

Identification of Osteoclast-specific Monoclonal Antibodies

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ABSTRACT Studies on the origin, identification, and characterization of osteoclasts have been difficult. This is in part due to a lack of definitive osteoclast markers and the similarity of these cells in form and function to cells of the mononuclear phagocyte system. To solve this problem, we inoculated isolated chick osteoclasts into mice to generate osteoclast-specific monoclonal antibodies. Supernatants from growth-positive hybridomas were screened by indirect immunofluorescent methods against cultured osteoclasts, monocyte-derived multinucleated giant cells, cultured monocytes, fibroblasts, and limb mesenchyme. Select hybridomas were cloned to produce 375 clones, which were analyzed as described above. Antibody from select clones was also reacted with paraffin sections of bone. In addition, two clones have been analyzed by enzyme-linked immunosorbent assay (ELISA) and Western blot analysis. Antibody binding from an osteoclast-specific clone and a clone reactive with osteoclasts, giant cells, and cultured monocytes (as determined by immunohistochemical assay) was confirmed by antibody-binding and titration curves quantitated by ELISA. The above studies demonstrate that osteoclast specific antigens exist, and that osteoclasts, giant cells, and cultured monocytes share common determinants not found on other cells screened.

The multinucleated osteoclast serves as the major cell type responsible for degradation of bone matrix, and its importance in bone growth, skeletal remodeling, and electrolyte homeostasis has been well established (1–3). The mechanisms and regulation of osteoclast-mediated bone resorption are not yet clearly understood; moreover, little is known about the recruitment and differentiation of osteoclast precursors (4, 5). It is generally believed that osteoclasts originate from cells belonging to the mononuclear phagocyte system (6–8). Although different, macrophages and osteoclasts share certain similarities in form and function, including membrane folding (9), positive acid phosphatase staining, activity of other lysosomal enzymes, and the ability to degrade connective tissue (2, 10). Chick-quail chimera studies (11, 12) have demonstrated a blood-borne origin for the osteoclast, and parabiosis and marrow transplantation have been successfully used to correct the osteoclast defect in osteopetrosis (13, 14). Together, these observations support the hypothesis that osteoclasts are polykaryons that derive from circulating mononuclear cells belonging to the monocyte-macrophage family.

In various cell populations, a seemingly homogeneous phenotype can now be divided into functional subpopulations with monoclonal antibodies raised to unique cell surface components. In this regard, mononuclear phagocytes have been shown to play important roles as antigen-presenting cells (15), cytotoxic effector cells (16), and also as secretory cells

(17). Recently, it has been demonstrated by the use of monoclonal antibody technology that one cell type may not be responsible for all functions. In this context, Sun and Lohmann-Matthes (18) employed monoclonal antibodies to identify functional subpopulations of macrophages. One population was responsible for natural killer activity, one for lymphokine-induced macrophage cytotoxicity, and one for neither function. Raffe et al. (19) described a monoclonal antibody reactive specifically with antigen-presenting macrophages. Therefore, if osteoclasts were a specialized subpopulation of macrophages, they would share some surface antigens with macrophages but possess a unique set of osteoclast specific cell surface components. However, to date no distinguishing markers have been identified.

A concern of many workers who deal with osteoclast development and function is related to the definition of the osteoclast. This results, in part, from a lack of definitive phenotypic markers for this cell as well as its similarity to other cells of the mononuclear phagocyte series. Populations of osteoclasts have been relatively inaccessible for study, and in vitro macrophage systems have been employed as surrogates to identify factors that are involved in osteoclast activity (20, 21). Recently, we developed an osteoclast isolation procedure that permits investigation of the osteoclast in vitro (22). In addition, we have described a cell culture system in which peripheral blood monocytes, when cultured under high

initial cell density, fuse and form multinucleated giant cells (MNGC).¹ The MNGC, although in some ways similar to cultured osteoclasts, differ in cell surface appearance and in ultrastructure. As a consequence, we have postulated that they are closely related to osteoclasts, but represent a different phenotype.

In this report, we describe results obtained using osteoclast isolated to produce osteoclast-specific monoclonal antibodies. In these studies, antibodies produced by select hybridomas show a variable spectrum of cell reactivity when screened against osteoclasts, monocyte-derived MNGC, macrophages, monocytes, and other phenotypes. From the results, it appears that monoclonal antibodies can be used to recognize osteoclast-specific cell surface antigens, and, furthermore, that osteoclasts, MNGC, and monocyte-derived macrophages share common antigenic determinants.

MATERIALS AND METHODS

Osteoclast Isolation and Mouse Immunization: Osteoclasts were isolated using a modification of the method of Osdoby et al. (22). White leghorn chick hatchlings were fed a low calcium diet (Purina) for at least 10 d to elevate osteoclast number (23). Chicks were killed and tibias dissected free of surrounding muscle and connective tissue. Bones were placed in Tyrode's balanced salt solution, pH 7.4, split, and the marrow removed. Marrow free bones were rinsed in a Ca-Mg free Tyrode's and subsequently incubated in Ca-Mg free Tyrode's containing 0.5% trypsin (Worthington Biochemical Corp., Freehold, NJ) and 0.03% EDTA for 30 min at 37°C. Bones were then agitated gently to remove loosely adherent cells, they were transferred to fresh trypsin solution for an additional 15 min at 37°C, and the trypsin action stopped with two washes of Ca-Mg free Tyrode's containing 5% fetal calf serum. Thereafter, tibias were incubated in 0.1% neutral red prepared in Tyrode's to visualize osteoclasts on bone. After several rinses with Tyrode's, osteoclasts were detached from bones by Ca-Mg Tyrode's buffer streaming, and the cells suspended in minimal essential medium containing 15% fetal calf serum.

