Journal of Cellular Biochemistry 71:559–568 (1998)

Multiple Myeloma Cells and Cells of the Human Osteoclast Lineage Share Morphological and Cell Surface Markers

Judy Faust, Pamela Hunt, Sheila Scully, and Victoria Shalhoub*

Department of Pathology, Amgen, Inc., Thousand Oaks, California 91320–1789

Abstract This study demonstrates that the multiple myeloma cell (MMC) in its plasma cell form is morphologically indistinguishable from human osteoclast-like cells that form in culture when peripheral blood mononuclear cells (PBMCs) are plated at high density in serum containing medium. MM has been described as a disease of B-cell lineage, monoclonal immunoglobulin (Ig) producing cells with unique properties: MM precursor cells lodge in bone, where they proliferate and differentiate into plasma cell tumors. Then, by some mechanism, presumably involving cytokines, these cells mediate an increase in neighboring osteoclast numbers and activity, leading to excessive bone erosion and hypercalcemia. Three days after plating PBMCs, tartrate resistant acid phosphatase- (TRAP-) blasts as well as TRAP+ cells, each with an eccentric nucleus, appear in culture. By day 10, TRAP+, vitronectin+ (VR+) cells, appear to be morphologically indistinguishable from multiple myeloma plasma cells (MMPCs) on cytocentrifuge preparations. These cells are CD19- and CD38++, as are MMCs reported by others. Other surface markers are also shared. Furthermore, Ig mRNA is demonstrated in the cytoplasm of cells at 8 days by in situ hybridization with the IgG FcA3 sequence. This novel finding is not unusual, in light of reports, demonstrating non-B-lineage Ig-producing cells. Thus, this study raises some serious questions about the true nature of MMCs. J. Cell. Biochem. 71:559–568, 1998.

Key words: plasma cell; CD19; CD38; naphthol AS-D chloroacetate esterase; B cells

Multiple myeloma (MM) is described in humans as a disease of B-cell lineage. It is characterized by accumulation in the bone marrow of B plasma cells that produce monoclonal immunoglobulin (Ig), osteolytic lesions, and hypercalcemia [Canellos, 1991; Jandl, 1996]. The loss of bone with disease progression results from increased osteoclastic bone resorption and eventual decreased bone formation [Bataille et al., 1991]. Multiple myeloma plasma cells (MMPCs) are believed to mediate increased numbers and activity of osteoclasts via MMPC, stromal, and hematological cell cytokine production [Torcia et al., 1996; Caligaris-Cappio et al., 1991; Bergui et al., 1993; Rettig et al., 1997] and are always found in close proximity to areas of osteoclastic bone destruction [Jandl, 1996; Barlogie et al., 1989]. Inhibitors of bone resorption, bisphosphonates [Greipp et al., 1985; Jandl, 1996; Berensen et al., 1996], and calcitonin [Rico et al., 1990] prevent and protect against the progression of osteolytic lesions. Several genetic lesions have been associated with MM, but to date the sequence of oncogenic events leading to the MM cell has not been identified [Zubler, 1997; Hallek et al., 1998].

MM is characterized by several unique properties. MM precursor cells home to the skeleton, and tumor masses are first seen within the bone marrow. The number of MMPCs in marrow correlates with the extent of bone destruction and osteoclast numbers [Thiele et al., 1988]. Abnormalities are rarely seen in plasma cells from the secondary lympoid organs, lymph nodes, and spleen and never in the gut, a rich source of plasma cells in humans [Lennert and Mohri, 1978]. MMPCs are CD19- and CD56+, whereas normal plasma cells from various tissues are CD19+ and CD56- [Harada et al., 1993, 1996].

Moreover, interleukin 1β (IL-1β, one of the most potent osteoclast activating factors, is produced by MMPCs [Lichtenstein et al., 1989] and found to be the major activity in conditioned medium of unfractionated myeloma bone

*Correspondence to: Victoria Shalhoub, Amgen, Inc., 15-2-B Amgen Center, Thousand Oaks, CA 91320–1789.

Received 26 June 1998; Accepted 1 July 1998

marrow cells [Torcia et al., 1996]. Furthermore, the hematological markers displayed by MMPCs are not restricted to one lineage and include erythroid, myeloid, and lymphoid markers [Ruiz-Arguelles and San-Miguel, 1994].

Recently we have followed the development of cells adopting the osteoclast phenotype (mono-, di-, or multinucleated, TRAP+, VR+, cathepsin K+, calcitonin receptor +) from precursor cells in normal peripheral blood mononuclear cells (PBMCs) in vitro on plastic and on bone [Faust et al., in press]. Within a few days of simply feeding with α minimum essential medium (αMEM) + 10% fetal bovine serum (FBS), almost homogeneous cultures of osteoclast-like cells, demonstrated by several criteria, are generated. Cells of the osteoclast lineage on cytocentrifuge preparations at different stages of development are morphologically indistinguishable from multiple myeloma cells (MMCs). They also appear in culture, at the same time after plating, as MMCs, identified in PBMC cultures from MM patients, by 6 days [Bergui et al., 1989]. Furthermore, elevated numbers of osteoclast precursors were found in the peripheral blood of MM patients [Gregoretti et al., 1995]. An analysis of MMPC, normal plasma cell, and osteoclast markers reveals that MMPCs alter their expression of several typical plasma cell markers (for example, loss of CD19) with the onset of the disease and adopt a profile of markers characteristic of not only MM cells but osteoclast-like cells as well.

MATERIALS AND METHODS

Tissue Culture and Cells

Peripheral blood mononuclear cells (PBMCs) obtained from HemaCare Corporation (Sherman Oaks, CA) by informed consent of volunteer donors, were Ficoll/Hypaque (F/H)-separated as described [Faust et al., in press]. The cells were cultured in αMEM + 10% FBS (Cansera, Rexdale, Ontario, Canada), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin sulfate at 1.5×10^6 cells/cm^2 in a 24-well plate and fed every 2–3 days by replacing half the medium during the first week and all the medium thereafter. At 3–14 days, cells were mechanically removed from the plates, in 1 ml medium supplemented with 100 µl of 10% FBS, by gentle scraping using a plastic pasteur pipette. Cytocentrifuge preparations were fixed with 2% paraformaldehyde for 10 min and stored at room temperature.

