American Journal of Pathology, Vol. 129, No. 3, December 1987

Retrovirus-Induced Osteopetrosis in Mice

Effects of Viral Infection on Osteogenic Differentiation in Skeletoblast Cell Cultures

JÖRG SCHMIDT, DVM, MARIA CASSER-BETTE, Dipl Biol, A. BEATRICE MURRAY, PhD, ARNE LUZ, MD, and VOLKER ERFLE, DVM

From the Abteilung für Molekulare Zellpathologie and Institut für Pathologie, Gesellschaft für Strahlen- und Umweltforschung (GSF), Neuherberg/München, Federal Republic of Germany

Newborn female strain NMRI mice were injected with a mouse retrovirus (OA MuLV) known to induce osteopetrosis. Primary skeletoblast cell cultures were established from humeri and calvaria of 3-day-old, 7-day-old, and 28-day-old animals. Infectious ecotropic MuLV was found in all humerus cultures from infected animals and in 7-day and 28-day calvaria cell cultures. Levels of alkaline phosphatase activity were markedly higher in cultures of calvaria and humeri from infected mice than in those from controls. *In vitro* infection of undifferentiated periosteal cells was followed by a decrease in cell growth and an increase in alkaline phosphatase activity. In contrast, differentiated osteoblast-like cells were barely susceptible to OA MuLV infection, and the virus did not influence their cell growth or differentiation. Electron-microscopic studies of skeletal tissue from infected old osteopetrotic mice showed virus particles associated with and budding from osteocytes and accumulated in devitalized osteocyte lacunae. The results indicate that progenitor cells of the osteoblastic lineage represent the target cells for OA MuLV in bone tissue, that virus infection induces an increase in osteoblastic activity, and that infected cells produce virus until full development of the disease. (Am J Pathol 1987, 129:503–510)

RECENTLY we isolated a murine leukemia virus, OA MuLV, from spontaneous osteomas in strain 101 mice,[1] which induces osteopetrosis, osteomas, and lymphomas after a latent period of 6–12 months. Several authors have observed that murine and avian leukemia viruses are capable of inducing skeletal changes such as new bone formation,[2,3] osteopetrosis, and osteomas.[4–8] The precise role played by retroviruses in the development of these skeletal anomalies is not yet clear, but it has been suggested that infection of osteoprogenitor cells may alter their differentiation program, leading to higher osteoblastic activity and increased cell growth.[9–12]

In previous studies we reported on virus production in osteoblasts and osteocytes in skeletal tissue of OA MuLV-infected newborn mice within 4 weeks after infection.[13] Here we report the results of an *in vitro* investigation using primary skeletoblast cell cultures from OA MuLV-infected osteopetrosis-prone mice and uninfected control mice.

The results suggest that OA MuLV primarily infects less differentiated precursor cells of the osteoblastic lineage, that infection induces increased expression of osteoblastic markers, and that infected cells produce virus until development of osteopetrosis.

Materials and Methods

Virus

OA MuLV was isolated from spontaneous osteomas in Strain 101 mice, cloned by three cycles of end-point dilution and further propagated in NIH 3T3 fibroblast cells. The biologic and molecular characterization of OA MuLV has been described elsewhere.[1,14] Fresh virus suspensions were filtered

Supported by EURATOM (Bio-D-366-81-D).
Accepted for publication July 6, 1987.
Address reprint requests to Dr. J. Schmidt, Abteilung für Molekulare Zellpathologie, Gesellschaft für Strahlen- und Umweltforschung (GSF), D-8042 Neuherberg /München, FRG.

through 0.22-μ Millipore filters before use. The virus titer was 1×10^5–5×10^6 infectious particles/ml.

Animals

Newborn female NMRI mice were obtained from the breeding colony of the Institut für Biologie, GSF. Fifteen newborn mice were given intraperitoneal injections of 2×10^5 infectious virus particles of OA MuLV (in 0.1 ml). Fifteen control littermates were injected with the same volume of Dulbecco's minimum essential medium (DMEM). Five infected and 5 control mice were killed 3, 7, and 28 days after injection. The spleens, humeri, calvaria, femurs, and lumbar vertebrae were removed aseptically and treated as described below. For long-term pathogenicity studies, 12 OA MuLV-infected and 14 control mice were checked on 6 days of the week over a maximum observation period of 2 years. Animals were killed when moribund and autopsied. Pathologic findings were evaluated as described previously.[1]

Cell Cultures

Humeri and calvaria were placed in cold medium, and single-cell suspensions were prepared for cell cultures. Humeri from 5 infected and 5 control mice were cleaned, freed from adherent connective tissue and muscle, pooled, and minced with scissors in 2 ml of DMEM. Tissues were digested three times (20 minutes each digest) at 37 C with trypsin/EDTA (0.05%/0.025%) in phosphate-buffered saline. After each digestion the released cells were collected by centrifugation, resuspended in DMEM, and seeded into 35-mm Petri dishes. The cells were cultured in DMEM and 10% fetal calf serum (FCS) GIBCO, Karlsruhe, FRG). Calvaria cells were obtained by fractionated dissociation (three times for 20 minutes each) in isotonic salt solution containing 0.2% collagenase (Sigma Chemical Co., St. Louis, Mo) and 0.1% hyaluronidase (Sigma). Cells obtained following each digestion (Fractions 1–3) were seeded into 60-mm Petri dishes and cultured in BGJ/b medium (GIBCO) with 10% FCS. NIH 3T3 mouse fibroblast cells and CCL-64 mink cells were cultured in DMEM and used to check for the presence of infectious virus particles in the supernatant of the humerus cell cultures.

