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Osteoclasts Formed by Measles Virus-Infected Osteoclast Precursors from hCD46 Transgenic Mice Express Characteristics of Pagetic Osteoclasts*

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ABSTRACT

Pagetic osteoclasts (OCLs) are abnormal in size and contain paramyxoviral-like nuclear inclusions that cross-react with antibodies to measles virus (MV). However, the role that MV infection plays in Paget's disease is unknown, because no animal model of Paget's disease is available. Therefore, we targeted a cellular MV receptor, human CD46 (hCD46), to cells in the OCL lineage in transgenic mice using the mouse tartrate-resistant acid phosphatase (TRAP) gene promoter. *In vitro* infection of OCL precursors from hCD46 transgenic mice with MV significantly increased OCL formation in bone marrow cultures. The numbers of TRAP-positive mononuclear cells and CFU-GM, the earliest identifiable OCL precursor, were also significantly increased. MV-infected OCLs formed from hCD46 marrow were increased in size, contained markedly increased numbers of nuclei, and had increased bone-resorbing capacity per OCL compared with OCLs formed from marrow of nontransgenic littermates. Furthermore, IL-6 and 24-hydroxylase messenger RNA expression levels were increased in MV-infected hCD46 transgenic mouse bone marrow cultures. Treatment of MV-infected hCD46 marrow cultures with a neutralizing antibody to IL-6 blocked the increased OCL formation seen in these cultures. These data demonstrate that MV infection of OCL precursors results in OCLs that have many features of pagetic OCLs, that the enhanced OCL formation is in part mediated by increased IL-6 expression induced by MV infection, and suggest that the hCD46 transgenic mouse may be a useful model for examining the effects of MV infection on OCL formation *in vivo*. (*Endocrinology* **142:** 2898–2905, 2001)

STUDIES OVER the past 30 yr have suggested a potential viral etiology for Paget's disease. The initial studies by Rebel and co-workers (1), using electron microscopic techniques, demonstrated nuclear and cytoplasmic inclusions in osteoclasts (OCLs) from patients with Paget's disease that were similar to paramyxoviral nucleocapsids. Mills and Singer (2) confirmed these findings and showed that these nuclear inclusions cross-reacted with antibodies that recognized measles virus (MV) or respiratory syncytial virus (RSV) nucleocapsid antigens. Similarly, Basle and co-workers (3), using *in situ* hybridization techniques, demonstrated MV messenger RNA sequences in over 90% of the OCLs and other mononuclear cells in pagetic bone specimens. Gordon and co-workers (4) reported the presence of canine distemper virus (CDV) nucleocapsid antigens in OCLs from patients with Paget's disease. These paramyxoviral-like nuclear inclusions are not unique to Paget's disease and have been reported in patients with familial expansile osteolysis (FEO) and rare patients with osteopetrosis, pycnodysostosis, otosclerosis, and oxalosis (5–7). In addition to containing viral-like nuclear inclusions, the abnormal OCLs in Paget's patients are markedly increased in number and size, have increased numbers of nuclei per OCL, and have an increased bone-resorbing capacity per cell (8).

We have previously identified MV nucleocapsid transcripts in bone marrow cells and peripheral blood derived monocytes from patients with Paget's disease (9). We further demonstrated that OCL precursors, including the earliest recognizable OCL precursor, the granulocyte-macrophage colony-forming unit (CFU-GM), as well as mature OCLs, from patients with Paget's disease, expressed MV nucleocapsid transcripts (10). We also found by RT-PCR analysis that peripheral blood samples from nine of ten patients with Paget's disease contain MV nucleocapsid transcripts, whereas none of the ten normals tested expressed MV nucleocapsid transcripts (9). Mee *et al.* (11) have demonstrated CDV nucleocapsid transcripts in affected bones from 100% of patients tested using *in situ* PCR techniques. They have further shown that infecting canine bone marrow with CDV results in development of multinucleated cells that share some of the phenotypic characteristics of pagetic OCLs, but that bones from dogs with CDV do not appear similar to

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Paget's disease. However, other investigators have been unable to detect paramyxoviral nucleocapsid transcripts in bone marrow cells obtained from patients with Paget's disease (12, 13). Furthermore, no infectious virus has been isolated from pagetic cells, and no full-length viral genes have been cloned from material obtained from Paget's patients. Thus, the role that MV infection plays in the abnormal OCL activity in Paget's disease remains controversial, and no *in vivo* model of Paget's disease is available for experimental studies. It is our hypothesis that MV infection of OCL precursors is in part responsible for the abnormal phenotype of pagetic OCLs.