HS-Sultan (ATCC CRL-1484) was obtained from ATCC (Rockville, MD). This cell line was obtained from a plasmacytoma of a 56-year-old male Caucasian who had IgG multiple myeloma. Cells were grown in RPMI and 10% fetal bovine serum.

Histochemical Analyses

Cytocentrifuge preparations were stained with Wright's/Giemsa. Parallel slides were stained for naphthol AS-D chloroacetate esterase (CAE) and αnaphthyl acetate esterase (NSE) activity (Sigma kit) in the presence of 18 mM sodium fluoride (NaF) (Sigma, St. Louis, MO). Monocytes have a NaF-sensitive NSE (NaF-S-NSE). Resistant cells (Na-R-NSE) stain dark brown to black. Another set of slides was stained for the osteoclast enzyme marker, tartrate resistant acid phosphatase (TRAP) (Sigma kit). All procedures were executed according to the guidelines supplied by the manufacturer.

Sections of a plasma cell myeloma from the sacrum of a 48-year-old woman were kindly provided by Dr. J. Mirra (Orthopaedic Hospital, Los Angeles, CA). The formalin-fixed and paraffin-embedded sections are described as composed of sheets of neoplastic plasma cells. There are focal areas where they are mixed with lymphocytes. There are also areas where there is hematopoietic marrow. The sections were deparaffinized in three changes of xylene, rinsed in ethanol, in decreasing concentrations from 100 to 70%, and placed in water till needed. They were then stained for NaF-R-NSE and CAE (Sigma kit). Control cytocentrifuge preparations of osteoclasts grown in PBMC cultures were treated similarly. These patient MMPCs were also stained for TRAP (Sigma kit) and CAE (Sigma kit) or subjected to immunocytochemical characterization (see below). Photographs were obtained with a NIKON MICROPHOT-FXA microscope and NIKON DX-DB2 camera using 10, 20, and 40× objectives.

Immunocytochemistry

CD19 monoclonal antibody, IgG1 isotype control and immunostaining kits, and LSAB-2 (red reaction product), were purchased from Dako (Carpinteria, CA). CD38 monoclonal antibody was purchased from Becton Dickinson (Mountain View, CA). All procedures were followed according to the guidelines specified by the supplier. CD19 and CD38 antibodies and isotype controls were used at 10 µg/ml.

In Situ Hybridization

Cytocentrifuge preparations of osteoclasts from day 8 PBMC cultures were fixed with acid alcohol, 2% paraformaldehyde, or acetone. In situ hybridization was performed according to Wilcox [1993] using ^{32}P-labelled riboprobes corresponding to nucleotides 1–717 of the human IgG, FcA3 sequence [Simonet et al., 1997]. Slides were counterstained with hematoxylin and eosin and photographed using darkfield illumination. Digital images were captured using Adobe Photoshop software.

RESULTS

Morphology and Surface Markers of Osteoclast-Like Cells Arising in Peripheral Blood Mononuclear Cell Cultures

A typical mixed population of mononuclear cells, including monocytes and lymphocytes from peripheral blood, were isolated using F/H and cultured on plastic in αMEM + 10% FBS at high density (day 1). Three days later (day 4), there was evidence of massive cell destruction. Many mononuclear cells with vacuolated cytoplasm and broken cell membranes can be seen. At this time, there is the emergence of three distinct populations of cells, which include TRAP- blasts, TRAP+ cells with an eccentric nuclei, and mitotic cells [Faust et al., in press].

By day 8, the initial monocyte population was replaced by cells, each of similar morphology (with a "fried-egg" appearance and an eccentric nucleus that are NaF-R-NSE+, TRAP+ cells, and VR+), and smaller lymphocytes with smaller, round, dark blue–staining nuclei. The cells with eccentric nuclei are CD38+ (Fig. 1) and VR+ [Faust et al., in press]. They were also CD19- (not shown), as reported for osteoclast lineage cells from a human osteoclastoma [James et al., 1996].

By 15 days, these TRAP+, VR+, cathepsin K+ (unpublished observations) cells from PBMC cultures had differentiated into mono-, di-, or multinucleated cells with the appearance of plasma cells (Fig. 2), a subset of which resorbed bone by 22 days [Faust et al., in press]. At 15 days (Fig. 2A) (and earlier times [not shown]), cells of low nuclearity (mainly 1–3 nuclei) and multinucleated cells were found in the presence of larger multinucleated cells with hair-like projections. The latter cells were reminiscent of the giant multinucleated cells with "flaming borders" that were found in association with MMPCs in bone marrow biopsies, most prevalent in patients with IgA myelomas [Jandl, 1996; Canellos, 1991]). Figure. 2B shows that these cells with plasma-cell-like morphology at 18 days were TRAP+. In older cells, TRAP could be seen in cytoplasmic granules, consistent with its lysosomal location. Figure 2C demonstrates that these 18 day cells, with a plasma-cell-like morphology, had an NaF-R-NSE, demonstrating that they were not typical monocytes. Cells with this morphology were also NSE+ (not shown). These cells were also naphthol AS-D chloroacetate esterase–positive (CAE+), demonstrating that they were not plasma cells (see below).