Cell suspensions were pelleted at 400 g for 5 min and resuspended in 50% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) prepared with Ca-Mg free Tyrode's. 40, 30, 20, and 10% Percoll fractions were sequentially overlaid on the 50% Percoll solution, and the gradients centrifuged in an HB-4 swinging bucket rotor at 400 g for 20 min at 22°C. The top layers containing osteoclasts and a population of mononuclear cells were harvested and rinsed in Ca-Mg-free Tyrode's. Cell suspensions were passed through 10- μ m mesh nylon screens that allowed the passage of small cells while retaining osteoclasts on the membrane. The nylon screens were then backwashed with minimal essential medium + 15% fetal calf serum and 70–90% enriched osteoclast cell populations were obtained.

Osteoclasts were placed in fresh minimal essential medium + 15% fetal calf serum and allowed to recover for 30 min before inoculating into mice. Approximately 2×10^6 osteoclasts per 0.1 ml of Tyrode's were injected into the retro-ocular sinus per mouse. This protocol was repeated every 2 wk until high antibody titers were achieved. Mice were then inoculated once more and 3 d later they were killed and their spleens harvested.

Fusion and Hybridoma Production: Spleen cells from immunized mice were fused with myeloma cells by the method of Galfre et al. (24). 50% polyethylene glycol 1500 (Fisher Scientific Co., Pittsburgh, PA) was used to fuse 2×10^8 spleen cells with 2×10^7 myeloma cells (25). Cells were transferred to 96-well tissue culture plates and maintained in Dulbecco's modified Eagle's medium containing 5% calf serum, 10% horse serum, 2% α -glutamine, 1 mM sodium pyruvate, and 1% antibiotic-antimycotic (Gibco Laboratories, Grand Island, NY) (CM medium). Cells were grown at 37°C in 92% air, 8% CO₂. After 24 h, the medium was changed to CM medium containing hypoxanthine, aminopterin, and thymidine. Medium was changed every third day until cells became confluent.

Initial Hybridoma Screening: Hybridoma growth-positive wells were screened to determine antibody specificity for osteoclasts. Isolated osteoclasts were inoculated into 24-well culture dishes containing 9-mm collagen coated coverslips and cell attachment was evident after 4 h at 37°C. Coverslips were then rinsed in Tyrode's balanced salt solution, pH 7.4, and fixed in 1% formaldehyde in phosphate-buffered saline, pH 7.4 (PBS). Hybridoma tissue

culture supernatants were then reacted with the cells for 1 h at 22°C, the coverslips were rinsed in PBS and reacted with fluorescein-conjugated anti-mouse IgG (Cappel Laboratories, Cochranville, PA) for 1 h at 22°C, and rinsed and mounted on slides. Positive and negative controls were used throughout all screening assays.

Hybridoma supernatants testing positive for osteoclasts were screened against day-6 cultured limb mesenchymal cells (26), monocytes cultured for 2 d, and monocyte-derived MNGC (22). In some cases hybridoma supernatants were screened against skin fibroblasts (27). All cell types were grown on or transferred to coverslips, allowed to attach for 4 h, and fixed and processed for antibody staining as already described.

Tissue Section Screening: Clonal tissue culture supernatants were concentrated by lyophilization to one-eighth their original volume and dialyzed against PBS. Paraffin sections of chick bone, liver, and whole 10-d chick embryos were prepared by the method of Saint-Marie (28), and fluorescent antibody staining was done as previously described. All clonal tissue culture supernatants were tested singly and, in some cases, in combination.

Production of Ascitic Fluid: BALB/c mice were primed by intraperitoneal injection of 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane) and two weeks later $2-5 \times 10^6$ hybrid cells from select clones were similarly injected. 7–14 d thereafter ascitic fluid was collected and centrifuged at 250 g for 10 min to remove cells and debris. Aliquots of ascites were snap frozen in liquid nitrogen and stored at -20°C until used.

Enzyme-linked Immunosorbent Assay: Ascites from two representative clones were analyzed by enzyme-linked immunoassay (ELISA). Cells were obtained as already described, spun down onto microtiter plates pretreated with poly-L-lysine, and fixed with 1% formaldehyde and 0.01% glutaraldehyde in PBS. After blocking with 1% BSA–10% horse serum, the cells were reacted with dilutions of ascites prepared in 1.0% BSA–10% horse serum in PBS for 1 h at 37°C. After a buffer wash, peroxidase-labeled affinity purified rabbit-anti-mouse IgG, prepared in 0.67% BSA-PBS, was added to all samples and allowed to react for 1 h at 37°C. Following several washings, the substrate ABTS (2,2-azinondi-C3-ethyl-benzthiazoline sulfonate) in cacodylate buffer containing hydrogen peroxide was added to all samples. After 1 h, the product formed was read at an absorption of 405 nm with a Dynatech ELISA reader (29). The absorbance reading was normalized against protein present (30). Similarly cell number was varied with a constant ascites concentration.

Western Blot Analysis: Whole cell homogenates of osteoclasts, MNGC, (10×10^6 cells) cultured monocytes, (25×10^6 cells), and limb mesenchyme (25×10^6 cells) were electrophoresed on 10% SDS polyacrylamide gels (31) and transferred to nitrocellulose using a Hoefer electrotransfer unit (Hoefer Scientific Instruments, San Francisco, CA). The method of Burnette (32) was used to transfer and visualize antigenic components to 121F antibody.