As an initial step to test the role of MV in Paget's disease *in vivo*, we targeted human CD46 (hCD46) to cells in the OCL lineage in transgenic mice. hCD46, also termed membrane cofactor protein (MCP), is a cellular receptor for MV (14). It is a 58-kDa type I membrane glycoprotein. Transcription of the gene encoding MCP results in six different messenger RNA (mRNA) isoforms due to alternative splicing (15). Although a murine homologue of hCD46, which has a 45% identity in the deduced protein sequence and 62% identity in the nucleotide sequence with hCD46, has been reported in spermatids during germ cell differentiation (16), murine cells are generally nonpermissive to MV infection. Therefore, we targeted human CD46 expression to OCL precursors of transgenic mice using the mouse tartrate-resistant acid phosphatase (TRAP) gene promoter and tested the capacity of MV infection of OCL precursors *in vitro* to induce a pagetic phenotype in OCLs.

Materials and Methods

mTRAP promoter-hCD46 transgene construct

An *Eco*RI DNA fragment (1.5 kb) encoding MCP-C1 complementary DNA (cDNA) (GenBank Accession No. X59406) isoform of hCD46 (generous gift from Dr. Thomas Atkinson, Washington University, St. Louis, MO) was subcloned into the pBSpKCR3 R1 vector (17) at the *Eco*RI restriction enzyme site, and the resulting plasmid construct was termed pKCMC1. We have previously described cloning and characterization of the mTRAP gene promoter region (18). An *Eco*RV and *Bgl*II DNA restriction fragment (1.8 kb) containing the 5′-flanking sequence of the murine TRAP gene, which was derived from the pBSmTRAP plasmid, was then subcloned into the pKCMC1 plasmid at the *Sma*I restriction site that was present upstream to the hCD46 cDNA. The resulting plasmid construct, pKCMC1TR7, was digested with *Xho*I to excise the mTRAP-hCD46 transgene, and the transgene DNA fragment was isolated by agarose gel electrophoresis, purified using Geneclean (Bio 101, La Jolla, CA) and used for microinjection studies.

Development of hCD46 transgenic mice

The mTRAP-hCD46 transgene, at a concentration of 2 μg/ml, was microinjected into the male pronucleus of fertilized one-cell mouse embryos, as described previously (19). The F2 embryos were obtained from matings of CB6F1 (C57BL/6× BALB/c) males and females (Harlan Sprague Dawley, Inc.; Indianapolis, IN). The injected embryos were reimplanted into pseudopregnant ICR female mice. The presence of the transgene in the resulting offspring was identified by Southern blot analysis of DNA purified from a small piece of tail tissue obtained at the time of weaning. Transgenic mice of subsequent generations were identified by PCR analysis of hCD46 mRNA expression.

RT-PCR analysis of hCD46 mRNA expression

Total RNA was isolated from the mouse bone marrow cells, highly purified human OCLs from giant cell tumors of bone, or from OCLs formed in bone marrow cultures from patients with Paget's disease (20) by the guanidinium isothiocyanate method using the RNAzol reagent (Tel-Test, Inc., Friendswood, TX) following the manufacturer's protocol. Approximately 3 μg total RNA from each sample was denatured at 65 C for 5 min and reverse transcribed using murine Moloney leukemia virus reverse transcriptase (9). The reaction volume was 20 μl and contained 5 mmol/liter $MgCl_2$, 50 mmol/liter KCl, 10 mmol/liter Tris-HCl (pH 8.3), 1 mmol/liter of each dNTP, 2.5 μmol/liter random hexamers, and 1 U ribonuclease inhibitor. The reaction was incubated for 15 min at 42 C, followed by inactivation of the reverse transcriptase at 94 C for 5 min. The cDNA products obtained were subjected to PCR amplification of hCD46 transcripts in a reaction mixture of 100 μl containing 2 mmol/liter of $MgCl_2$, 50 mmol/liter KCl, 10 mmol/liter Tris HCl (pH 8.3), 0.2 mmol/liter of each dNTP, 2.0 U Ampli *Taq* DNA polymerase, 0.1 μmol/liter of sense (5′-CAG CGA CAC AAT TGT CTG TGA CAG-3′) and antisense (5′-GGT TGA TTT AGT CTG GTA AGT GGC-3′) hCD46 gene specific primers (21), respectively. β-actin transcripts were amplified in all the samples analyzed using gene specific primers as reported previously (10). The PCR was carried out by incubating the samples at 94 C for 1 min followed by 45 cycles of 94 C for 1 min and 60 C for 1 min, with a final extension for 5 min at 60 C. The amplified products were electrophoresed on a 1.2% agarose gel with a 123 bp DNA ladder (BRL) as a size marker. Bands were visualized by ethidium bromide staining.