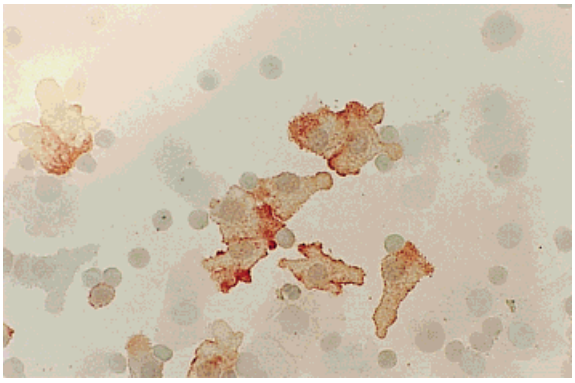

Fig. 1. CD38 antigen is expressed in preosteoclasts/osteoclasts generated from PBMCs at day 8 in culture. Preparations from the same experiment yield TRAP+, NaF-R-NSE+, VR+ cells of identical morphology, demonstrating an osteoclast phenotype. A parallel slide incubated with control IgG showed no staining (not shown). Not all cells of identical morphology are positive, likely due to cells in culture representing different stages of osteoclast development. 40×.

Naphthol AS-D Chloroacetate Esterase in Osteoclast-Like Cells and MM Cells

Tissue sections consisting of sheets of neoplastic plasma cells from the bone marrow of a MM patient were examined for CAE (Fig. 3A), an enzyme present in and cells of the myeloid lineage (myeloblasts, monocytes, and promonocytes) but absent in normal plasma cells, lymphoblasts, and lymphocytes (Sigma handbook, procedure 90). A positive red to orange enzyme reaction product was demonstrated in control 15 day osteoclast-like cells (Fig. 3B), generated from PBMCs in culture. CAE was present in many patient MMPCs (Fig. 3B). This indicated that CAE+ MMPCs acquired this enzyme, that CAE- MMPC were contaminated with CAE+

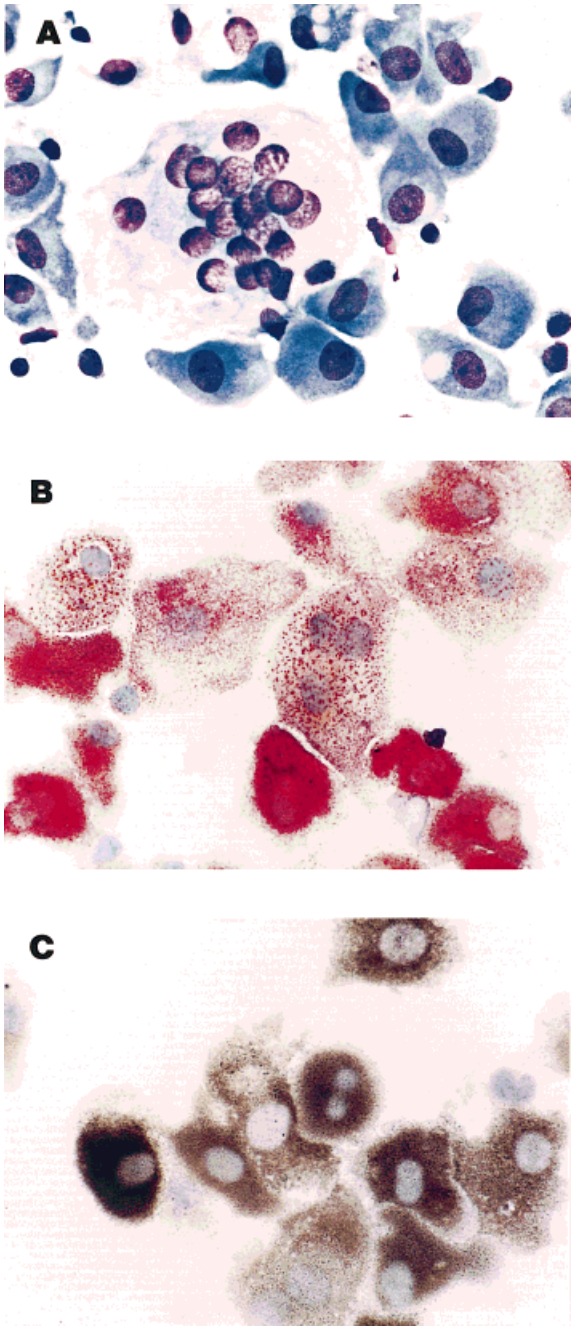

Fig. 2. Morphological and histochemical analysis of osteoclastic cells grown in culture for at least 15 days. **A:** Wright's/Giemsa staining reveals the coexistence of mononuclear cells with plasma cell morphology and multinuclear cells resembling "flaming cells" of multiple myeloma [Jandl, 1996] in 15 day cultures. **B:** Tartrate resistant acid phosphatase staining of plasma-cell-like osteoclasts formed in 18 day cultures. Note the presence of a positive-staining cell with three nuclei (arrow). **C.** The presence of sodium fluoride–resistant αnaphthyl-acetate-esterase (NaF-R-NSE) in plasma-cell-like cells in 18 day cultures. Note the presence of a very positive cell with two nuclei (arrow). 40×.

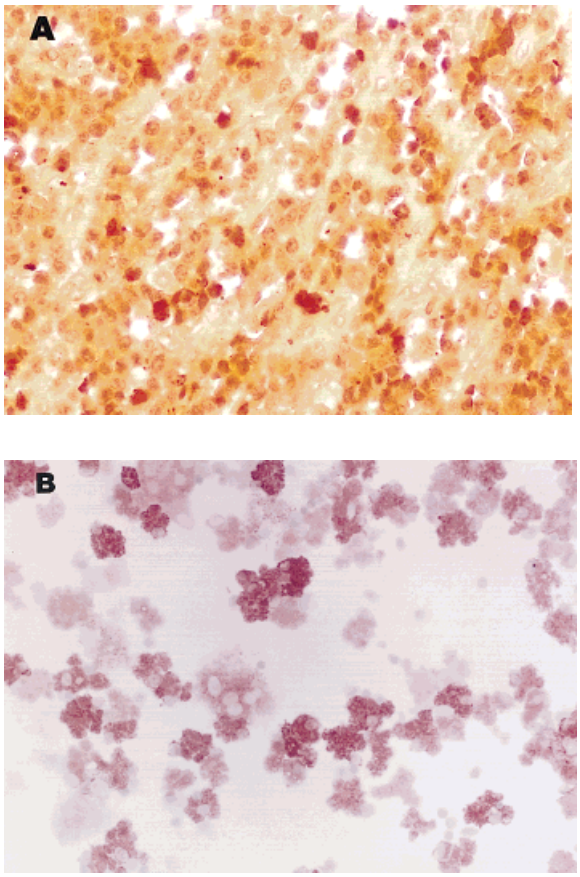

Fig. 3. Naphthol AS-D chloroacetate esterase (CAE) is positive in MMPC from patient bone marrow. **A:** MMPCs from patient bone marrow, demonstrate the presence of variable levels of CAE. **B:** CAE expression in osteoclast-like cells that develop from human PBMCs at day 15 in culture. A positive red reaction indicates the presence of CAE in human osteoclast-like cells. Negative T cells on the same slide serve as negative controls. 20×.