RESULTS

Cell Isolation and Culture

The procedure for osteoclast isolation used in these studies yields cell preparations enriched 70–90% for the bone resorbing cells. Typically 1×10^6 osteoclasts are obtained from each bird, routinely 10^7 osteoclasts are harvested per preparation and ~75% of isolated osteoclasts attach to culture dishes within 4 h after inoculation. The cells flatten out on culture dishes, remain multinucleated, exhibit multiple microtubular organizing centers (not shown), and display a plasma membrane profile with numerous processes and microvilli projecting from the surface (Fig. 1A). Osteoclasts cultured on collagen-coated surfaces do not spread out but maintain a rounded configuration comparable to that observed on the bone surface (Fig. 2A). Aside from cell shape, these cells are essentially identical morphologically to those cultured in the absence of collagen.

Peripheral blood monocytes isolated by Ficoll-hypaque separation techniques and seeded at high initial cell densities attach to culture dishes within 24 h. These cells fuse in culture and by day 6 MNGC predominate (Fig. 1B). Although possessing multiple nuclei, these cells lack the ultrastructural complexity of isolated cultured osteoclasts (22). For example, MNGC have fewer mitochondria and polysomes, and, when grown under identical culture conditions to osteoclasts,

¹ Abbreviations used in this paper: ELISA, enzyme-linked immunosorbent assay; MNGC, multinucleated giant cells; UV, ultraviolet.

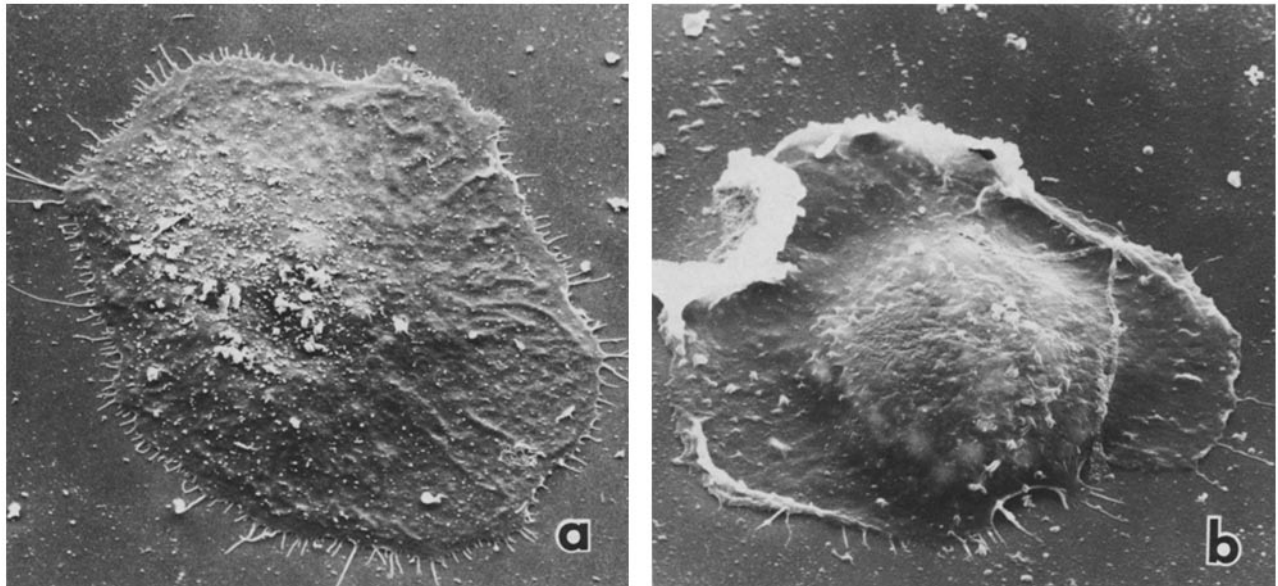


FIGURE 1 (a) Scanning electron micrograph of isolated osteoclast cultured for 6 d on tissue culture plate. Note small microvilli projecting up from cell surface and numerous processes radiating off the plasma membrane. $\times 1,000$. (b) Scanning electron micrograph of monocyte-derived MNGC cultured for 6 d under same conditions of cell in a. Note membrane ruffling but fewer membrane processes and microvilli in comparison to cultured osteoclast. $\times 1,350$. (Scanning electron micrographs prepared as outlined in reference 29.)

MNGC do not exhibit the numerous microvilli and cell processes that are found on the cell surface of cultured osteoclasts.

Hybridoma Clonal Analysis

Select hybridomas from fusion 1 were cloned, generating 108 clones, similarly 267 clones were derived from fusion 2. Fig. 2, *A* and *B*, are photomicrographs of osteoclasts grown on collagen-coated coverslips and reacted with antibody produced by clone 121F. Fig. 2*B* is the same field as Fig. 2*A* but viewed with ultraviolet (UV) illumination to detect fluorescent antibody. Osteoclasts exhibit intense fluorescence while the small cells and matrix display background fluorescence. Likewise, flattened osteoclasts not grown on collagen stain positive with antibody, although the antibody localization is not evenly distributed over the cell surface (Fig. 2, *C* and *D*).

Of the 375 clones that were produced and tested, 30 clones from 21 primary hybridomas elaborated osteoclast-specific antibodies (including clone 121F), and 10 additional clones produced antibody reactive with osteoclast and MNGC only. 10 other antibody-producing clones reacted with MNGC and cultured monocytes only, whereas antibody from five other clones were reactive with osteoclast MNGC, and cultured monocytes (including clone 29C). It was possible that primary clones are not true clonal populations but rather heterogeneous hybridoma populations producing more than one antibody species. Such a possibility could account for cross reactivity between phenotypes. However, recloning of these primary clones have not altered their cell reactivity pattern.