Primary cultures of periosteal cells were prepared by scraping off the periosteal and endosteal cell layer of calvaria from untreated newborn NMRI mice, dissociating the cells as described above, and seeding them at a density of 10,000 cells/sq cm into 60-mm Petri dishes. Primary cultures of osteoblastlike cells were prepared by culturing the Fraction 2 cells (see above) obtained after dissociating the remaining part of the calvaria. The cells were cultured in DMEM supplemented with 10% FCS. All cell cultures were incubated in a humidified atmosphere with 5% CO_2. The medium was changed at 3-day intervals. Cell counts were carried out in a Coulter Counter Model ZM (Coulter Electronics, Hialeah, Fla).

Virus Detection

The presence of viral core protein p30 was used as a marker of virus infection in the cell cultures. The p30 was identified by the indirect immunoperoxidase method (IIP) as described by Nexø[15] with anti-OA MuLV p30 antibody raised in rabbits. Endogenous peroxidase activity was eliminated by preincubation with 0.005% H_2O_2 in methanol (30 minutes, room temperature). The level of infection was determined either by counting the number of infectious centers per dish or by dividing the number of p30-positive cells by the total cell number in 10 random areas of the culture dishes. These areas were limited by a rectangular field superimposed on the cells by a phototubus lens in an inverted Leitz microscope. The cells were counted at 125× magnification. The presence of infectious MuLV in humerus cell cultures was determined in NIH 3T3 mouse fibroblast cells and CCL-64 mink cells after treatment with cell-free humerus cell culture supernatants for 24 hours.

Alkaline Phosphatase (ALP) Determination

The effect of virus infection on osteoblastic differentiation *in vitro* was investigated in first-passage cells with ALP as a marker. The monolayers were washed with cold phosphate-buffered saline (PBS), fixed (10 minutes) with cold buffered formaldehyde (4%), washed with PBS (3×), and incubated in a substrate solution containing naphthol-AS-MX phosphate (Sigma) dissolved in dimethylformamide (1%, wt/vol), and fast blue BB salt (Serva, Heidelberg, FRG) buffered with Tris-HCl (50 mM, pH 8.3, 0.1%, wt/vol). ALP-positive cells were determined quantitatively. Polyclonal antibody to the bone-liver-kidney–specific isoenzyme of ALP isolated from the rat osteoblastic osteosarcoma cell line ROS 17/2.8[16] was a generous gift from Dr. G. A. Rodan, MSD, West Point, Pennsylvania. NIH 3T3 fibroblast cells and cryostat sections of rat kidney were used as controls.

For quantitative determination of specific ALP activity the cells were washed with PBS, scraped off with a rubber policeman in 250 μl of Tris-HCl (50 mM, pH 9.8), and stored at −20°C. Before the ALP assay the samples were thawed and the cells disrupted on a

Vortex. The assay was carried out according to Mössner et al.[17] Briefly, 20 μl of the homogenates containing 0.1% Triton X-100 were mixed with 180 μl of substrate solution (diethanolamine, 1 M, pH 9.8; $MgCl_2$, 0.5 mM; *p*-nitro-phenylphosphate, 15 mM). Serial dilutions of ALP (Type VII-NLA, Sigma) were used as standard. The assay was carried out in ELISA microtiter plates (Dynatech, Nürtingen, FRG). After incubation for 20 minutes at room temperature, the test was measured in an SLT 210.2 ELISA reader (Salzburg Electronic Industries, Salzburg, Austria) at 404.7 nm. The protein content in the samples was determined by a dye binding assay described by Spector[18] using Coomassie brilliant blue G (Serva) in phosphoric acid. Twenty microliters of the samples were mixed with 230 μl of the dye reagent in ELISA microtiter plates and the test measured at 577 nm as described above. Bovine serum albumin (Sigma) in PBS was used as standard.

Electron Microscopy

Sections of femora from infected osteopetrotic and 14-month-old control mice were prepared as described elsewhere in detail[19] and scanned in a Zeiss EM10CR electron microscope.

Results

Skeletoblast Cell Cultures From Mice Infected as Newborns

Separate monolayer cultures were established from each of the three fractions obtained after fractionated trypsinization (three times for 20 minutes each) of the pooled and minced humeri and calvaria from infected and control mice at the three selected time points, with the exception of calvaria from 28-day-old mice. The latter were dissociated individually, but cultures could only be established from Fraction 1 cells. The cultures varied in morphologic features. Fraction 1 cells were predominantly fibroblastic, Fraction 3 cells predominantly epitheloid, and Fraction 2 cells a mixture of the types in Fractions 1 and 3 (Figure 1A and B). The number of differentiated osteoblastlike cells increased in both humerus and calvaria cell cultures from Fraction 1 to Fraction 3. This was shown by an increase in the number of epitheloid–cuboidal cells with high alkaline phosphatase (ALP) activity (Figure 1C and D). The specific alkaline phosphatase activity in cell lysates from Fractions 1 to 3 of 7-day humeri was also lowest for Fraction 1 cultures and highest for Fraction 3 cultures (Table 1).