non-MM cells (which must be non lymphoid cells, since lymphoid cells are CAE-), or that CAE+ MMPCs were true MM cells, not of plasma cell origin. CAE staining was variable within cells of identical morphology in our cultures and probably reflected the stage of differentiation, as discussed for TRAP, Na-R-NSE, and VR [Faust et al., in press]. A positive control slide of mouse bone marrow cells demonstrated that the majority of the cells were, as expected, CAE+ granulocytes (not shown).

TRAP Expression in Patient MMCs and Osteoclasts

To determine if MMPCs from a MM patient express osteoclast marker proteins, tissue sections consisting of sheets of plasma cells from a

MM patient were assayed for the presence of TRAP (Fig. 4). The bright red mononuclear and large multinucleated TRAP+ osteoclasts were seen on the sections adjacent to bone (Fig. 4A),

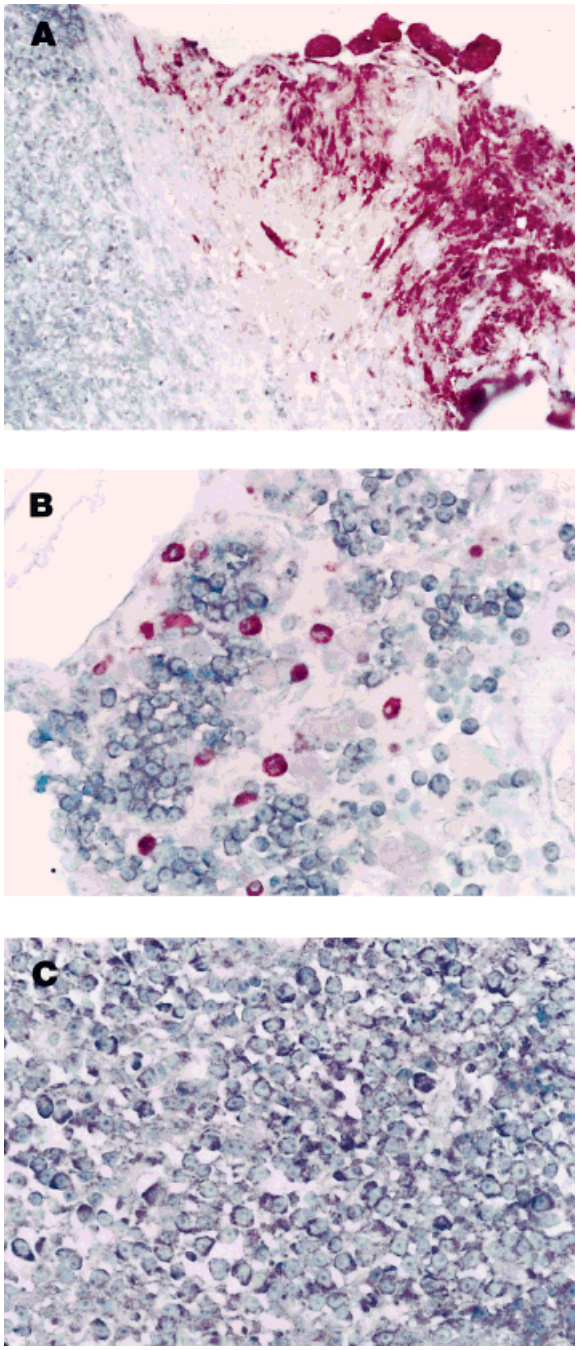

Fig. 4. The presence of TRAP in representative histological sections of MMPCs from an MM patient. **A:** A patient sample with some attached bone fragments demonstrates the presence of multinucleated osteoclasts near the bone surface (arrow). 10×. **B:** A higher power view of the area where MMPCs are mixed with mononuclear TRAP+ cells (arrow) and pale purple/pink-staining cells. 20×. **C:** MMPCs further away from the bone are negative for TRAP. 20×.

not further away (Fig. 4C). This was not unexpected, since TRAP is an inducible enzyme reported to be synthesized upon contact with a bone or plastic surface [reviewed in Drexler and Gignac, 1994; Baron et al., 1986]. Purple/pink staining in some mononuclear cells adjacent to bone could represent the activation of developing and/or mature osteoclasts (Fig. 4B) on sections stained for TRAP. MM cells near bone and further away were VR+ (not shown).

