors would provide important information concerning the regulation of the osteoclast differentiation. FACS also have great advantages in purifying specific cell lineage.

Although multinucleated osteoclasts adhering to the bone surface have been identified to be osteoclasts only from their morphological criteria, mononuclear osteoclast precursors are hard to identify only from the morphological point of view. In the case of osteoporosis (Kleerekoper and Avioli, 1993), osteolysis accompanied by operations of total hip displacement (Kadoya et al., 1995), and bone resorption of the alveolar bone accompanied by periodontal disease (Bhaskar, 1986), osteoclastic bone resorption has been shown to be actually involved, but little information has been obtained concerning the localization of osteoclast precursors in bone tissues in each case. The specific antibody recognizing cells in osteoclast lineage will enable the convenient detection of osteoclast precursors *in situ* in these cases accompanying osteoclastic bone resorption.

To date, over 130 cell surface antigens have been identified on human white blood cells. These antigen molecules have been given their own numbers after «CD» («cluster of differentiation»). Several lineage selective or specific markers are identified in each cell lineage. CD3, CD20, and CD68 are the specific markers for T cells, B cells, and macrophages, respectively. However, a specific marker of the osteoclasts has not been found. Some of the lineage specific or selective CD antigens have been elucidated to be important molecules in lymphopoiesis or the activation of lymphocytes. CD40 is the cell surface molecule expressed on B cells. As the monoclonal antibody specific to CD40 mediates a variety of effects on B lymphocytes, including cell proliferation, it was hypothesized that there should be a natural ligand for the CD40 expressed on B cells. Finally the CD40 ligand was found to be a novel molecule expressed on the cell surface of the activated T cells (Armtiage et al., 1992). This discovery revealed the fundamental mechanism underlying the T cell-dependent B cell proliferation observed in the germinal center of the lymphoid tissues. Kishihara et al. (1993) revealed that CD45, generally expressed on white blood cells, has the essential role in the signal transduction mediated by antigen receptors in lymphocytes. Thus, antigen molecules defined by specific monoclonal antibodies,

which are expressed on a specific cell lineage, are expected to have some important roles in the differentiation or the activation of some cell lineages. These immunological search for the key molecules in the differentiation of the specified cell lineage to analyze the mechanism of the differentiation at the molecular level will become possible when the cDNA of the antigen molecule is cloned. The recent development of mutant mice lacking in a specific gene by using gene targeting enables us to clarify the actual function of the antigen molecules *in vivo*.

We review the trials of developing lineage-specific monoclonal antibodies in the field of the cell biology of the osteoclasts.

Searching for osteoclast-specific antigens

Oursler et al. (1985) were the first to report the production of osteoclast-specific monoclonal antibodies. They established a monoclonal antibody designated as 121F which reacted to avian osteoclasts. Further biochemical analysis of the antigen revealed it to be a manganate superoxide dismutase involved in the removal of the radicals (Oursler et al., 1991a,b). This antigen can be induced by the presence of calvaria-conditioned media (Oursler and Osdoby, 1988) and the function of this antigen is considered to be the protection of active osteoclasts from harmful super oxide-free radicals produced during bone resorption.

Horton et al. (1985) produced a monoclonal antibody designated as 13C6 or 23C6, specific to multinucleated giant cells in cyrosections of the human osteoclastoma tissues (giant cell tumor). Further biochemical analysis revealed that this antigen was the vitronectin receptor (Davies et al., 1989), the integrin which is the heterodimer of the αv chain and $ß_3$ chain. Although the vitronectin receptor is expressed in various cell lineages (Athanasou et al., 1990), the high expression of this integrin in osteoclasts is considered to have an important role in the functional regulation of the osteoclast. These monoclonal antibodies inhibit bone resorbing activity of the osteoclasts *in vitro* (Chambers et al., 1986). Reinholt et al. (1990) proposed a direct interaction of this integrin and bone matrix osteopontin in the area of the osteoclast clear zone. Although others did not show the clear localization of the vitronectin receptor in the clear zone, biochemical data demonstrated that the osteopontin, a bone matrix protein having an RGD sequence, binds vitronectin receptors expressed on osteoclasts followed by stimulating the phosphatidyl-inositol-3-hydroxyl kinase activity (Hruska et al., 1995). These findings suggests that the binding of osteopontin to the vitronectin receptor expressed on osteoclasts is important in the modulation of the activity of the osteoclasts. Hultenby et al. (1995) demonstrated that the ligand of $\alpha_v ß_3$ integrin is the osteopontin in the osteoclast clear zone in osteopetrotic (*ia/ia*) rats. Hu et al. (1995) recently showed that elevation of the extracellular calcium level suppresses the cell adhesion to osteopontin by attenuating binding affinity for integrin $\alpha_v ß_3$. Thus, vitronectin receptors expressed on osteoclasts first found by Horton et al. (1985) are now considered to be one of the essential cell surface molecules in the regulation of the osteoclast activity.