The cell cultures established from humeri 3, 7, and 28 days after infection showed expression of viral p30 protein with approximately 40 infectious centers per dish. Little variation was observed between cultures established from different cell fractions. Infectivity studies using NIH 3T3 (ecotropic virus) cells and CCL-64 mink (xenotropic virus) cells revealed the presence of infectious ecotropic viruses in the supernatants harvested from all fractions of humerus cells established from infected mice. Because NMRI mice do not harbor endogenous ecotropic proviral sequences,[14] this indicates replication of the OA MuLV injected at birth.

Viral p30 protein expression was detected in the calvaria cultures established 7 and 28 days after infection but not in those established 3 days after infection or from controls. At Day 7 after infection about 60% of the cells in Fraction 1 cultures were p30-positive, about 30% of cells in Fraction 2 cultures, but only about 5% in Fraction 3 cultures.

The effect of OA MuLV on cell growth was investigated by determining the total protein content of the culture dishes from 7-day humeri. Growth of Fraction 1 and that of Fraction 2 cells from the infected and control groups was very similar. However, Fraction 3 cells from infected mice showed markedly reduced growth in comparison with those from control mice (Table 1).

The effect of OA-MuLV on osteogenic differentiation was investigated with alkaline phosphatase activity, determined histochemically and quantitatively, as a marker. Increasing numbers of ALP-positive cells were observed in parallel with increasing cell fraction number in 7-day-old humerus cultures from both infected and control mice. In Fraction 1, only a few cells showed ALP activity, whereas in Fraction 3, 5% of the cells showed moderate to high ALP expression. Quantitative analysis showed the increase in ALP activity to be considerably more marked in the cultures from infected animals. Whereas Fraction 1 cells from both control and infected mice had specific activities of less than 0.01 U/mg protein, Fraction 2 cells had specific activities of 0.01 and 0.04 U/mg protein, and Fraction 3 cells specific activities of 0.03 U/mg and 0.08 U/mg, respectively (Table 1).

A similar increase in the number of ALP-positive cells from Fraction 1 to Fraction 3 was observed in calvaria cultures from 7-day-old mice. Quantitative analysis of ALP activity was determined in pooled samples of Fraction 1–3 cells. The specific ALP activity in cultures from controls was 0.043 U/mg protein, and in those from infected mice, 0.146 U/mg protein.

In Vitro Infection of Skeletoblast Cell Cultures

Subconfluent osteoblast precursor cells (periosteal) and osteoblastlike cells obtained from calvaria of un-