CFU-GM colony formation in murine bone marrow cultures

Bone marrow from mouse tibiae that had been aseptically removed was obtained by flushing with 1 ml α-MEM using a tuberculin syringe fitted with a 27-gauge needle. The bone marrow-derived cells were washed twice, resuspended in α-MEM-10% FCS and depleted of cells adherent to plastic by incubating the marrow cell suspension in sterile 10-cm tissue culture dishes for 2 h. The nonadherent bone marrow cells (10^5/ml) were plated on 35-mm tissue culture dishes (Falcon, Lincoln Park, NJ) in 1 ml 1.5% methylcellulose (Aldrich Chemical Co., Inc. Milwaukee, WI) supplemented with 20% FCS, 0.1% BSA and 100 pg/ml recombinant murine GM-CSF. The cultures were incubated at 37 C in an atmosphere of 5% CO_2-air for 7 days. The number of CFU-GM colonies (>40 cells) formed were scored using a microscope (22).

Osteoclast formation in murine bone marrow

Nonadherent marrow cells (2.0×10^6 cells/ml), prepared as described above, were plated in 48-well plates in α-MEM-10% FCS supplemented with 10^{-8} M to 10^{-11} M 1,25-$(OH)_2D_3$ in the presence or absence of 100 ng/ml of a neutralizing antibody to murine IL-6 (R&D Systems, Minneapolis, MN) as described by Takahashi *et al.* (23). The cells were cultured for 7 days at 37 C in a humid atmosphere of 5% CO_2-air. The cultures were then fixed with 4.5 mM citric acid, 2.25 mM sodium citrate, 3 mM sodium chloride, 3% formaldehyde/acetone and were washed twice in distilled water. The cultures were then stained for TRAP activity using an acid phosphatase staining kit (Sigma, St. Louis, MO). The TRAP(+) multinucleated cells (MNC) containing three or more nuclei were counted with an inverted microscope.

Bone resorption assay

For bone resorption assays, murine marrow cultures described above were overlaid onto sterile sperm whale dentin slices (generously provided by the U.S. Fish and Wildlife Service). At the end of the 7-day culture period, the dentin slices were removed and the cells fixed in 2% glutaraldehyde and stained for TRAP activity. The number of TRAP(+) multinucleated cells on each dentin slice was scored, and the number of resorption pits and area of the dentin resorbed was determined with an inverted microscope using Java image analysis software (Jandel Scientific, Corte Madrona, CA), as previously described (24).

Immunocytochemical staining for hCD46 expression in osteoclasts

For immunocytochemical studies, OCL-like cells formed in transgenic mouse bone marrow cultures were fixed with 2% formaldehyde for 20 min and washed with PBS. To reduce the nonspecific binding of

antibodies, the cells were treated with 1% BSA in PBS for 30 min. The cells were then incubated with a goat antiserum raised against hCD46 (Research Diagnostics, Inc., Flanders, NJ) or preimmune serum at 1:200 dilution in PBS for 1 h and washed three times with PBS. The cells were then incubated with biotinylated antigoat IgG and stained using an ABC kit from Vector Laboratories, Inc. (Burlingame, CA) following the manufacturer's protocol.