Nijweide et al. (1985) reported the production of a panel of monoclonal antibodies reactive to quail osteoclasts. One of the antibodies designated as OC6.9 recognizes the polarity of the osteoclasts, but further biochemical studies have not been reported. Hentunen et al. (1991) produced a series of monoclonal antibodies recognizing chicken osteoclasts. The monoclonal antibody K20 has been shown to inhibit osteoclastic bone resorption via cell surface 150 KDa membrane protein expressed on osteoclasts. Alternatively, their monoclonal antibody K5 is interesting because it does not react to all osteoclast preparations, suggesting that this antibody recognizes a subpopulation of chicken osteoclasts. Nelson et al. (1991) also produced monoclonal antibodies recognizing multinucleated giant cells in osteoclastoma tissues. James et al. (1991) reported the production of monoclonal antibodies which selectively react to human osteoclast-like multinucleated cells formed *in vitro*. Kukita and Roodman (1989) previously reported the production of a monoclonal antibody which reacts to baboon osteoclasts. Biochemical studies concerning these antigens have not been reported.

Searching for osteoclast-selective antigens by use of the commercially available anti-CD antigen monoclonal antibodies

As numerous monoclonal antibodies recognizing human CD antigens are commercially available, cell surface phenotypes of human osteoclasts have been extensively examined (Horton and Helfrich, 1992). In human osteoclasts the expression of CD9 (distributed in non T non B acute lymphoblastic leukemia cells, platelets, monocytes and granulocytes), CD13 (amino peptidase N: distributed in monocytes, granulocytes, acute myelogenous leukemic cells, chronic myelocytic leukemia cells, tonsilar macrophages, and intestine), CD15a (distributed in granulocytes, acute myelogenous leukemic cells), CD44 (hyaruronic acid receptor/ distributed in T cells, B cells, granulocytes, monocytes, erythrocytes) (Athanasou and Quinn, 1990), CD45 (white blood cell common antigen bearing tyrosine-phosphatase: distributed in T cells, B cells granulocytes, and monocytes), CD49b (very late antigen α_2 chain/VLAα_2: distributed in platelets, activated T cells), CD51 (vitronectin receptor α chain/integrin α_v: distributed on platelets), CD54 (intercellular adhesion molecule-1/ICAM-1: distributed in white blood cells), CD61 (vitronectin receptor ß chain/integrin $ß_3$: distributed in platelets), and CD71 (transferrin receptor: distributed in activated T cells, activated macrophages, reticulocytes, other white blood cells) have been identified. Expression of CD51 (integrin α_v) and CD61

(integrin β_3) has also been confirmed in mouse, rat, and chicken osteoclasts (Horton and Chambers, 1986). Nesbitt et al. (1993) demonstrated that integrin α_2, α_v, β_1, and β_3 are expressed on human osteoclasts while α_4, α_5, α_6, α_8, α_L, α_M, α_x, gpIIb, β_2, β_4, β_6 and β_8 are not expressed in these cells. CD68, the cytoplasmic antigen expressed in macrophages, is also expressed in human osteoclasts (Athanasou and Quinn, 1990).

Among these cell surface molecules expressed on human osteoclasts, CD51 and CD61 form heterodimers to act as the vitronectin receptor as already described in this review. The functions of CD9, CD13, and CD45 are not known. CD49b is the integrin α_2 and this molecule functions as the collagen receptor when it is heteromerized with integrin β_1 expressed on osteoclasts. CD71 is the transferrin receptor and this molecules is generally expressed on proliferating cells requiring iron for growth. The reason why osteoclasts, which do not proliferate, express CD71 is supposed to be related to their high requirement of iron owing to the high level of the tartrate-resistant acid phosphatase activity (Horton and Helfrich, 1992). This enzyme requires iron for expressing enzyme activity.

Introduction of an immunological approach for analyzing osteoclast differentiation

Here we introduce our strategy for studying osteoclast differentiation by use of immunological methods.

As it is difficult to obtain a large number of isolated osteoclasts from bone, we first established an *in vitro* culture system for forming osteoclast-like multinucleated cells (MNCs) from rat bone marrow cells in the presence of 1α, 25-dihydroxyvitamin D_3 (Hata et al., 1992). We further modified this culture system to form a large number of osteoclast-like MNCs having high bone resorbing activity and calcitonin sensitivity by the addition of the heat-treated conditioned medium of the rat osteosarcoma cell line ROS17/2.8 (Kukita et al., 1993a,b). We also established a culture condition for forming macrophage polykaryons in the presence of a high concentration of phorbor-12-myristate-13-acetate diester (Kukita et al., 1992).