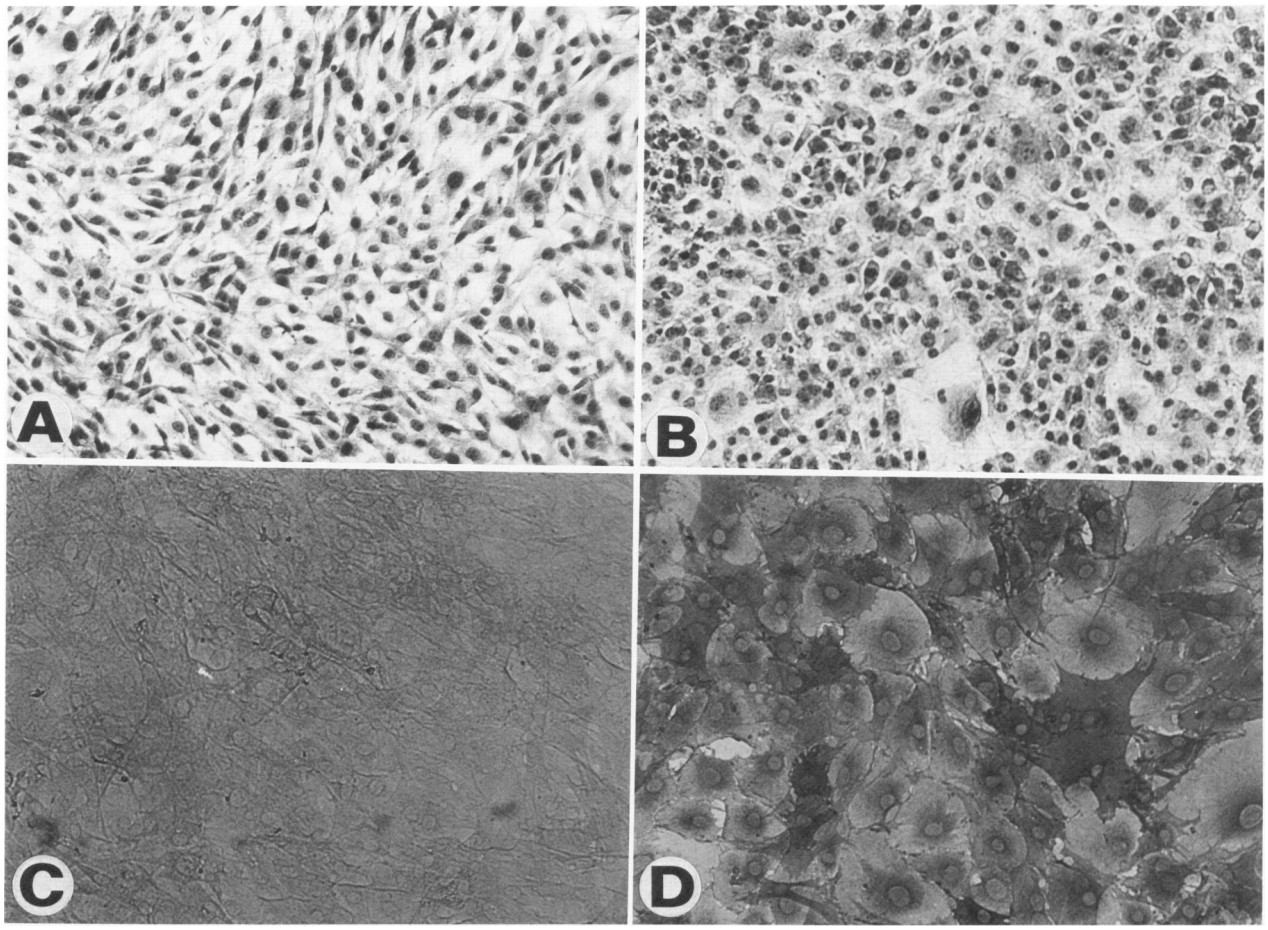

Figure 1—Morphology and ALP activity staining of Fraction 1 (**A** and **C**) and Fraction 3 (**B** and **D**) cell cultures established from mouse calvaria. Fraction 1 (**A**) and fraction 3 (**B**) cells were cultured for 4 days, fixed with ethanol and stained with Giemsa solution (10%, 10 minutes). Parallel cell cultures (**C** and **D**) were fixed with acetone/methanol (1 : 1, −20 C), incubated with rabbit antibody to the bone-liver-kidney-specific isoenzyme of ALP, horseradish-labeled goat anti-rabbit antibody, and 4-chloro-2-naphthol substrate. Note the strong reaction along the cell membranes in **D**. (×250)

treated newborn mice were infected with serial dilutions of OA MuLV. The periosteal cultures, which contained few ALP-positive cells, showed a high level of infection (Table 2, Figure 2A and C); whereas the cultures of osteoblastlike cells, with a high number of ALP-positive cells, were only slightly infected (Figure 2B and D).

Periosteal and osteoblastlike calvaria cell cultures were infected with OA MuLV, passaged once, and reinfected after attachment of the cells and twice later at 6-hour intervals. This protocol impaired cellular differentiation, increased the susceptibility to virus infection, and resulted in homogeneously infected periosteal and osteoblastlike cell cultures, as shown immunohistochemically with anti-OA MuLV p30

Table 1—ALP Activity in Cell Cultures Established From Humeri 7 Days After Infection of Newborn NMRI Mice

	Humerus cell cultures	
	Protein content (μg/p35 dish)*	ALP activity (U/mg protein)
Control mice		
Fraction 1 cells	112.5	<0.01
Fraction 2 cells	275	0.01
Fraction 3 cells	650	0.03
Infected mice		
Fraction 1 cells	136	0.01
Fraction 2 cells	319.5	0.04
Fraction 3 cells	150	0.08

*Specific ALP activity and protein content were determined in duplicate cultures (mean of duplicate assays).

Table 2—Infectivity of OA MuLV in Primary Periosteal and Osteoblastlike Cell Cultures

Virus titer (infectious particles/ml)	% cells infected*	
	Periosteal cell culture	Osteoblastlike cell culture
8×10^5	NT	5.6 ± 2.5
8×10^3	10.7 ± 1.6	1.1 ± 0.5
8×10^1	6.9 ± 1.6	0.2 ± 0.4

*See Table 3 legend. NT, not tested.