Tissue Section Screening

Antibody from 107 clones were reacted with paraffin sections prepared from low calcium chick bones. 37 clones produced antibody that reacted only with osteoclasts and gave fluorescent staining intensity comparable to that observed in isolated cells. This specificity was determined by examining

the endosteal surface of bones with intact marrow cavities (Fig. 3, *A* and *B*) or the periosteal surface where osteoclasts and periosteal fibroblasts are present (Fig. 3, *C* and *D*). In both cases, the fluorescent pattern in tissue sections was localized to osteoclasts when antibody from clone 121F was used. Rarely, a few mononucleated or binucleated cells in the marrow demonstrated weak fluorescence. These cells were usually closely associated with osteoclasts. Moreover, in areas of cartilage hypertrophy and resorption multinucleated cells (chondroclasts) displayed positive fluorescent antibody binding with antibody from clone 121F (Fig. 4, *A* and *B*). The cartilage cells and matrix exhibited no antibody reactivity and in other sections not shown peripheral muscle tissue around bone was also nonreactive with 121F antibody.

An additional set of clones demonstrated antibody reactivity that was specific for osteoclasts but exhibited weak fluorescence. 32 clones displayed antibody binding to osteoclasts and foci of marrow cells (Fig. 4, *C* and *D*) and an additional 14 clones were nonspecific. In osteoclast-specific clones tested against liver sections there was no reactivity evident and in whole embryos displaying a wide spectrum of tissue types the only reactivity of 121 antibody was associated with osteoclasts in bone.

ELISA

To corroborate the immunohistochemical observations on antibody specificity and cell reactivity antibody dilution curves were generated using a more sensitive peroxidase ELISA. Two antibody-producing clones were used in these studies: an osteoclast-specific clone, 121F9, and a clone-producing antibody 29C, that is reactive with osteoclast, MNGC, and cultured monocytes. Fig. 5*A* illustrates the results of the antibody dilution curve for clone 121F9. It is clear that with increasing concentrations of ascites from 121F9 there is a concomitant increase in antibody binding to osteoclasts. However, saturation levels were not obtained at these