Hata et al. (1994) immunized mouse spleen cells *in vitro* with osteoclast-like MNCs which had been replated on dentine slices and already commenced resorption. After hybridoma formation according to the standard protocol, antibody screening was performed by immunohistochemical methods using cryosections of mandible obtained from neonatal rats. In these sections, numerous osteoclasts were observed in mandibles surrounding the tooth germ. By use of this immunohistochemical method, we selected and obtained a monoclonal antibody Ch1 recognizing the polarity of the osteoclasts. The peripheral region of the active osteoclasts facing the bone surface was specifically stained by this monoclonal antibody Ch1. More detailed observations by use of the confocal laser scanning microscopy revealed that the Ch1 antigen was localized in the ruffled border and clear zone. We have also shown that expression of the Ch1 antigen is markedly induced by the presence of the calcified matrices. Although the biochemical properties of this antigen have not been elucidated, this antigen may be involved in the ruffled border formation.

Maeda et al. (1994) have immunized mouse spleen cells *in vitro* with osteoclast-like MNCs and have obtained monoclonal antibody reacting with secretory products of osteoclast-like MNCs as well as the cell surface of the osteoclasts. Biochemical analysis using Western blotting revealed that this monoclonal antibody, designated as HOK1, specifically reacts with the purified osteopontin. Localization of osteopontin recognized by this monoclonal antibody HOK1 was detected in resorption lacunae formed by osteoclast-like MNCs. These observations demonstrated the actual involvement of osteopontin in bone remodeling.

Kukita et al. (1994a,b) immunized mouse spleen cells with lightly fixed osteoclast-like MNCs having high calcitonin sensitivity and high bone resorbing activity (Kukita et al., 1993a,b). In the screening process of the monoclonal antibodies, we have selected antibodies reacting with the osteoclast-like MNCs bearing high calcitonin sensitivity but not reacting with the macrophage polykaryons. The obtained monoclonal antibody designated as Kat1 is highly specific to rat osteoclasts. As the monoclonal antibody Kat1 does not stain rat NRK cells expressing the vitronectin receptor (CD51/CD61) and it does not stain rat primary kidney epithelial cells expressing the calcitonin receptor, the Kat1-antigen recognized with the monoclonal antibody Kat1 (Kat1-antigen) is likely to be a novel molecule expressed on the cell surface of rat osteoclasts. To estimate the function of the Kat1-antigen, we have examined the biological activity of this antibody. Pretreatment of osteoclast-like MNCs with the monoclonal antibody Kat1 significantly elevated the affinity of the calcitonin receptor without altering the number of receptors when ^{125}I-labeled calcitonin binding properties to osteoclast-like MNCs was investigated by Scatchard analysis. The monoclonal antibody Kat1 markedly augmented the sensitivity of osteoclast precursors to calcitonin in the system of osteoclast-like cell formation. The monoclonal antibody Kat1 significantly inhibited dentine resorption by osteoclast-like MNCs. These findings indicated that the Kat1-antigen is a novel cell surface molecule specifically expressed on rat osteoclasts and this molecule is involved in the functional modulation of the calcitonin receptor and also in the regulation of osteoclastic bone resorbing function. Recent morphological studies concerning the localization of Kat1-antigen *in vivo* revealed that the expression of this antigen is initiated in preosteoclasts, mononuclear precursors of the osteoclasts, and the highest expression of this antigen is observed in the activated osteoclasts. However, the level of the Kat1-antigen expression is

dramatically suppressed during the degeneration of osteoclasts because the Kat1-antigen cannot be detected in post-functional osteoclasts (Kukita et al., 1994b).

We have prepared other monoclonal antibodies having different reactivities against rat osteoclasts. Some of them react with cytoplasmic antigens expressed in rat osteoclasts and others react with nuclear antigens expressed in these cells (Maeda et al. unpublished data).

The monoclonal antibody Kat1, which recognizes the lineage-specific cell surface antigen expressed on osteoclasts, is especially useful in enriching cells in osteoclast-lineage. After enrichment of cells in osteoclast-lineage (osteoclast-like MNCs and their mononuclear precursors) by use of the magnetic cell separator system using the monoclonal antibody Kat1 as the primary antibody, we extracted mRNAs from these cells and constructed the cDNA expression library (Kukita et al. unpublished data). We screened the cDNA library of the osteoclast-like MNCs by using several antibodies recognized rat osteoclasts and obtained several unique cDNA clones (Kukita et al. unpublished data). Gene expression of mRNAs encoded by the obtained cDNA clones in bone tissues was examined by use of the *in situ* hybridization technique and selective expression of some genes in rat osteoclasts was obtained. Detailed molecular analysis of the cDNAs is under the study in our laboratories.