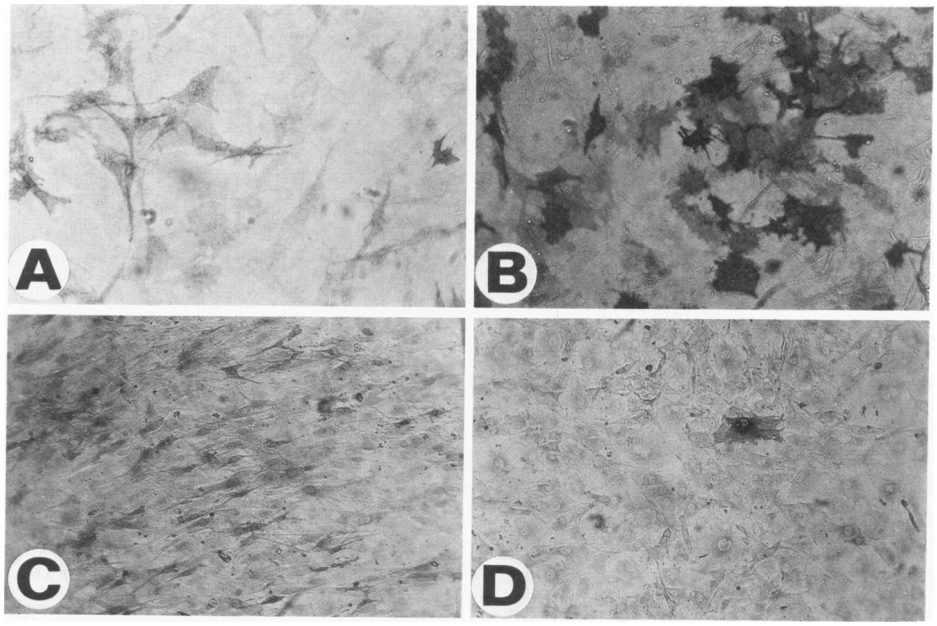

Figure 2—Reciprocal relationship between osteogenic differentiation and virus infection in periosteal and osteoblastlike cells. **A** and **B**— ALP activity (histochemistry) in periosteal cells (**A**) and osteoblastlike cells (**B**) in 10-day-old cultures. **C** and **D**— OA MuLV infection of periosteal cells (**C**) and osteoblastic cells (**D**). Cells were infected with OA MuLV and immunolabeled with anti-OA MuLV p30 antibody 24 hours later. (×125)

antibody (data not shown). A fourfold increase was observed in the number of ALP-positive cells in the infected periosteal cell cultures; the specific ALP activity was also higher than in uninfected, control cells, particularly after 5 days in culture (Figure 3). The number of ALP-positive cells in the osteoblastlike cell cultures remained constant (Table 3). Cell growth of infected periosteal cells was reduced to a level comparable to that of the differentiated osteoblastlike cells (Figure 4).

Long-Term Pathogenicity of OA MuLV

After an observation period of up to 24 months, 7 of 12 infected mice developed roentgenographically detectable osteopetrosis in lumbar vertebrae, os ilium, or the femurs, and 2 mice developed histologically detectable osteopetrosis in lumbar vertebrae. No comparable osseous lesions were found in the 14 mock-treated controls. These results were similar to those in previous investigations.[1,14]

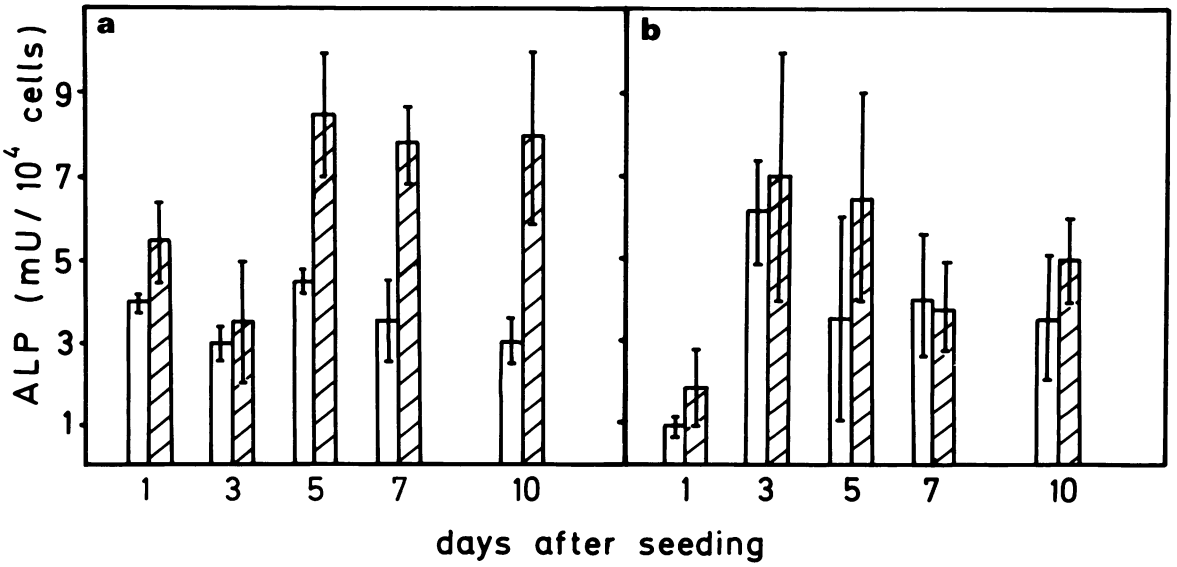

Figure 3—ALP activity in cell homogenates of control and OA MuLV-infected (100%) periosteal (**a**) and osteoblastlike (**b**) cell cultures. *Bars* represent means of quadruplicate determinations ±SD. *Open bars*, controls; *hatched bars*, OA MuLV-infected cells.

Table 3—ALP Activity in Control and OA MuLV-Infected Periosteal and Osteoblastlike Cell Cultures

Cell culture	ALP-positive cells (%)*
Periosteum, control	3.6 ± 0.8
Periosteum, infected	12.3 ± 3.1
Osteoblastlike, control	16.0 ± 4.6
Osteoblastlike, infected	14.0 ± 6.8

*Quantitative analysis was carried out in random areas of Petri dishes. Figures represent means of 10 determinations ± standard deviation.