MV infection and immunostaining for nucleocapsid protein expression in osteoclasts

Live MV were isolated from an infected patient by coculture of patient peripheral blood monocytes with PHA stimulated cord blood mononuclear cells (25). Bone marrow cells were infected at a multiplicity of infection (MOI) of 0.5 by incubating in α-MEM-1% FCS for 90 min at 37 C. Cells were washed in α-MEM and cultured to form OCL-like cells as described. Immunocytochemical stains for MV nucleocapsid protein expression in OCLs was performed using human sera containing high titers of antibody against MV at 1:200 dilution. Alkaline phosphatase conjugated goat antihuman IgG at 1:500 dilution was used as secondary antibody and the cells stained under conditions as described above.

Electron microscopy

hCD46 transgenic mouse bone marrow cells were infected with MV and cultured for 7 days to form OCL-like cells as described (23). At the end of the culture period, the culture media were removed and adherent cells were fixed *in situ* for 60 min at 4 C in 4% formaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, secondarily fixed with 1% osmium tetroxide for 30 min and dehydrated through propylene oxide. The cells were scraped from glass chamber slides and placed in centrifuge tubes before embedding in poly Bed 812 (Polysciences, Inc., Pittsburgh, PA). Ultrathin sections were stained with uranyl acetate and Reynolds lead before images were captured, using a Philips 208 with AMT digital system (Advanced Microscopy Technologies, Inc., Danvers, MA).

Statistical analysis

The mean ± SE of the means for culture results were compared using a one-way ANOVA for repeated measures and were considered significant at $P < 0.05$.

Results

Development of mTRAP-hCD46 transgenic mice

Because multiple isoforms of the hCD46 gene are expressed, we identified the most abundant hCD46 isoform expressed in human OCLs, to determine which would be most appropriate for constructing the hCD46 transgene. Up to four isotypes of hCD46 mRNA were detected in samples of purified human OCLs (Fig. 1). MCP-C_1 was the largest isoform of CD46 highly expressed in OCLs. OCLs also demonstrated high levels of expression for an aberrantly spliced transcript of MCP, which has a lower molecular weight than the MCP-C_1 isoform. We could not detect any significant differences in hCD46 mRNA phenotyping in OCLs derived from pagetic bone marrow cultures compared with normals (data not shown). Therefore, a cDNA encoding the MCP-C_1 isoform of hCD46 was used to construct the transgene shown in Fig. 2. The mTRAP-hCD46 transgene was used to generate two hCD46 founder mice, each with approximately three copies of the transgene (data not shown), and lines of mice were established from each. No phenotypic abnormalities were observed in either the founders or offspring of either line.

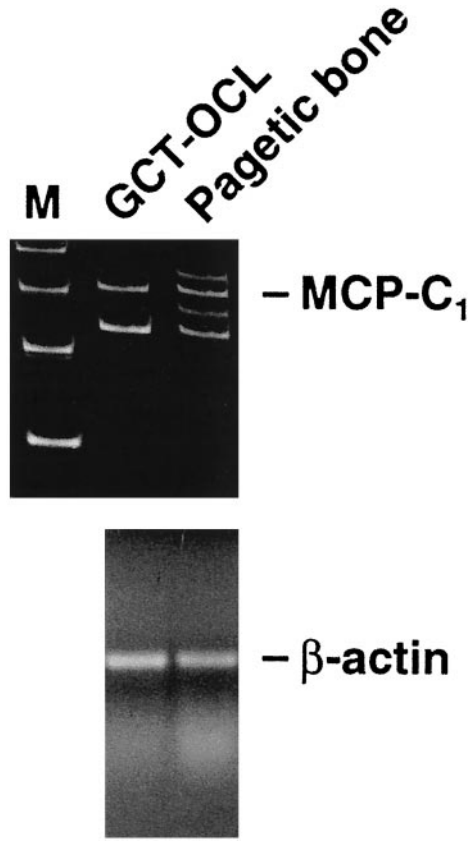

FIG. 1. Analysis of hCD46 mRNA expression in purified human osteoclasts. Total RNA was isolated from human osteoclastoma-derived osteoclasts (GCT-OCL) and obtained from an involved bone of a Paget's disease patient. RT-PCR analysis was performed as described in *Materials and Methods*. The MCP-C_1 isoform is the predominant form of hCD46 expressed in osteoclasts.