Future perspectives

To clarify the molecular mechanism of the osteoclast differentiation, it is essential to search for molecules involved in each process of the osteoclast-differentiation. If some antigen molecule specifically or selectively expressed on osteoclasts is found and if this molecule is shown to be involved in the regulatory process of the differentiation or the activation of the osteoclasts, detailed investigation of the molecule should provide important information for developing an ideal clinical therapy in the future. Before entering into such clinical applications, actual functions of the molecule should be analyzed in detail by use of the *in vivo* system. Recent progress in establishing mutant mice lacking a specific gene enables bone cell biologists to examine the actual role of the antigen molecule involved in the regulation of the differentiation or the activation of the osteoclasts *in vivo*. Alternatively, the technology in preparing the transgenic mice provides a system of regulated overexpression of the specified molecule. These gene-targeted animals will be interesting animal models of various human bone diseases.

Acknowledgments. We thank Dr. David Roodman and Dr. Gegrory Mundy of the University of Texas Health Science Center San Antonio (USA) for their continuous encouragement. We thank Dr. Takeshi Watanabe of Medical Institute of Bioregulation of Kyushu University (Japan) for helpful discussions. We also thank Dr. Tadahiko Iijima of Kyushu University and Dr. Osamu Kohashi of Saga Medical School (Japan) for encouragement.

References

Athanasou N.A. and Quinn J. (1990). Immunophenotypic differences between osteoclasts and macrophage polykaryons: immunohistological distinction and implications for osteoclast ontogeny and function. J. Clin. Pathol. 43, 997-1003.

Athanasou N.A., Quinn J., Horton M.A. and McGee J.O'D. (1990). New sites of cellular vitronectin receptor immunoreactivity detected with osteoclast-reacting monoclonal antibodies 13C2 and 23C6. Bone Miner. 8, 7-22.

Armitage R.J., Fanslow W.C., Strockbine L., Sato T.A., Clifford K.N., Macduff B.M., Anderson D.M., Gimpel S.D., Davis-Smith T., Maliszewski C.R., Clark E.A., Smith C.A., Grabstein K.H., Cosman D. and Spriggs M.K. (1992). Molecular and biological characterization of a murine ligand for CD40. Nature 357, 80-82.

Baron R. (1989). Molecular mechanisms of bone resorption by the osteoclast. Anat. Rec. 224, 317-324.

Baron R., Neff L., Brown W., Courtoy P.J., Louvard D. and Farquhar M.G. (1988). Polarized secretion of lysosomal enzymes: Co-distribution of cation-independent mannose-6-phosphate receptors and lysosomal enzymes along the osteoclast exocytic pathway. J. Cell Biol. 106, 1863-1872.

Baumhueter S., Singer M.S., Henzel W., Hemmerich S., Renz M., Rosen S.D. and Lasky L.A. (1993). Binding of L-selectin to the vascular sialomucin CD34. Science 262, 436-438.

Bhaskar S.N. (1986). Maxilla and mandible (alveolar process). In: Orban's oral histology and embryology. 10th ed. Bhaskar S.N. (ed). CV Mosby Company. St. Louis. pp 232-252.

Boyce B.F., Yoneda T., Lowe C., Soriano P. and Mundy G.R. (1992). Requirement of $pp60^{c\text{-}src}$ expression for osteoclasts to form ruffled borders and resorb bone in mice. J. Clin. Invest. 90, 1622-1627.

Burger E.H. and Nijweide P.J. (1991). Cellular origin and theories of osteoclast differentiation. In: The osteoclast/bone. Vol. 2. Hall B.K. (ed). CRC Press. Boca Raton. pp 31-59.

Chambers T.J. (1992). Regulation of osteoclast development and function. In: Biology and physiology of the osteoclast. Rifkin B.R. and Gay C.V. (eds). CRC Press. Boca Raton. pp 105-128.

Chambers T.J., Fuller K., Darby J.A., Pringle J.A.S. and Horton M.A. (1986). Monoclonal antibodies against osteoclasts inhibit bone resorption in vitro. Bone Miner. 1, 127-135.

Chambers T.J., Owens J.M., Hattersley G., Jat P.S. and Noble M.D. (1993). Generation of the osteoclast-inductive and osteoclastogenic cell lines from the *H-2K^{b}tsA58* transgenic mouse. Proc. Natl. Acad. Sci. 90, 5578-5582.

Davies J., Warwick J., Totty N., Philip R., Helfrich M. and Horton M. (1989). The osteoclast functional antigen, implication in the regulation of bone resorption, is biochemically related to the vitronectin receptor. J. Cell Biol. 109, 1817-1826.