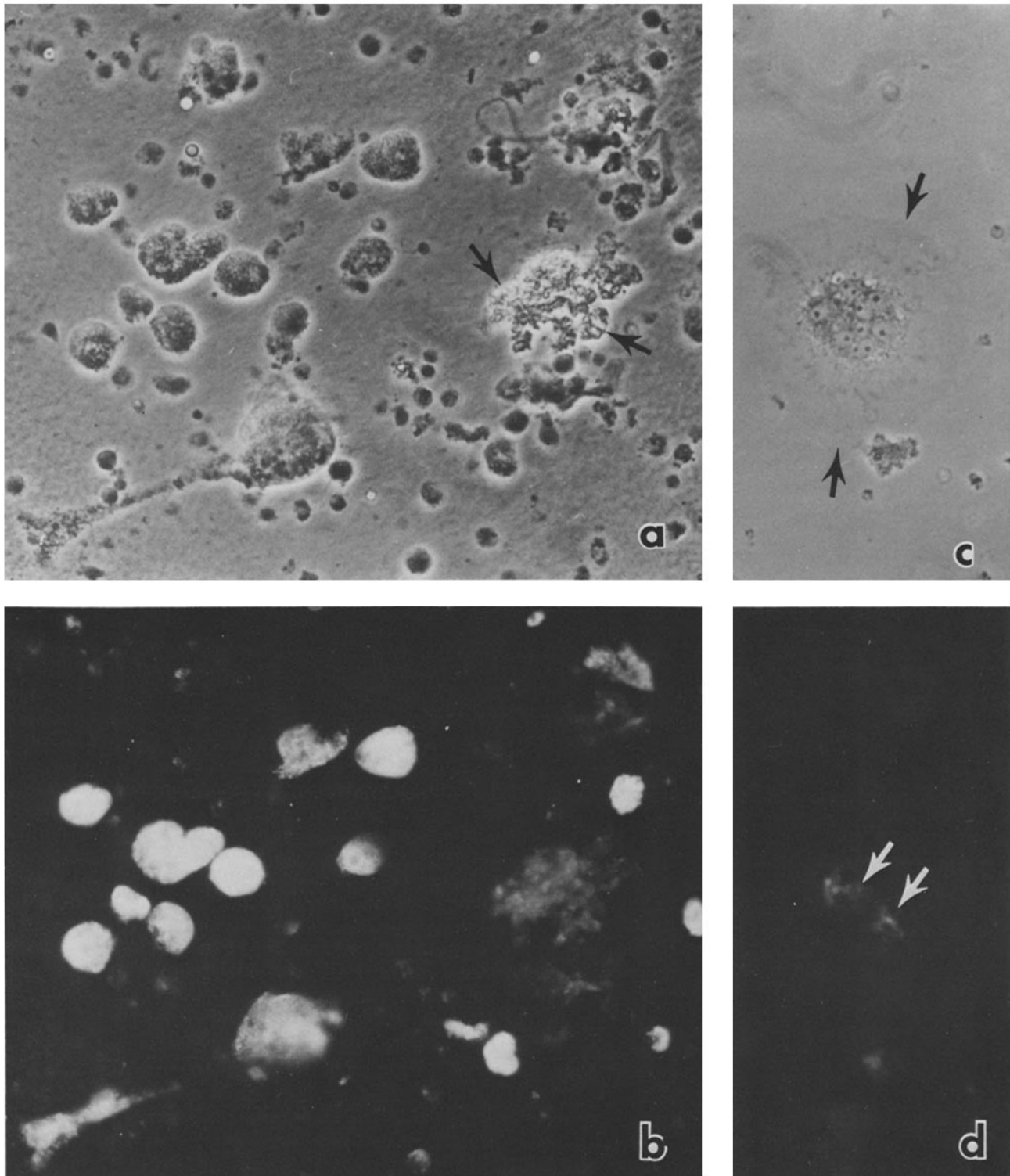


FIGURE 2 (a) Phase-contrast photomicrograph of osteoclasts and smaller cells plus matrix debris (arrows) grown on collagen-coated coverslips and reacted with 121F antibody followed by fluoresceinated anti-mouse IgG. Note elongated osteoclast. Small cells are intact or represent fragments of damaged osteoclasts. $\times 273$. (b) Same field as a viewed by UV illumination to illustrate fluorescent staining on osteoclasts and a few smaller cells or fragments. Most small cells are negative for antibody binding. $\times 273$. (c) Osteoclast grown on glass coverslip without collagen to illustrate flattened morphology and multinucleation. $\times 273$. (d) Same field as c visualized by UV illumination to detect antibody binding unevenly distributed on cell surface (arrows). $\times 273$.

concentrations. It is also apparent that day-2 cultured monocytes and limb mesenchymal cells exhibit low level binding at all concentrations of ascites—levels representative of non-specificity. The MNGC antibody-binding curve shows a pattern similar to that observed with other nonosteoclastic cells.

However, there is a slight increase in binding at the 1:4,000 ascitic dilution. The significance of this observation remains to be established.

The dilution curve for antibody produced by clone 29C is depicted in Fig. 5 B. ELISA confirms the immunohistochem-

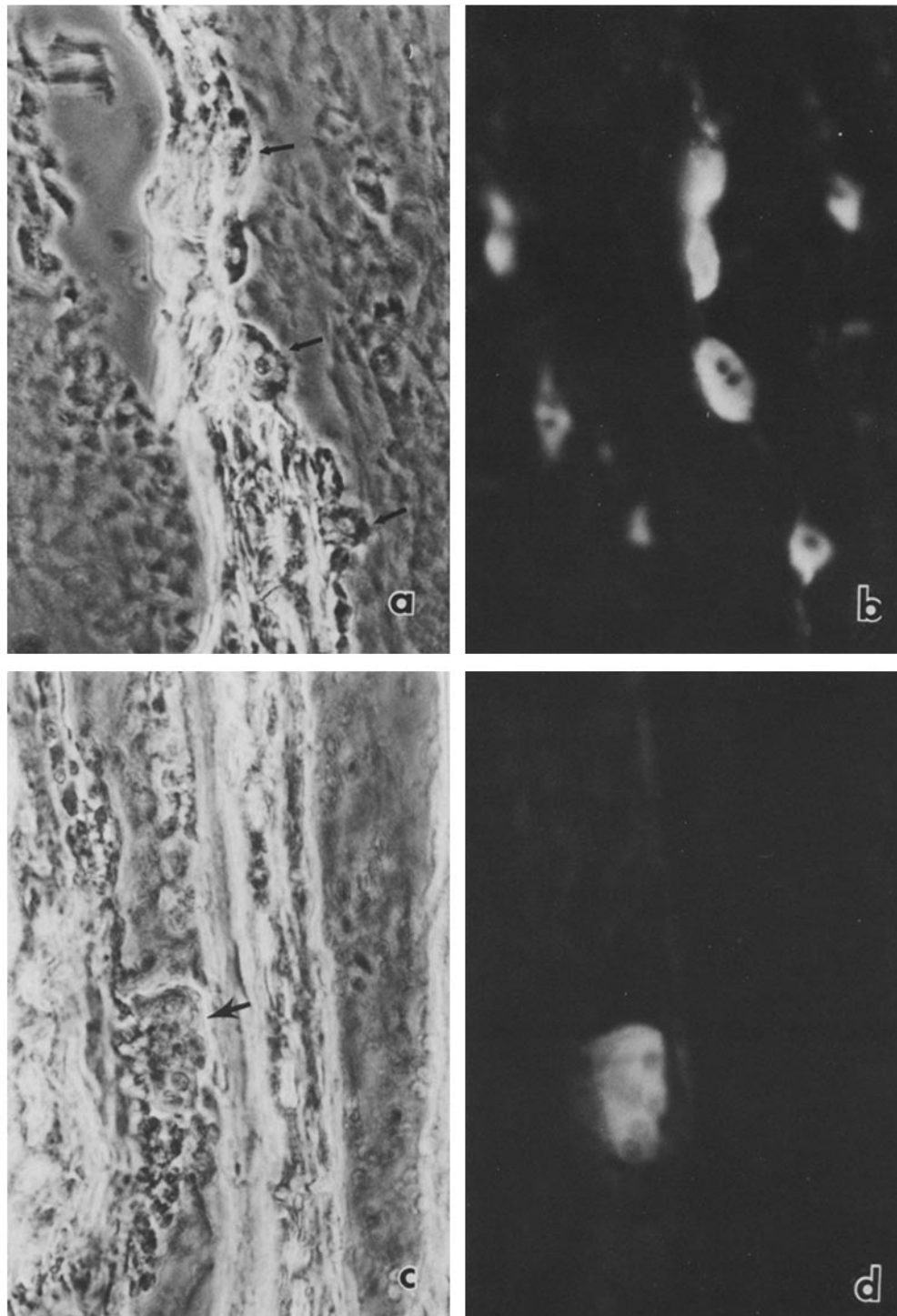


FIGURE 3 Phase-contrast photomicrograph of section from low calcium chick tibia illustrating osteoclasts (arrows) on body trabecula surrounded by marrow cells. $\times 420$. (b) Same field as a viewed with UV illumination to illustrate fluorescent pattern of 121F antibody binding to osteoclasts on body trabeculae and on tissue pulled away from bone surface. $\times 420$. (c) Phase-contrast photomicrograph of section from low calcium chick tibia in the periosteal region. One prominent multinucleated osteoclast (arrow) is evident. $\times 420$. (d) Same field as c viewed by UV illumination to illustrate antibody binding to osteoclasts from antibody testing osteoclast specific in cell analysis. Note negative image of nuclei in this micrograph and in b. $\times 420$.

ical observations. Antibody binding to osteoclasts and MNGC increase with increasing concentration of ascites. Like the immunohistochemical results, monocytes maintained in culture show an increase in antibody binding but the binding levels are greatly reduced. Limb mesenchymal cells do not

bind antibody from clone 29C.