Electron Microscopy

Immature and mature C-type virus particles were found associated with osteocytes in the distal femurs of osteopetrotic mice. Virus was observed budding from the membranes of osteocytes, and large numbers of mature and pleomorphic virus particles were also found accumulated in devitalized osteocyte lacunae within osteopetrotic tissue. Virus particles were not observed in bone tissue from the control mice (Figures 5A and B).

Discussion

Osteopetrosis is a general description of a disease associated with the excessive production of new bone tissue or decreased resorption of bone tissue. So far, two essentially different forms have been described.[20–24] The inherited form, observed in mutant mice and rats, is primarily caused by impairment of osteoclasis with phenotypically altered osteoclasts, followed by reduced remodeling during bone growth and disfigurement of the normal osseous form and architecture.[23,25] The second form is induced by the avian retrovirus MAV-2(O) and is characterized by the displacement of the medullary cavity of long bones with newly synthesized and appositionally deposited compact bone, apparently as a consequence of osteoblast infection.[8,9,11] The OA MuLV-induced osteopetrosis appears to be similar to the second form. Our ultrastructural analysis shortly after infection of newborn mice showed virus expression exclusively in osteoblasts and osteocytes,[13] suggesting that cells of this lineage represent primary targets in skeletal tissue. Virus infection in chicken is thought to affect the functional properties of osteoblasts and osteocytes,[24] and it seems likely that a similar mechanism is involved in osteopetrosis induction in mice by OA MuLV. This hypothesis was tested in skeletal cell cultures established from neonatally infected mice and in primary cell cultures from bone tissue infected *in vitro*.

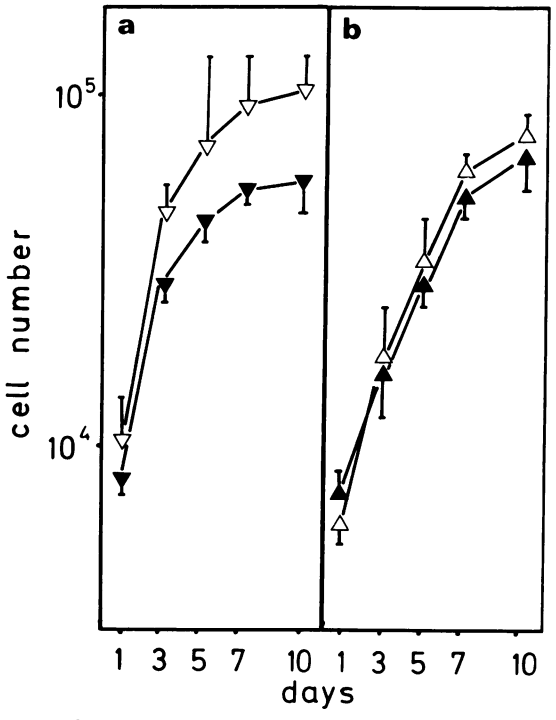

Figure 4—Growth of control and OA MuLV-infected (100%) periosteal (**a**) and osteoblastlike (**b**) cells; 10^4 cells were seeded in multiwell dishes (Nunc). Medium was changed at 3-day intervals. Data represent mean of quadruplicate determinations ± standard deviation. ▽ △, control cells; ▼ ▲, OA MuLV-infected cells.

Late fractions of sequentially dissociated mouse calvaria have been reported to exhibit osteoblastic properties.[26–28] This was confirmed in our experiments, in which a high expression of alkaline phosphatase, the marker enzyme for differentiated osteoblasts,[27,29] was found in cells of Fraction 3 cultures from humeri and calvaria. These cells also reacted strongly with the antibody to the bone-liver-kidney–specific isoenzyme of ALP. ALP activity in humerus and calvaria cultures established from infected mice was considerably higher than that in cultures from control mice, indicating a specific enhancement of osteoblastic activity as a consequence of viral infection. This increase in osteogenic differentiation was also associated with a lower protein concentration in the cultures of the infected cells, indicative of reduced cell growth.[30]

The undifferentiated osteoblast precursor cells in Fraction 1 cultures from calvaria from infected animals showed a higher proportion of virus-infected cells than did the differentiated osteoblast-like cells in Fraction 3. Thus, undifferentiated osteoblast precursor cells seem likely to represent the primary target population for OA MuLV in skeletal tissue. The ultrastructural evidence of virus production by osteocytes in osteopetrotic tissue from 14-month-old infected mice suggests the maintenance of the osteoinductive effect of the virus in the bone matrix-producing cell until full development of the disease.