Duong L.T., Tanaka H. and Rodan G.A. (1994). VCAM-1 involvement in osteoblast-osteoclast interaction during osteoclast differentiation. J. Bone Miner. Res. 9, s131 (Abstract).

Eiseman E. and Bolen J.B. (1990). *src*-Related tyrosine kinases as signaling components in hematopoietic cells. Cancer Cells 2, 303-310.

Felix R. Cechini M.G. and Fleisch H. (1990a). Macrophage colony stimulatin factor restores *in vivo* bone resorption in the *op/op* osteopetrotic mouse. Endocrinology 127, 2592-2594.

Felix R., Cechini M.G., Hofstetter W., Elford P.R., Stutzer A. and Fleisch H. (1990b). Impairment of macrophage colony-stimulating factor

production and lack of resident bone marrow macrophages in the osteopetrotic *op/op* mouse. J. Bone Miner. Res. 5, 781-789.

Fuller K., Owens J.M., Jagger C.J., Wilson A., Moss R. and Chambers T.J. (1993). Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. J. Exp. Med. 178, 1733-1744.

Gallatin W.M., Weissman I.L. and Butcher E.C. (1983). A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature 304, 30-34.

Gay C.V., Faleski E.J., Schraer H. and Schraer R. (1974). Localization of carbonic anhydrase in avian gastric mucosa, shell gland and bone by immunohistochemistry. J. Histochem. Cytochem. 22, 819-825.

Goto T., Tsukuba T., Ayasaka N., Yamamoto K. and Tanaka T. (1992). Immunocytochemical localization of cathepsin D in the rat osteoclast. Histochemistry 97, 13-18.

Grigoriadis A.E., Wang Z-Q., Cecchini M.G., Hofstetter W., Felix R., Fleisch H.A. and Wagner E.F. (1994). C-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. Science 266, 443-448.

Hata K., Kukita T., Akamine A., Kukita A. and Kurisu K. (1992). Trypsinized osteoclast-like multinucleated cells formed in rat bone marrow cultures efficiently form resorption lacunae on dentine. Bone 13, 139-146.

Hata K., Kukita T., Akamine A., Kukita A., Kurisu K. and Iijima T. (1994). A novel monoclonal antibody recognizing a unique antigen of rat osteoclasts induced by the calcified matrices. Histochemistry 101, 347-354.

Hentunen T.A., Lakkakorpi P.T., Rautiala T. and Väänänen H.K. (1991). Inhibition of bone resorption by a monoclonal antibody that reacts with a 150 kD membrane protein in chicken osteoclasts. J. Bone Miner. Res. 6, 1091-1097.

Hodgkinson C.A., Moore K.J., Nakayama A., Steingrimsson E., Copeland N.G., Jenkins N.A. and Arnheiter H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell 74, 395-404.

Hofstetter W., Wetterwald A., Cecchini M.C., Felix R., Fleisch H. and Mueller C. (1992). Detection of transcripts for the receptor for macrophage colony-stimulating factor, *c-fms*, in murine osteoclasts. Proc. Natl. Acad. Sci. 89, 9637-9641.

Horton M.A. and Chambers T.J. (1986). Human osteoclast-specific antigens are expressed by osteoclasts in a wide range of non-human species. Br. J. Exp. Pathol. 67, 95-104.

Horton M.A. and Helfrich M.H. (1992). Antigenic markers of osteoclasts. In: Biology and physiology of the osteoclasts. Rifkin B.R. and Gay C.V. (eds). CRC Press. Boca Raton. pp 33-54.

Horton M.A., Lewis D., McNulty K., Pringle J.A.S. and Chambers T.J. (1985). Monoclonal antibodies to osteoclastomas (Giant cell bone tumors): definition of osteoclast-specific cellular antigens. Cancer Res. 45, 5663-5669.

Hoyland J.A., Freemont A.J. and Sharpe P.T. (1994). Interleukin-6, IL-6 receptor, and IL-6 nuclear factor gene expression in Paget's disease. J. Bone Miner. Res. 9, 75-80.

Hu D.D., Hoyer J.R. and Smith J.W. (1995). Ca^{2+} suppresses cell adhesion to osteopontin by attenuating binding affinity for integrin alpha v beta 3. J. Biol. Chem. 270, 9917-9925.

Hultenby K., Reinholt F.P., Heinegard D., Andersson G. and Marks S.C.Jr. (1995). Osteopontin: a ligand for the alpha v beta 3 integrin of the osteoclast clear zone in osteopetrotic (*ia/ia*) rats. Ann. N.Y. Acad. Sci. 760, 315-318.