IgG mRNA Expression in Osteoclast-Like Cells in Culture

MMPCs are classified by their production of Ig protein and mRNA, characteristics of B cells. However, non-B-lineage cells are found that express Igs [Stavnezer et al., 1986; Akashi et al., 1991]. One report [Stavnezer et al., 1986] demonstrated mRNA and protein expression of Ig lambda light chain by the HL60 cell line—transformed cells of the myeloid lineage. Another report (Akashi et al., 1991) demonstrated PBMC-derived IgG-producing myelomonocytic cells (with lymphoid and myeloid antigens) from a patient exhibiting both myelomonocytic leukemia and MM. PBMCs from this patient in suspension culture produced colonies of macrophages and IgG+ plasmacytoid cells, while bone marrow mononuclear cells (BMMC) in long-term methylcellulose assays produced colonies of CD14+ myelomonoblasts, macrophages, and IgG+ plasma cells. The presence of osteoclasts in these cultures was not assessed. Hence, we examined the possible presence of IgG, FcA3 mRNA expression in our plasma-cell-like osteoclasts in culture at different times (8 days and 15 days) by in situ hybridization using preparations made from cell cultures by day 8 [Faust et al., in press]. At these times, few T and B lymphocytes remained (as demonstrated by CD3 and CD19 immunostaining of parallel preparations), and an abundance of cells with osteoclast phenotype were present (as demonstrated by 23c6 antibody, TRAP, NaF-R-NSE expression) [Faust et al., in press]. Antisense IgG, FcA3 probe (Fig. 5A) but not sense probe (Fig. 5B) hybridized to transcripts in the cytoplasm of a subset of cells with an eccentric nucleus and abundant cytoplasm in 8 day cultures (Fig. 5). The specificity of the reaction was demonstrated by the absence of antisense hybridization to transcripts in the T-cell population in these cultures. Patient MMPCs served as a positive control, demonstrating the expected presence of cytoplasmic transcripts (Fig. 5C).

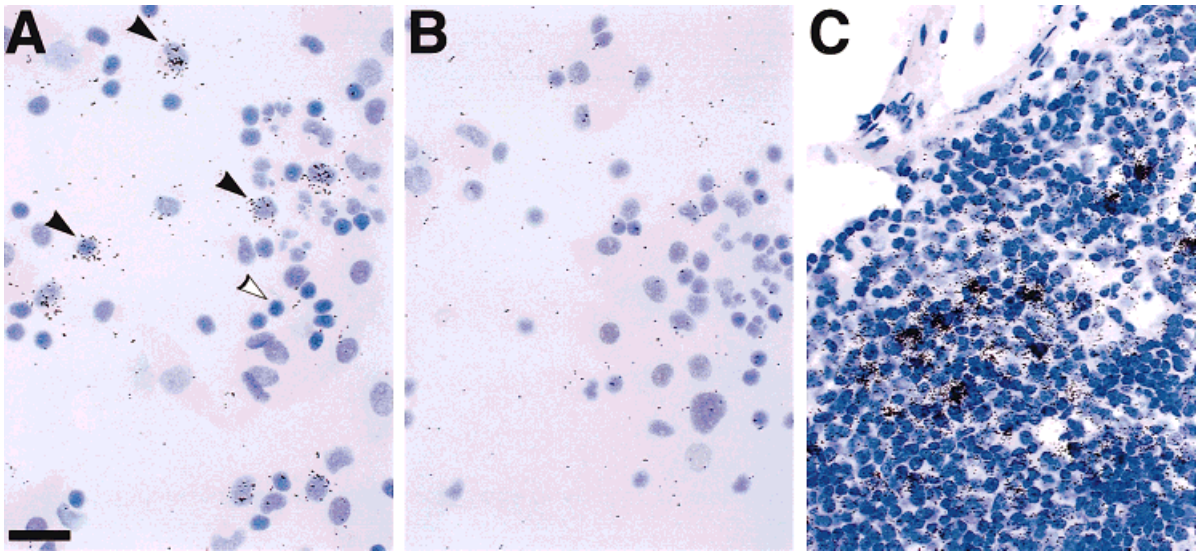

Fig. 5. The presence of IgG, FcA3 containing mRNA in cells from 8 day cultures by in situ hybridization. **A:** Antisense probe to IgG, FcA3 showing a positive reaction over the cytoplasm of cells with similar euchromatic nuclear morphology (dark arrowheads). The negative T cells are characterized by smaller, darker staining, round nuclei (white arrowhead). **B:** Sense probe demonstrates the absence of specific transcripts. **C:** Positive control slide showing labelling over MMPCs from an MM patient. Bar represents 25 μm.

Areas of heavy staining possibly represented transcripts over focal areas of lymphocytes (see Materials and Methods for a description of patient samples). However, mature osteoclasts on the patient sections as well as in the 15 day cultures were negative for IgG, FcA3 mRNA (not shown). This may relate to loss of expression as cells differentiate, as in the case of HL60s cells, where surface Ig lambda chains and lambda RNA were lost upon differentiation into monocytes/macrophages with phorbol ester treatment [Stavnezer et al., 1986].

TRAP- Blasts Appear Early in Osteoclast-Like Cell Cultures

Finally, we found that the morphology of the blasts that appear early in culture (Fig. 6A) and that of an MM cell line associated with the more aggressive form of the disease [Jandl, 1996] (HS-Sultan, ATCC-CRL 1484) (Fig. 6B) are indistinguishable: Both are large and round with nucleoli [Jandl, 1996]. The early appearance of these blasts precedes the accumulation of abundant (40% of cells in culture by day 15) TRAP+, VR+ cells in culture [Faust et al., in press].

DISCUSSION

This is the first report to compare the morphology of osteoclast-like cells on cytocentrifuge preparations with that of MM plasma cells. This comparison is based on our recently developed PBMC culture system, which has allowed characterization of a dominant population of adherent cells arising with osteoclast phenotype over a period of 2 weeks [Faust et al., in press]. In these cultures of normal PBMCs, osteoclast-like cells are morphologically indistinguishable from MMCs and appear within the same time frame as MMCs in cultures of MM PBMCs, albeit under slightly different experimental conditions of added cytokines [Bergui et al., 1989]. Although the precise relationship of the blasts to the fried-egg and plasma-cell-like cells with osteoclast features is yet to be determined, there is no doubt about the preosteoclastic/osteoclastic nature of the latter two cell types that appear concomitantly and/or subsequent to the appearance of blasts in culture [Faust et al., in press]. Furthermore, in the presence of the osteoclast maturation factor, osteoprotegerin ligand (OPGL/ODF/TRANCE/RANKL) [Simonet et al., 1997; Lacey et al., 1998; Matsuzaki et al., 1998], PBMC-derived osteoclast-like cells resorb more than 60% of