In experiments in which cell number was varied, the 121F antibody exhibited increased binding with increase in osteoclast number. Minor elevation in antibody binding was detectable when other cell types were tested (Fig. 5C). The 29C

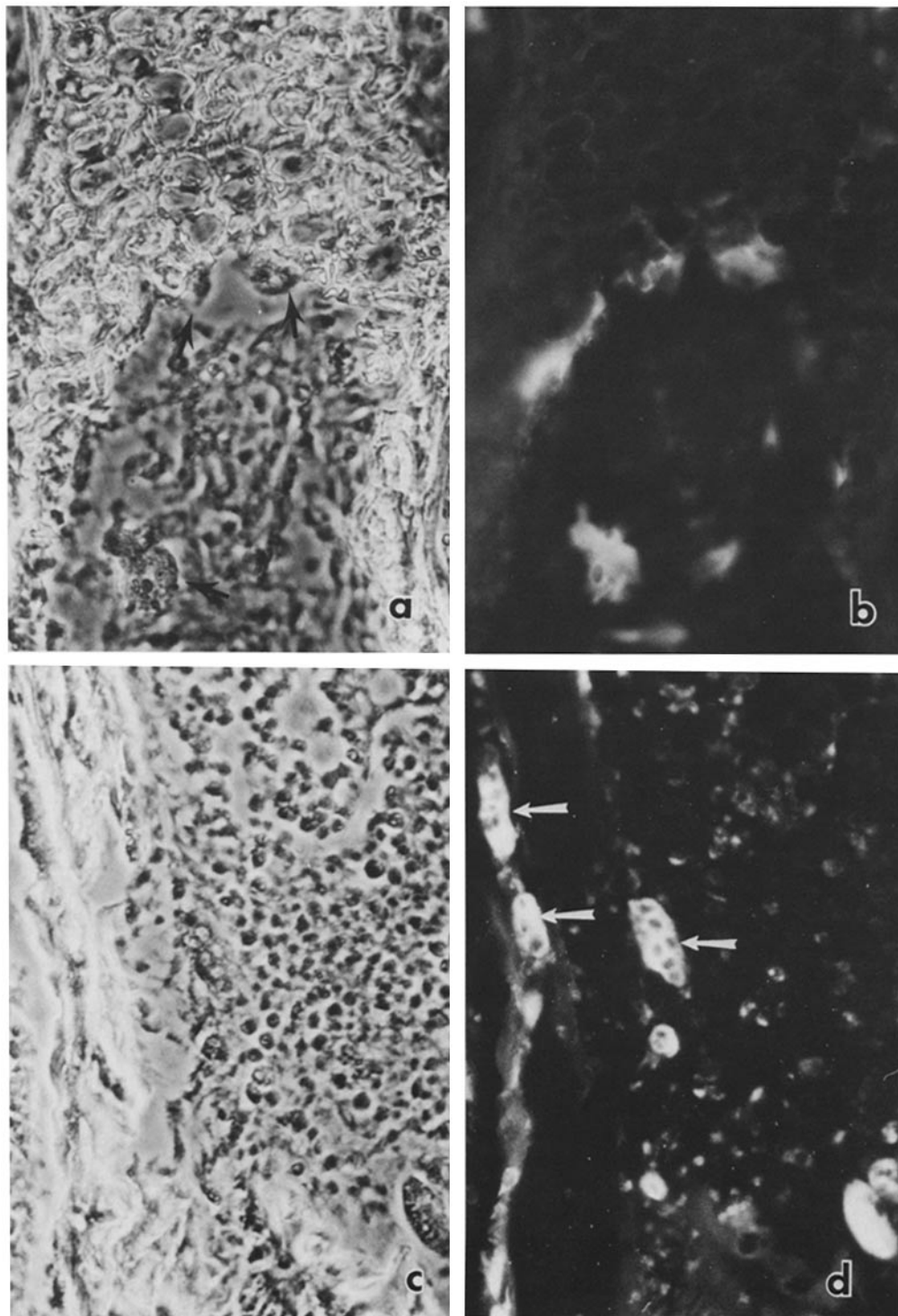


FIGURE 4 (a) Phase-contrast photomicrograph of tibia section from chick maintained on low calcium diet. Area of photomicrograph illustrates region of cartilage erosion by chondroclasts (arrows). Note multinucleated cell not attached to matrix (arrowhead). $\times 395$. (b) Same field as a viewed by UV illumination to reveal fluorescent pattern of 121F antibody binding to chondroclasts and multinucleated cell not associated with matrix. $\times 395$. (c) Phase-contrast photomicrograph of tibia section from chick maintained on low calcium diet. Bone surface is at the left and marrow makes up most of the field. $\times 490$. (d) Same field as c viewed by UV illumination to reveal fluorescent pattern of 29C antibody binding to osteoclasts (arrows) and foci of marrow cells. $\times 490$.

antibody binding to cells rose relative to increases in osteoclast and MNGC number. However, no binding was detectable on cultured monocytes or limb mesenchyme at the ascites concentration used (1:8,000) (Fig. 5D).