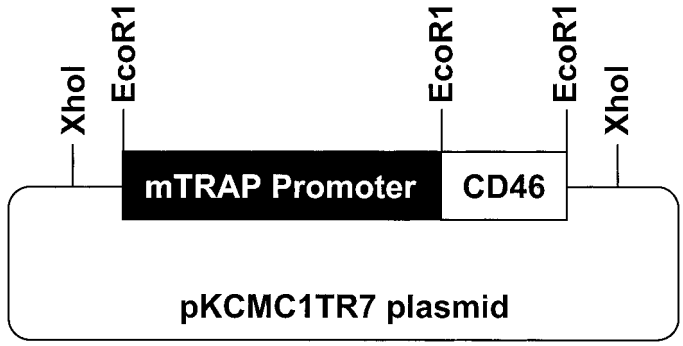

FIG. 2. Map of the mTRAP-hCD46 transgene plasmid. The mTRAP-hCD46 transgene contains the 5′ flanking region of the mTRAP gene extending from 1294 bp upstream of the major transcription initiation site, to the T of the ATG translation start codon and includes the intron in the 5′ untranslated region (18). This promoter directs the expression of a cDNA encoding the MCP-C_1 isoform of hCD46. This cDNA has been inserted into a fragment of the rabbit β-globulin gene, containing part of exon 2, the second intron, and exon 3, which provides an intron and polyadenylation site for efficient transgenic mRNA expression (17).

Analysis of hCD46 transgene expression

Because both lines were comparable in terms of hCD46 mRNA expression, line 1 was used for all subsequent studies.

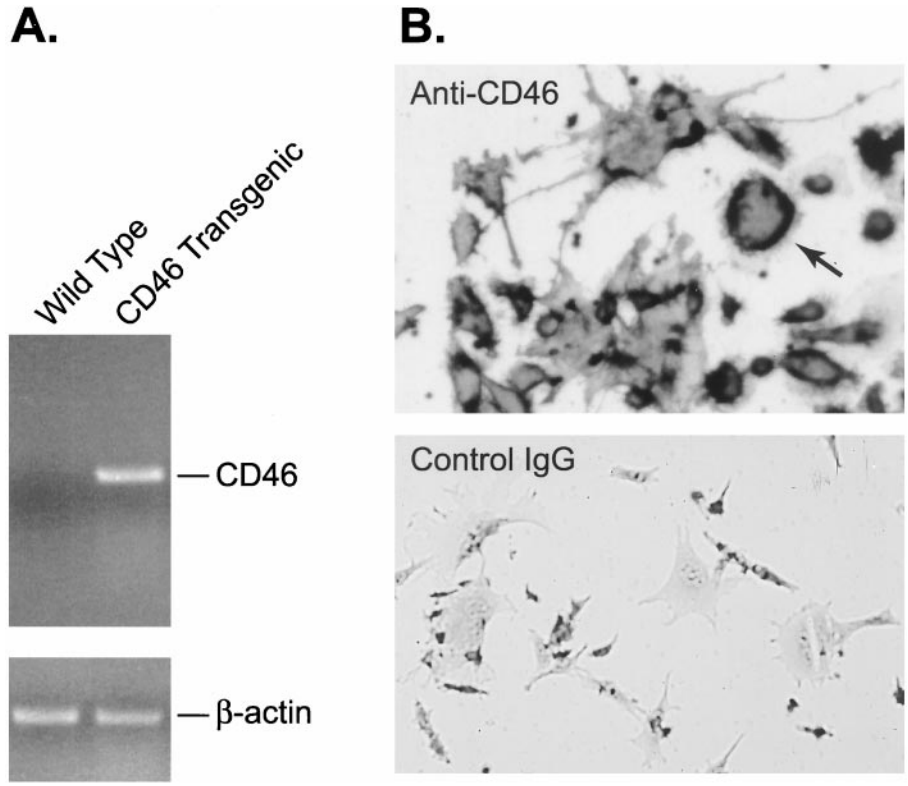

FIG. 3. mTRAP-hCD46 transgene expression in mouse bone marrow cell cultures. A, RT-PCR analysis of mTRAP-hCD46 mRNA expression in wild-type and hCD46 transgenic mouse bone marrow cells. RT-PCR analysis was performed as described in *Materials and Methods*. Only hCD46 mice expressed the transgene. B, Immunocytochemical staining for hCD46 in osteoclast like cells formed in hCD46 transgenic mouse bone marrow cultures. Immunocytochemical staining was performed as described in *Materials and Methods*. A positive reaction is denoted by the dark color. Control cultures were stained with mouse IgG of the same isotype. Magnification, 200×.