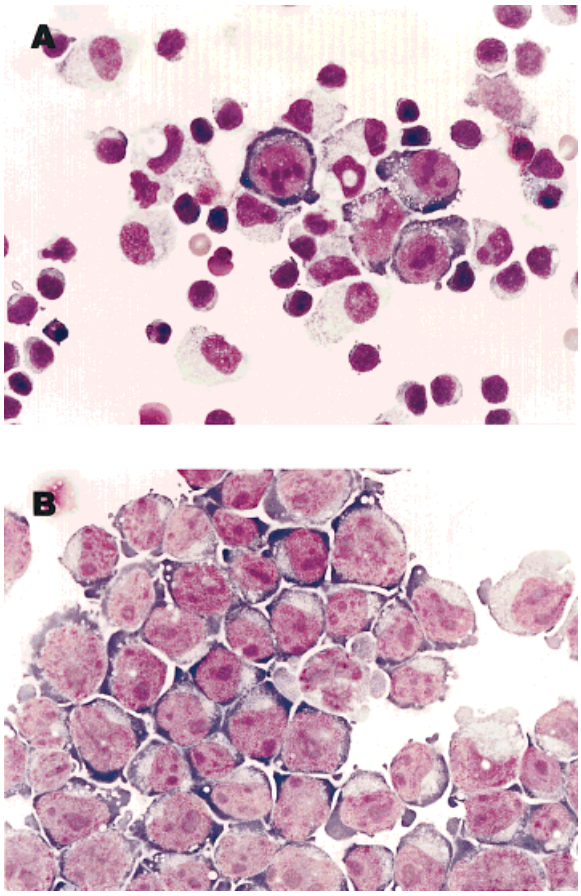

Fig. 6. Morphology of osteoclastic cells grown in culture from PBMCs. **A:** Wright's/Giemsa staining of PBMCs grown in culture for 3 days (day 4) showing the presence of a blast cell population among cells of heterogeneous nuclear morphology. **B:** Wright's/Giemsa staining of cells grown in culture from a MM cell line (HS-Sultan, ATCC CRL-1484) demonstrating identical blast cell morphology as in A. 40×.

bone slice surfaces and on cytocentrifuge preparations are identical in morphology and markers to the osteoclast-like cells grown in the absence of the factor in Figure 2 [Shalhoub et al., in press].

Several studies have demonstrated human osteoclasts of low nuclearity in vivo [van Riet and Van Camp, 1993] and in vitro [Caligaris-Cappio et al., 1991; James et al., 1996; Sarma and Flanagan, 1996]. Furthermore, mono- and polynuclear osteoclasts that were positive for I^{125} calcitonin in BM stromal cell cultures from MM patients are demonstrated but not compared with the plasma cells obtained from patient biopsies [Caligaris-Cappio et al., 1991].

To our knowledge, this is also the first report to examine the presence of CD19 and CD38 on osteoclast-like cells in culture. These markers have been used to define and select, respectively, MMPCs. In this report, we show that CD38 (an antigen on several cell types [Wijdenes et al., 1996]) is also present on osteoclast-like cells. Hence, BM cells selected with this antigen could be contaminated with or even enriched for cells of the osteoclast lineage. Osteoclast-like cells generated in vitro in this study are CD19-. This is consistent with a previous report that demonstrated CD19- human cells of the osteoclast lineage from an osteoclastoma [James et al., 1996]. However, normal plasma cells taken from various tissues are CD19+ [Harada et al., 1993]. Also, MMPCs are CD56+, while normal plasma cells are CD56- [van Riet and Van Camp, 1993]. Normal donor osteoclasts and MM osteoclasts stain weakly for CD56 [Gregoretti et al., 1994]. Both MMPCs and osteoclasts express very high levels of MMP-9 [Barille et al., 1997], although this is not an unusual marker for other transformed cell types. There are no reports of normal plasma cells expressing MMP-9. CD45 is reported to be present on immature and primitive MMCs as well as normal plasma cells [Schneider et al., 1997] and osteoclasts [Athanasou and Quinn, 1990].

Preliminary studies demonstrate IgG, FcA3 mRNA in osteoclast-like cell cultures by in situ hybridization (Fig. 5) and RNase protection assays (study in progress). Why these day 8 cells in culture express IgG, FcA3 mRNA is not known, since few B cells are present. Current studies are addressing this issue. It could be due to a common B cell/osteoclast progenitor that expresses lymphoid features before final commitment to the osteoclast lineage. The ability of the MMCs to synthesize IgG mRNA could relate to a transformation event at this stage before final commitment to the osteoclast lineage, as discussed for IgG expressing transformed HL60 cells. Rearranged genes have been proposed to be or to not be associated with the expression of IgG mRNA in the case of Ig producing HL60s [Stavnezer et al., 1986, and references therein]. The situation with respect to Ig producing normal cells in culture is not known. This point deserves further investigation.

Alternatively, these results could relate to the in vitro conditions. Nevertheless, whether normal osteoclast lineage cells in vivo do or do not express Igs has no bearing on the possible existence of neoplastic Ig producing osteoclasts.

In support of all these observations is the description of MMCs as expressing not only B-cell markers but several haematopoietic markers, including myelomonocytic, erythroid, and megakaryocytic markers, [references in Bakkus et al., 1995; Ruiz-Arguelles and San-Miguel, 1994]. Also, MMPCs are reported to be different from normal plasma cells in that they may produce lower levels of Ig [Werner-Favre and Barnet, 1993].

One other study supports the existence of Ig producing cells of the osteoclast lineage [Akashi et al., 1991]. The non-B-lineage CD14+, PB, myelomonocytic cells from a patient with both myelomonocytic leukemia and MM PBMC produced IgG in culture supernatant. Because the same rearrangement was found on Southern blots of a immunoglobulin heavy chain gene in both BMMC containing myeloma cells and myelomonocytic cells and CD14+ myelomonocytic cells in peripheral blood, it was concluded that common leukemic progenitors give rise to both myeloma and myelomonocytic leukemia cells, and this was attributed to lineage infidelity. The results of this study and the fact that macrophages and osteoclasts have a common progenitor support the possibility that the patient PBMC myelomonocytes, IgG+ plasmacytoid cells, BMMC myelomonoblasts, and IgG+ plasma cells belong to the same lineage.