Western Blot Analysis

Whole cell homogenates electrophoresed on 10% SDS polyacrylamide gels, transferred to nitrocellulose, and reacted with

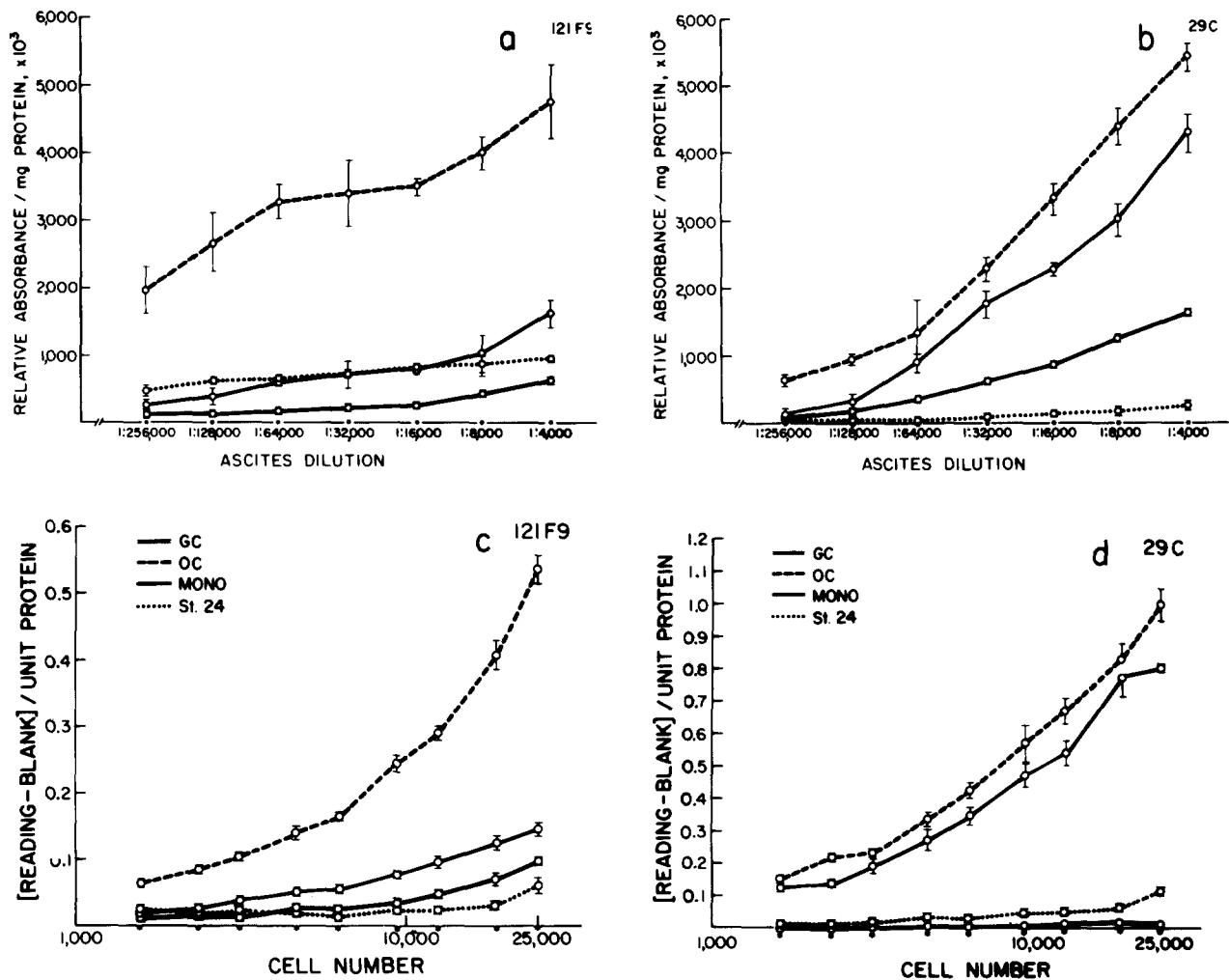


FIGURE 5 (A) Ascites dilution curve for ascites produced from 121F9 hybridoma. The cell number was constant, i.e., 25,000, and the ascites concentration was changed. Broken line represents values of osteoclasts, double line represents values for MNGC, solid line represents cultured monocytes, and dotted line represents values obtained with cultured limb mesenchyme. Data is represented as absorbance per milligram protein and bars represent standard deviation. (B) Ascites dilution curve for ascites produced from 29C hybridoma. (C) Ascites concentration was kept constant and cell number varied for analysis of 121F antibody binding. Data indicate correlation with increase in cell number and 121F9 antibody binding to osteoclasts. (D) Cell number varies for analysis of 29C antibody binding. Osteoclast and MNGC binding increased with increase in cell number.

121F antibody reveal results consistent with the immunohistochemical cell screening and ELISA data. The osteoclast lane displays antibody recognition of antigen possessing apparent molecular weights of 96,000, 91,000, and 45,000. The relationship of these components has yet to be determined. No antigen was recognized in the MNGC, cultured monocytes, or mesenchymal cell lanes (Fig. 6), although the protein staining pattern showed much more protein in these lanes compared to the osteoclasts (not shown).