RT-PCR analysis of total RNA derived from freshly isolated hCD46 transgenic mouse bone marrow cells demonstrated expression of hCD46 mRNA (Fig. 3A). In contrast, bone marrow cells derived from wild-type littermates did not express hCD46 mRNA. Immunocytochemical staining further confirmed expression of the hCD46 protein in OCL-like cells formed in hCD46 transgenic mouse marrow cultures (Fig. 3B).

MV infection of osteoclasts from hCD46 transgenic mice

Freshly isolated bone marrow cells from the hCD46 transgenic mouse were infected with MV *in vitro* and then cultured to form OCL-like cells (23). Immunostaining of multinucleated cells formed in the hCD46 transgenic mouse bone marrow cultures demonstrated expression of MV nucleocapsid protein (Fig. 4A), confirming MV infection of OCL precursors. Furthermore, on electron microscopic analysis, MV particles were present in the nuclei of OCLs from hCD46 transgenic mice (Fig. 4B). MV nucleocapsid protein could not be detected in OCLs in marrow cultures from nontransgenic littermates (data not shown).

Phenotypic analysis of MV-infected osteoclasts

TRAP(+) OCLs formed in MV infected hCD46 transgenic mouse marrow cultures were significantly increased in size and contained increased numbers of nuclei per OCL compared with wild-type littermates (Fig. 5). The numbers of TRAP(+) mononuclear cells were also significantly increased in hCD46 mouse bone marrow cultures. As shown in Fig. 6A, quantitation of these data demonstrated that MV infection of hCD46 transgenic mouse marrow cultures induced a 2-fold increase in OCL numbers compared with uninfected cultures. In contrast, MV infection did not increase OCL formation in wild-type mouse marrow cultures. The hCD46 transgene did not adversely affect OCL formation compared with wild-type mouse marrow cultures. Furthermore, hCD46 transgenic mouse marrow cells that were infected with MV formed significantly more CFU-GM colonies than noninfected hCD46 marrow cells (134 ± 4 *vs.* 76 ± 7; $P < 0.05$).

OCLs formed in hCD46 mouse bone marrow cultures infected with MV contained 3-fold more nuclei per OCL compared with wild-type littermates (Fig. 6B). The number of nuclei per OCL in hCD46 mice and wild-type littermate bone marrow cultures that were not infected with MV did not differ significantly.

Bone resorption capacity of MV-infected osteoclasts

In addition, OCLs formed by hCD46 transgenic mouse bone marrow cells infected with MV demonstrated a significantly increased bone resorption capacity (Fig. 7A). The area resorbed by OCLs formed by hCD46 transgenic mouse bone marrow cells infected with MV was significantly increased compared with uninfected or wild-type littermates (Fig. 7B).

IL-6 levels in hCD46 mouse bone marrow cultures

IL-6 levels were measured in conditioned media obtained from MV-infected hCD46 transgenic mouse bone marrow cells cultured for 48 h as well as from marrow cultures containing OCLs using a commercial ELISA kit (R&D, Minneapolis, MN). As shown in Fig. 8A, MV infection of hCD46 transgenic mouse bone marrow cells demonstrated a 9-fold