Hruska K.A., Rolnick F., Huskey M., Alvarez U. and Cheresh D. (1995). Engagement of the osteoclast integrin alpha v beta 3 by osteopontin stimulates phosphatidylinositol 3-hydroxyl kinase activity. Endocrinology 136, 2984-2992.

Ishimi Y., Miyaura C., Jin C.H., Akatsu T., Abe E., Nakamura Y., Yamaguchi A., Yoshiki S., Matsuda T., Hirano T., Kishimoto T. and Suda T. (1990). IL-6 is produced by osteoblasts and induces bone resorption. J. Immunol. 145, 3297-3303.

James I.E., Walsh S., Dodds R.A. and Gowen M. (1991). Production and characterization of osteoclast-selective monoclonal antibodies that distinguish between multinucleated cells derived from different human tissues. J. Histochem. Cytochem. 39, 905-914.

Jilka R.L., Hangoc G., Girasole G., Passeri G., Williams D.C., Abrams J.S., Boyce B., Broxmeyer H. and Manolagas S.C. (1992). Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257, 88-91.

Johnson R.S., Spiegelman B.M. and Papaioannou V. (1992). Pleitropic effects of a null mutation in the *c-fos* proto-oncogene. Cell 71, 577-586.

Kadoya Y., Al-Saffar N., Revell P.A., Kobayashi A., Scott G. and Freeman M.A.R. (1995). Histomorphometry of the bone surface in aseptic loosening of total joint arthroplasties. Orthop. Res. Soc. Ann. Meet. 41, 746 (Abstract).

Kishihara K., Penninger J., Wallace V.A., Kuding T.M., Kawai K., Wakeham A., Timms E., Pfeffer K., Ohashi P.S., Thomas M.L., Furlonger C., Paige C.J. and Mark T.W. (1993). Normal B lymphocyte development but impaired T cell maturation in CD45-exon 6 protein tyrosine phosphatase-deficient mice. Cell 74, 143-156.

Kleerekoper M. and Avioli L.V. (1993). Evaluation and treatment of postmenopausal osteoporosis. In: Primer on the metabolic bone diseases and disorders of mineral metabolism. 2nd ed. Favus M.J. (ed). Raven Press. New York. pp 223-229.

Kodama H., Yamasaki A., Nose M., Niida S., Ohgame Y., Abe M., Kumegawa M. and Suda T. (1991a). Congenital osteoclast deficiency in osteopetrotric (*op/op*) mice is cured by injections of macrophage colony stimulating factor. J. Exp. Med. 173, 269-272.

Kodama H., Nose M., Niida S. and Yamasaki A. (1991b). Essential role of macrophage colony-stimulating factor in the osteoclast differentiation supported by stromal cells. J. Exp. Med. 173, 1291-1294.

Kodama H., Yamasaki A., Abe M., Niida S., Hakeda Y. and Kawashima H. (1993). Transient recruitment of osteoclasts and expression of their function in osteopetrotic (*op/op*) mice by a single injection of macrophage colony-stimulating factor. J. Bone Miner. Res. 8, 45-50.

Kukita A., Kukita T., Hata K., Kurisu K. and Kohashi O. (1993a). Heat-treated osteoblastic cell (ROS17/2.8)-conditioned medium induces the formation of osteoclast-like cells. Bone Miner. 23, 113-127.

Kukita A., Kukita T., Shin J.-H. and Kohashi O. (1993b). Induction of mononuclear precursor cells with osteoclastic phenotypes in a rat bone marrow culture system depleted on stromal cells. Biochem. Biophys. Res. Commun. 196, 1383-1389.

Kukita T. and Roodman G.D. (1989). Development of a monoclonal antibody to osteoclasts formed in vitro which recognizes mononuclear osteoclast precursors in the marrow. Endocrinology 125, 630-637.

Kukita T., Kukita A., Hata K. and Kurisu K. (1992). 12-O-Tetradecanoylphorbol-13-acetate inhibits osteoclast-like cell

differentiation in rat bone marrow cultures by inducing macrophage polykaryons. Endocrinology 130, 577-584.

Kukita T., Kukita A., Nagata K., Maeda H., Kurisu K., Watanabe T. and Iijima T. (1994a). Novel cell-surface Ag expressed on rat osteoclasts regulating the function of the calcitonin receptor. J. Immunol. 153, 5265-5273.

Kukita T., Kukita A., Xu L.X., Maeda H. and Iijima T. (1994b). A novel osteoclast differentiation antigen modulating the functional regulation of rat osteoclasts. J. Bone Miner. Res. 9, s156 (Abstract).

Kumegawa M. (1987). The bone II-The first cinematographic record of osteoclast formation. J. Bone Miner. Res. 2, 269 (Abstract).