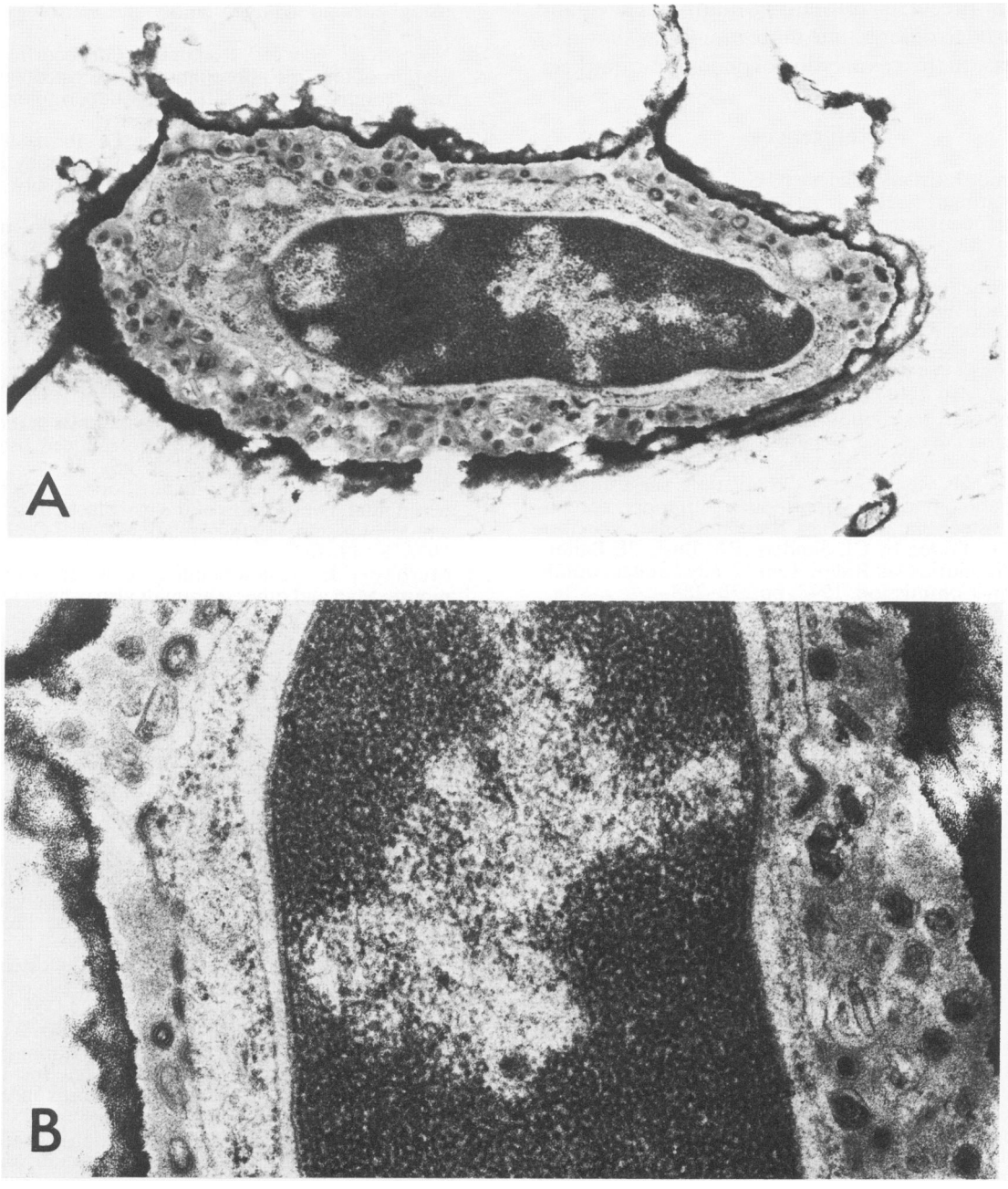

Figure 5—Distal femur from a 14-month-old infected mouse with osteopetrosis. **A**—Osteocyte in lacuna filled with pleomorphic immature and mature virus particles. (×17,800) **B**—Detail of **A** showing budding, immature, and atypical mature virus particles. (×52,400)

In vitro infection of primary periosteal and osteoblastlike cell cultures established from calvaria of newborn NMRI mice supported the findings obtained following *in vivo* infection. The infection rate in periosteal cells was higher than that in osteoblastlike cells, further supporting the observation of a reciprocal relationship between osteoblastic differentiation and susceptibility to virus infection. A similar effect has been observed in permanent cultures of differentiating rat and mouse osteoblastic cell cultures.[31] Further, infection of periosteal cells was followed by a reduction in cell growth and an increase in phenotypic marker expression, indicating the induction of higher osteoblastic activity.

The functional role of OA MuLV in osteopetrosis induction is not yet clear. It appears, however, that

osteoblast precursor cells are primarily infected and are induced to differentiate more rapidly, as shown by an increase in their expression of phenotypic markers.