Large multinucleated cells with ill-defined borders and hair-like projections are similar in morphology to the giant multinucleated cells with "flaming border" in MM [Jandl, 1996] and could represent non-bone-resorbing polykaryons [Sarma and Flanagan, 1996] produced by the fusion of aging mono- or polynuclear osteoclast-like cells or macrophages [reviewed in Connor et al., 1995; MacDonald et al., 1987; Zheng et al., 1991; Flanagan et al., 1992].

The presence of CAE in MMPCs of similar morphology on patient specimens (Fig. 5) is consistent with the staining characteristics of osteoclast-like cells (Fig. 6) in our cultures. These data imply that the MMPCs do not display the enzyme activity predicted for normal plasma cells. (It is also further confirmation that the cells generated in our PBMC cultures, shown to be cells of the osteoclast lineage by a variety of criteria, are not plasma cells).

It is also interesting that the cytokines that stimulate MMPC growth and that are produced in the bone marrow of MM patients (by MMPCs, stromal cells, activated T cells) are also major osteoclast activating factors—IL-1β, tumor necrosis factor β (TNFβ), IL-3, macrophage colony stimulating factor (M-CSF), IL-6 [Torcia et al., 1996; Caligaris-Cappio et al., 1991; Bergui et al., 1993].

Athough the above similarities between osteoclast-like cells and the MMCs may be circumstantial (in view of an as yet unidentified mechanism to explain the critical oncogenic events in MMPCs and the fact that non-B-lineage cells express Igs), these preliminary observations raise questions about the origin and precise nature of multiple myeloma cells.

In conclusion, characterization of cells at various stages of osteoclast development has led to the hypothesis that MM may be a disease of osteoclasts and/or their precursors. This hypothesis, if proven could explain several of the unique properties of MM cells that have puzzled researchers for years.

ACKNOWLEDGMENTS

We thank Dr. Colin Dunstan for review of this manuscript.

REFERENCES

Akashi K, Harada M, Shibuya T, Kimura K, Sagawa K, Yoshikai Y, Teshima T, Kikuchi M, Niho Y (1991): Simultaneous occurrence of myelomonocytic leukemia and multiple myeloma: Involvement of common leukemic progenitors and their developmental abnormality of "lineage infidelity." J Cell Physiol 148:446–456.

Athanasou NA, 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.

Bakkus M, van Riet I, de Greef C, van Camp B, Thielemans K (1995): The clonogenic precursor cell in multiple myeloma. Leukemia and Lymphoma 18:221–229.

Barille S, Akhoundi C, Collette M, Mellerin M-P, Rapp M-J, Harousseau J-L, Bataille R, Amiot M (1997): Metalloproteinases in multiple myeloma: Production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. Blood 90:1649–1655.

Barlogie B, Epstein J, Selvanayagam P, Alexanian R (1989): Plasma cell myeloma: New biological insights and advances in therapy. Blood 73:865–879.

Baron R, Neff L, Van PT, Nefussi J-R, Vignery A (1986): Kinetic and cytochemcial identification of osteoclast precursors and their differentiation into multinucleated osteoclasts. Am J Pathol 122:368–378.

Bataille R, Chappard D, Marcelli C, Dessauw P, Baldet P, Sany J, Alexandre C (1991): Recruitment of new osteoblasts and osteoclasts is the earliest critical event in the pathogenesis of human multiple myeloma. J Clin Invest 88:62–66.

Berensen JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, Lipton A, Keller A, Ballester O, Kovacs MJ, Backlock HA, Bell R, Simeone J, Reitsma DJ, Heffernan M, Seaman J, Knight RD (1996): Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. N Engl J Med 334:488–493.

Bergui FM, Gregoretti MG, Ghia P, Aimo G, Lindley IJD, Caligaris-Cappio F (1993): Cytokines involved in the progression of multiple myeloma. Clin Exp Immunol 92:27–31.

Bergui L, Schena M, Gaidano G, Riva M, Caligaris-Cappio F (1989): Interleukin 3 and interleukin 6 synergistically promote the proliferation and differentiation of malignant plasma cell precursors in multiple myeloma. J Exp Med 170:613–618.

Caligaris-Cappio F, Bergui L, Gregoretti MG, Gaidano G, Gaboli M, Schena M, Zallone AZ, Marchisio PC (1991): Role of bone marrow stromal cells in the growth of human multiple myeloma. Blood 77:2688–2693.

Canellos PC (1991) "Atlas of Diagnostic Oncology." New York, London: Gower Medical Publishing.

Connor JR, Dodds RA, James IE, Gowen M (1995): Human osteoclast and giant cell differentiation: The apparent switch from nonspecific esterase to tartrate resistant acid phosphatase activity coincides with the in situ expression of osteopontin mRNA. J Histochem Cytochem 43: 1193–1201.

Drexler HG, Gignac SM (1994): Characterization and expression of tartrate-resistant acid phosphatase (TRAP) in hematopoietic cells. Leukemia 8:359–368.

Faust J, Lacey DL, Hunt P, Burgess TL, Scully S, Van G, Eli A, Qian Y-X, Shalhoub V (in press): Osteoclast markers accumulate on cells developing from human peripheral blood mononuclear precursors. J Cell Biochem.

Flanagan AM, Horton MA, Dorey EL, Collins DA, Evely RS, Moseley JM, Firkin FC, Chambers TJ, Helfrich MH, Martin TJ (1992): An assessment of the ability of human bone marrow cultures to generate osteoclasts. Int J Exp Pathol 73:387–401.

Gregoretti MG, Gottardi D, Ghia P, Bergui L, Merico F, Marchisio PC, Caligaris-Cappio F (1994): Characterization of bone marrow stromal cells from multiple myeloma. Leuk Res 18:675–682.