Kurachi T., Morita I. and Murota S. (1993). Involvement of adhesion molecules LFA-1 and ICAM-1 in osteoclast development. Biochim. Biophys. Acta 1178, 259-266.

Kurihara N., Suda T., Miura Y., Nakauchi H., Kodama H., Hiura K., Hakeda Y. and Kumegawa M. (1989). Generation of osteoclasts from isolated hematopoietic progenitor cells. Blood 74, 1295-1302.

Lakkakorpi P.T., Horton M.A., Helfrich M.H., Karhukorpi E.-K. and Väänänen H.K. (1991). Vitronectin receptor has a role in bone resorption but does not mediate tight sealing zone attachment of osteoclasts to the bone surface. J. Cell Biol. 115, 1179-1186.

Lasky L.A. (1992). Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 258, 964-969.

Littlewood A.J., Russell J., Harvey G.R., Hughes D.E., Russell R.G.G. and Gowen M. (1991). The modulation of the expression of IL-6 and its receptor in human osteoblasts *in vitro*. Endocrinology 129, 1513-1520.

Lowik C.W.G.M., van der Pluijm G., Bloys H., Hoekman K., Bijvoet O.L.M., Aarden L.A. and Papapoulos S.E. (1989). Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogenesis. Biochem. Biophys. Res. Commun. 162, 1546-1552.

MacDonald B.R., Mundy G.R., Clark S., Wang E.A., Kuehl T.J., Stanley E.R. and Roodman G.D. (1986). Effects of human precombinant CSF-GM and highly purified CSF-1 on the formation of multinucleated cells with osteoclast characteristics in long-term bone marrow cultures. J. Bone Miner. Res. 1, 227-233.

Maeda H., Kukita T., Akamine A., Kukita A. and Iijima T. (1994). Localization of osteopontin in resorption lacunae formed by osteoclast-like cells: a study by a novel monoclonal antibody which recognizes rat osteopontin. Histochemistry 102, 247-254.

Matsumoto H.N., Tamura M., Denhardt D.T., Obinata M. and Noda M. (1995). Establishment and characterization of bone marrow stromal cell lines that support osteoclastogenesis. Endocrinology 136, 4084-4091.

Mbalaviele G., Chen H., Boyce B.F., Mundy G.R. and Yoneda T. (1995). The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow. J. Clin. Invest. 95, 2757-2765.

Mcsheehy P.M.J. and Chambers T.J. (1986). Osteoblast-like cells in the presence of parathyroid hormone release soluble factor that stimulates osteoclastic bone resorption. Endocrinology 119, 1654-1659.

Minkin C. (1982). Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif. Tissue Int. 34, 285-290.

Miyauchi A., Alvarez J., Greenfield E.M., Teti A., Grano M., Colucci S., Zambonin-Zallone A., Ross F.P., Teitelbaum S.L., Cheresh D. and Hruska K.A. (1991). Recognition of osteopontin and related peptides by an αvß3 integrin stimulates immediate cell signals in osteoclasts. J. Biol. Chem. 266, 20369-20374.

Nelson P.N., Pringle J.A.S. and Chambers T.J. (1991). Production and characterization of new monoclonal antibodies to human osteoclasts. Calcif. Tissue Int. 49, 317-320.

Nesbitt S., Nesbit A., Herfrich M. and Horton M. (1993). Biochemical characterization of human osteoclast integrins. J. Biol. Chem. 268, 16737-16745.

Nicholson G.C., Moseley J.M., Sexton P.M., Mendelsohn F.A.O. and Martin T.J. (1986). Abundant calcitonin receptors in isolated rat osteoclasts. J. Clin. Invest. 78, 355-360.

Nijweide P.J., Vrijheid-Lammers T., Mulder R.J.P. and Blok J. (1985). Cell surface antigens on osteoclasts and related cells in the quail studied with monoclonal antibodies. Histochemistry 83, 315-324.

Oursler M.J. and Osdoby P. (1988). Ostoeclast development in marrow cultured in calvaria-conditioned media. Dev. Biol. 127, 170-178.

Oursler M.J., Li L. and Osdoby P. (1991a). Purification and characterization of an osteoclast membrane glycoprotein with homology to manganese superoxide dismutase. J. Cell. Biochem. 46, 219-233.

Oursler M.J., Collin-Osdoby P., Li L., Schmitt E. and Osdoby P. (1991b). Evidence for an immunological and functional relationship between superoxide dismutase and a high molecular weight osteoclast plasma membrane glycoprotein. J. Cell. Biochem. 46, 331-344.

Oursler M.J., Bell L.V., Clevinger B. and Osdoby P. (1985). Identification of osteoclast-specific monoclonal antibodies. J. Cell Biol. 100, 1592-1600.