References

1. Schmidt J, Erfle V, Pedersen FS, Rohmer H, Schetters H, Marquart K-H, Luz A: Oncogenic retrovirus from spontaneous osteomas: I. Isolation and biological characterization. J Gen Virol 1984, 65:2237–2248
2. Van Gorp LHM, Swaen GJV: Myelofibrosis in rats experimentally infected with a murine leukemia virus. J Pathol 1969, 97:235–240
3. Wlodarsky K, Kobus M, Luczak M, Dolowy J: Virally induced periosteal osteogenesis in mice. Calcif Tissue Int 1981, 33:135–142
4. Finkel MP, Reilly CA Jr, Biskis BO, Greco IL: Bone tumour viruses, Bone—Certain Aspects of Neoplasia (Colston Papers, No. 24). Edited by CHG Price, FGM Ross. London, Butterworths, 1973, pp 353–364
5. Finkel MP, Reilly CA Jr: Observations suggesting the viral etiology of radiation-induced tumours, particularly osteogenic sarcomas, Radionucleotide Carcinogenesis. Edited by CL Sanders, RH Bush, JE Ballon, DD Mahlum. Oak Ridge, Tenn, United States Atomic Energy Commission, 1973, pp 278–288
6. Merwin RM, Redmon LW: Skeletal and reticular tissue disorders produced in mice by agent(s) from sarcoma 37. J Natl Cancer Inst 1969, 43:365–376
7. Smith RE, Moscovici C: The oncogenic effects of nontransforming viruses from avian myeloblastosis virus. Cancer Res 1969, 29:1356–1366
8. Smith RE, Morgan JH: Pathogenesis of osteopetrosis induced by rapid and slow onset plaque isolates of an avian osteopetrosis virus. Metab Bone Dis Rel Res 1984, 5:289–298
9. Boyde A, Banes AJ, Dillaman RM, Mechanic GL: A morphological study of an avian bone disorder caused by myeloblastosis-associated virus. Metab Bone Dis Rel Res 1978, 1:235–242
10. Schmidt EV, Smith RE: Avian osteopetrosis virus induces proliferation of cultured bone cells. Virology 1981, 111:275–282
11. Schmidt EV, Crapo JD, Harrelson JM, Smith RE: A quantitative histologic study of avian osteopetrotic bone demonstrating normal osteoclast numbers and increased osteoblastic activity. Lab Invest 1981, 44:164–173
12. Banes AJ, Mechanic GL: Collagen and noncollagen protein synthesis in chick limb bud cells infected with a virus that causes osteoblastoma. Metab Bone Dis Rel Res 1981, 3:81–89
13. Murray AB, Schmidt J, Rieke L: Retrovirus-induced osteopetrosis in mice: Ultrastructural evidence of early virus production in osteoblasts and osteocytes. Am J Pathol 1986, 124:319–323
14. Leib-Mösch C, Schmidt J, Etzerodt M, Pedersen FS, Hehlmann R, Erfle V: Oncogenic retrovirus from spontaneous murine osteomas: II. Molecular cloning and genomic characterization. Virology 1986, 150:96–105
15. Nexø BA: A plaque assay for murine leukemia virus using enzyme coupled antibodies. Virology 1977, 77:849–852
16. Majeska RJ, Nair BC, Rodan GA: Glucocorticoid regulation of alkaline phosphatase in the osteoblastic osteosarcoma cell line ROS 17/2.8. Endocrinology 1985, 116:170–179
17. Mössner E, Boll M, Pfleiderer G: Purification of human and bovine alkaline phosphatases by affinity chromatography. Hoppe-Seylers Z Physiol Chem 1980, 361:543–549
18. Spector T: Refinement of the Coomassie blue method of protein quantitation. Anal Biochem 1978, 86:142–146
19. Murray AB, Rieke L: Identification of the position of selected cells in large sections of oriented material following electron microscopy. Stain Technol 1986, 61:375–376
20. Holmes JR: Avian osteopetrosis. Natl Cancer Inst Monographs 1964, 17:63–79
21. Marks SC Jr: Pathogenesis of osteopetrosis in the ia rat: Reduced bone resorption due to reduced osteoclast function. Am J Anat 1973, 138:165–190
22. Marks SC Jr: Tooth eruption and bone resorption: Experimental investigation of the ia (osteopetrotic) rat as a model for studying their relationships. J Oral Pathol 1976, 5:149–163
23. Murphy HM: A review of inherited osteopetrosis in the mouse: Man and other mammals also considered. Clin Orthop 1969, 65:97–109
24. Smith RE: Avian osteopetrosis. Curr Top Microbiol Immunol 1982, 101:75–94
25. Milhaud G, Labat M-L: Thymus and osteopetrosis. Clin Orthop 1978, 135:260–271
26. Wong GL: Characterization of subpopulations of OC and OB bone cells obtained by sedimentation at unit gravity. Calcif Tissue Int 1982, 33:67–75
27. Wong GL, Kocur BA: Differential serum dependence of cultured osteoclastic and osteoblastic bone cells. Calcif Tissue Int 1983, 35:778–782
28. Wong GL, Cohn DV: Target cells in bone for parathormone and calcitonin are different: Enrichment for each cell type by sequential digestion of mouse calvaria and selective adhesion to polymeric surfaces. Proc Natl Acad Sci USA 1975, 72:3167–3171
29. Rodan GA, Rodan SB: Expression of the osteoblastic phenotype, Bone and Mineral Research. Edited by WA Peck. Amsterdam, Elsevier, 1984, pp 244–285
30. Zeytinoglu FN, DeLellis RA, Gagel RT, Wolfe H, Tashjian AH Jr. Establishment of a calcitonin-producing rat medullary thyroid carcinoma cell line: I. Morphological studies of the tumor and cells in culture. Endocrinology 1980, 107:509–515
31. Schmidt JM, Casser-Bette M, Rodan GA, Baumgartner-Decker C, Erfle V: Bone tumor inducing retroviruses and osteoblastic cells in vitro: Target cell specificity and effects of viral infection, Current Advances in Skeletogenesis III. Edited by S Hurwitz, J Sela. Pergamon Press, 1987 (In press)

Acknowledgments

We thank E. I. Closs for ALP activity determinations. The expert technical assistance of C. Baumgartner-Decker and L. Rieke is gratefully acknowledged.