DISCUSSION

In this report we detail the results of experiments with osteoclast-specific monoclonal antibodies that were generated in response to chick osteoclasts as immunogen. Immunohistochemical methods and ELISA have been successfully used to identify hybridomas producing antibodies to osteoclasts. Moreover, cell and tissue screening protocols have enabled us to select and identify antibody-producing hybridomas that synthesize antibody to osteoclast antigens not detectable on the closely related monocyte, macrophage, MNGC, or marrow cell. Other hybridomas derived from the same spleen

population have been shown to produce antibody cross-reactive with osteoclasts, MNGC, and cultured monocytes. These same antibodies react with osteoclasts and foci of marrow cells in bone sections while those antibodies specific for osteoclasts in cell isolates only recognize osteoclasts in tissue sections.

Western blot analysis has successfully identified the antigenic determinant to clone 121F. The Western blot pattern includes a 45,000-mol-wt component. However, in preliminary studies using immunoaffinity chromatography to purify 121F antigen, no 45,000-mol-wt species is found. Therefore, this component may represent a breakdown product from the 96,000- or 91,000-mol-wt species.

In the tissue sections of bone, antibody binding did not appear to be restricted to the cell surface but localized throughout the cell. Two explanations can account for this staining pattern. First, the antigenic component may be on the surface but not restricted to the membrane. Second, and in a related fashion, it is known that the osteoclast membrane involutes deep into the cytoplasm, and such membrane folding may account for the apparent cytoplasmic localization.

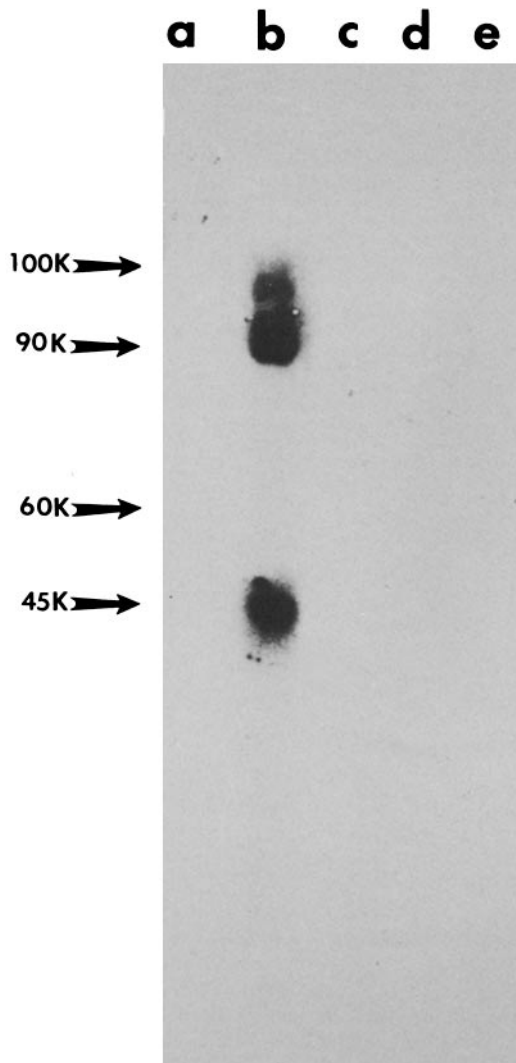


FIGURE 6 Western blot pattern of 10% SDS polyacrylamide gel transferred to nitrocellulose and reacted with 121F9 antibody and iodinated anti-mouse IgG. (Lane A) Molecular weight markers; (lane B) osteoclast homogenates; (lane C) MNGC homogenates; (lane D) monocyte homogenates; and (lane E) mesenchymal cell homogenates.

The localization of osteoclast-specific antibodies in bone sections was occasionally found to include a few mononuclear cells. These mononuclear cells have yet to be identified, but these cells may represent mononuclear osteoclasts, osteoclast progenitors, or even osteoclast fission products. In a number of reports, in which monoclonal antibodies have been raised to specific phenotypes, it has been found that the antibodies also recognize the immediate precursor population. This was shown to be the case for B-lymphocyte monoclonal antibodies (33).

These observations demonstrate that monoclonal antibodies to cell surface components can be used to identify osteoclasts. These cell surface markers can likewise serve as powerful tools to discriminate osteoclasts from other giant cells and mononuclear phagocytes and quantitate osteoclast numbers in bone. Perhaps as important, the pattern of hybridoma and clonal antibody reactivity to the phenotypes tested suggests that osteoclasts, MNGC, and monocyte-derived macrophages share antigenic determinants not found on other cells

screened. Such results support the hypothesis that osteoclasts, macrophages, and monocytes are in some way related. The osteoclast-specific and the phenotypic cross-reactive antibodies should prove to be valuable to help clarify the functional and developmental relationship between osteoclasts and other cells of the mononuclear phagocyte family.

Haythorn (34) suggested that osteoclasts were members of a family of MNGC found throughout many organs. He further suggested that the unique phenotypic features of the different multinucleated cells was dependent on local environmental factors affecting these cells. This concept is consistent with our present understanding of tissue macrophage heterogeneity (18, 19). Therefore, it is quite possible that the bone environment in some way imparts the unique phenotypic qualities associated with osteoclasts.

The bone environment is a complex milieu known to contain growth factors, and bioactive matrix components that individually or together help regulate fracture repair and bone remodeling (35). Components of bone have been shown to influence osteoclast precursor recruitment (36), but less is known in regard to how the bone environment influences osteoclast cytodifferentiation. The antibodies described in this report should now make it possible to more competently examine how specific components of the osseous environment influence osteoclast development.

Monoclonal antibody technology has been successfully used to identify phenotypes and discriminate subpopulations of closely related cell populations that it was not possible to segregate before. We have applied this technology to demonstrate that osteoclasts in all likelihood represent a unique MNGC that can be discriminated from other cells of the mononuclear phagocyte system.

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