Gregoretti MG, Bergui L, Aragno M, Cremona O, Marchisio PC, Caligaris-Capio F (1995) Osteoclast precursors circulate in the peripheral blood of patients with aggressive multiple myeloma. Leukemia 9:1392–1397.

Greipp PR, Raymond NM, Kyle RA, O'Fallon WM (1985): Multiple-myeloma: Significance of plasmablastic subtype in morphological classification. Blood 65:305–310.

Hallek M, Bergsagel PL, Anderson KC (1998): Multiple myeloma: Increasing evidence for a multistep transformation process. Blood 91:3–21.

Harada H, Kawano M, Huang N, Harada Y, Iwato K, Tanabe O, Tanaka H, Sakai A, Asaoku H, Kuramoto A (1993): Phenotypic difference of normal plasma cells from mature myeloma cells. Blood 81:2658–2663.

Harada Y, Kawano MM, Huang N, Mahmoud MS, Lisukov IA, Mihara K, Tsujimoto T, Kuramoto A (1996): Identification of early plasma cells in peripheral blood and their clinical signficance. Br J Haematol 92:184–191.

James IE, Dodds RA, Lee-Rykaczewski E, Eichman CF, Connor JR, Hart TK, Maleeff BE, Lackman RD, Gowen M (1996): Purification and characterization of fully functional human osteoclast precursors. J Bone Miner Res 11:1608–1618.

Jandl JH (1996) Multiple myeloma and related plasma cell dyscrasias. In Jandl JH (ed.): "Blood: Textbook of Hematology." New York: Little, Brown and Company, Vol 2, pp 1042–1097.

Lacey DL, Timms E, Tan H-L, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Caparelli C, Eli A, Qian Y-X, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ (1998): Osteoprotegerin (OPG) ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176.

Lennert K, Mohri N (1978): Histopathology and diagnosis of non-Hodgkin's lymphoma. In Lennert K, Mohri N, Stein H (eds.): "Malignant Lymphomas Other Than Hodgkin's Disease." Berlin: Springer-Verlag, pp 263.

Lichtenstein A, Berensen J, Norman D, Chang M-P, Carlile A (1989): Production of cytokines by bone marrow cells obtained from patients with multiple myeloma. Blood 74:1266–1273.

MacDonald BR, Takahashi N, McManus LM, Holahan J, Mundy GR, Roodman GD (1987): Formation of multinucleated cells that respond to osteotropic hormones in long term human bone marrow cultures. Endocrinology 120:2326–2333.

Matsuzaki K, Udagawa N, Takahashi N, Yamaguchi K, Yasuda H, Shima N, Morinaga T, Toyama Y, Yabe Y, Higashio K, Suda T (1998): Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. Biochem Biophys Res Commun 246:199–204.

Rettig MB, Ma HJ, Vescio RA, Pold M, Schiller G, Belson D, Savage A, Nishikubo C, Wu C, Fraser J, Said JW, Berenson JR (1997): Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. Science 276:1851–1854.

Rico H, Hernandez ER, Diaz-Mediavilla J, Alvarez A, Martinez R, Espinos D (1990): Treatment of multiple myeloma with nasal spray calcitonin: A histomorphometric and biochemical study. Bone Miner 8:231–237.

Ruiz-Arguelles GJ, San-Miguel JF (1994): Cell surface markers in multiple myeloma. Mayo Clin Proc 69:684–690.

Sarma U, Flanagan AM (1996): Macrophage colony-stimulating factor induces substantial osteoclast generation and bone resorption in human bone marrow cultures. Blood 88:2531–2540.

Schneider U, van-Lessen A, Huhn D, Serke S (1997): Two subsets of peripheral blood plasma cells defined by differential expression of CD45 antigen. Br J Haematol 97: 56–64.

Shalhoub V, Faust J, Boyle WJ, Dunstan CR, Kelley M, Kaufman S, Scully S, Van G, Lacey DL (in press): Osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) effects on osteoclast formation from human peripheral blood mononuclear cell precursors. J Cell Biochem.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang M-S, Luthy R, Nguyen HQ, Shimomoto G, DeRose M, Elliott R, Colombero A, Tan H-L, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Amgen EST program, Boyle WJ (1997):

Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309–319.

Stavnezer J, Al-Katib A, Koziner B (1986): Expression of immunoglobin lambda light chain by the promyelocytic cell line HL-60. J Immunol 137:3978–3982.

Thiele J, Arenz B, Klein H, Vierbuchen M, Zankovich R, Fischer R (1988): Differentiation of plasma cell infiltrates in the bone marrow. A clinicopathological study on 80 patients including immunohistochemistry and morphometry. Virchows Arch A Pathol Anat Histopathol 412:553–562.

Torcia M, Lucibello M, Vannier E, Fabiani S, Miliani A, Guidi G, Spada O, Dower SK, Sims JE, Shaw AR, Dinarello CA, Garaci E, Cozzolino F (1996): Modulation of osteoclast-activating factor activity of multiple myeloma bone marrow cells by different interleukin-1 inhibitors. Exp Hematol 24:868–874.

van Riet I, Van Camp B (1993): The involvement of adhesion molecules in the biology of multiple myeloma. Leukemia and Lymphoma 9:441–452.

Werner-Favre C, Barnet MT (1993): High IgE secretion capacity of human plasma cells. Eur J Immunol 23:2038–2040.

Wijdenes J, Vooijs WC, Clement C, Post J, Morard F, Vita N, Laurent P (1996): A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br J Haematol 94:318–323.

Wilcox JN (1993): Fundamental principles of in situ hybridization. J Histochem 41:1721–1723.

Zheng MH, Papadimitriou JM, Nicholson GC (1991): A quantitative cytochemcial investigation of osteoclasts and multinucleate giant cells. Histochem J 23:180–188.

Zubler RH (1997): Key differentiation steps in normal B cells and in myeloma cells. Semin Hematol 34:13–22.