Reinholt F.P., Hultenby K., Oldberg A. and Heinegard D. (1990). Osteopontin- a possible anchor of osteoclast to bone. Proc. Natl. Acad. Sci. 87, 4473-4475.

Rifkin B.R., Vernillo A.T., Kleckner A.P., Auszmann J.M., Rosenberg L.R. and Zimmerman M. (1991). Cathepsin B and L activities in isolated osteoclasts. Biochem. Biophys. Res. Commun. 179, 63-69.

Rodan G.A. and Martin T.J. (1981). Role of osteoblasts in hormonal control of bone resorption-A hypothesis. Calcif. Tissue Int. 33, 349-351.

Ross F.P., Chappel J., Alvarez J.I., Sander D., Butler W.T., Farach-Carson M.C., Mintz K.A., Robey P.G., Teitelbaum S.L. and Cheresh D.A. (1993). Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin αvß3 potentiate bone resorption. J. Biol. Chem. 268, 9901-9907.

Soriano P., Montgomery C., Geske R. and Bradley A. (1991). Targeted disruption of the c-*src* proto-oncogene leads to osteopetrosis in mice. Cell 64, 693-702.

Springer T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76, 301-314.

Stamper H.B. and Woodruff J.J. (1976). Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules. J. Exp. Med. 144, 828-833.

Streeter P.R., Rouse B.T.N. and Butcher E.C. (1988a). Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. J. Cell Biol. 107, 1853-1862.

Streeter P.R., Berg E.L., Rouse B.T.N., Bargatze R.F. and Butcher E.C. (1988b). A tissue-specific endothelial molecule involved in lymphocyte homing. Nature 331, 41-46.

Suda T., Takahashi N. and Martin T.J. (1992). Modulation of osteoclast

differentiation. Endocrine Rev. 13, 66-80.

Takahashi N., Akatsu T., Udagawa N., Sasaki T., Yamaguchi A., Moseley J.M., Martin T.J. and Suda T. (1988). Osteoblastic cells are involved in osteoclast formation. Endocrinology 123, 2600-2602.

Takahashi N., Udagawa N., Akatsu T., Tanaka H., Shionome M. and Suda T. (1991). Role of colony-stimulating factors in osteoclast development. J. Bone Miner. Res. 6, 977-985.

Tanaka S., Neff L., Levy J.B. and Baron R. (1995). Identification of a 120 KDa protein which associates with and requires c-*src* to be phosphorylated in osteoclasts. J. Bone Miner. Res. 10, s157 (Abstract).

Thomas S.M., Soriano P. and Imamoto A. (1995). Specific and redundant roles of Src and Fyn in organizing the cytoskeleton. Nature 376, 267-271.

Udagawa N., Takahashi N., Katagiri T., Tamura T., Wada S., Findlay D.M., Martin T.J., Hirota H., Taga T., Kishimoto T. and Suda T. (1995). Interleukin (IL)-6 induction of osteoclast differentiation depends on IL-6 receptors expressed on osteoblastic cells but not on osteoclast progenitors. J. Exp. Med. 182, 1461-1468.

Vaananen H.K. (1984). Immunohistochemical localization of carbonic anhydrase isoenzymes I and II in human bone, cartilage and giant cell tumor. Histochemistry 81, 485-487.

Väänänen H.K. and Parvinen E.K. (1983). High active isoenzyme of carbonic anhydrase in rat calvaria osteoclasts, immunohistochemical study. Histochemistry 78, 481-485.

Väänänen H.K., Karhukorpi E.-K., Sundquist K., Wallmark B., Roininen I., Hentunen T., Tuukkanen J. and Lakkakorpi P. (1990). Evidence for the presence of a proton pump of the vacuolar H^+-ATPase type in the ruffled borders of osteoclasts. J. Cell Biol. 111, 1305-1311.

Vaan Norden C.J.F., Vogels I.M.C. and Smith R.E. (1989). Localization and cytophotometric analysis of cathepsin B activity in unfixed and undecalcified cryostat sections of whole rat knee joints. J. Histochem. Cytochem. 37, 617-624.

Warshawsky H., Goltzman D., Rouleau M.F. and Bergeron J.J.M. (1980). Direct in vivo demonstration by radioautography of specific binding sites for calcitonin in skeletal and renal tissues of the rat. J. Cell Biol. 85, 682-694.

Wucherpfennig A.L., Li Y.-P., Stetler-Stevenson W.G., Rosenberg A.E. and Stashenko P. (1994). Expression of 92 KD type IV collagenase/gelatinase B in human osteoclasts. J. Bone Miner. Res. 9, 549-556.

Yoshida H., Hayashi S., Kunisada T., Ogawa M., Nishikawa S., Okamura H., Sudo T., Shultz L.D. and Nishikawa S. (1990). The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 345, 442-444.