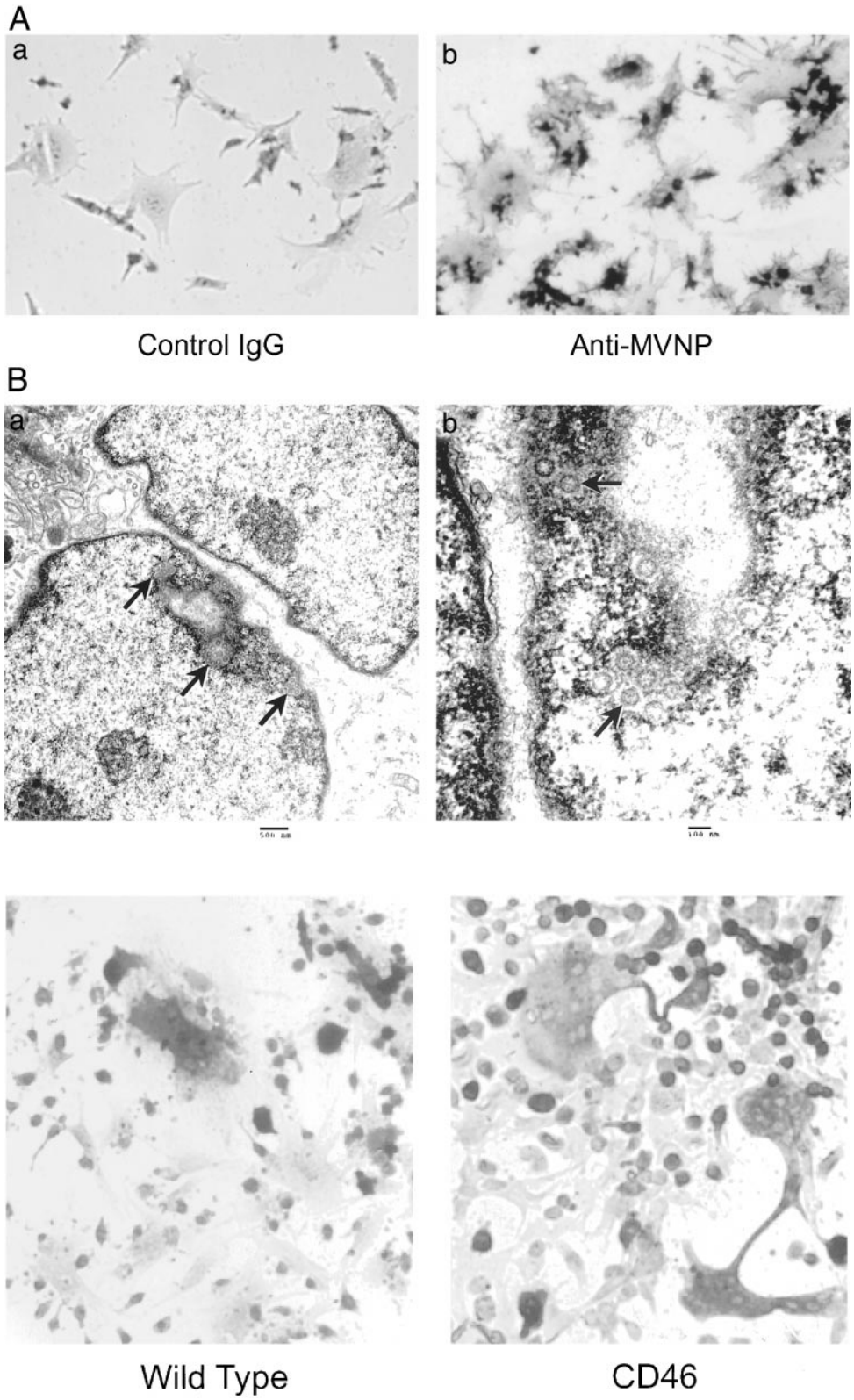

FIG. 4. A, *top panel*: Immunostaining of MV nucleocapsid protein expression in MV-infected osteoclast-like cells formed in hCD46 mouse bone marrow cultures. Immunocytochemical staining was performed as described in *Materials and Methods*. A positive reaction is denoted by the dark color. Mouse IgG of the same isotype was used for control cultures. Magnification 200×. B, *bottom panel:* Electron microscopy of MV-infected OCLs. Electron micrographs of osteoclasts formed in marrow cultures from hCD46 mouse marrow cells infected with MV. MV particles in the nuclei are shown by the *arrow*. Similar results were seen in two experiments.

FIG. 5. MV infection increases TRAP-positive cells in hCD46 transgenic mouse bone marrow cultures. Marrow cells from hCD46 mice and nontransgenic littermates were infected with MV and cultured with 1,25-$(OH)_2D_3$ (10^{-8} M) as described in *Materials and Methods*. Similar results were seen in three independent experiments. Magnification 200×.

increase in IL-6 production. In addition, IL-6 levels in MV-infected hCD46 transgenic mouse bone marrow cells cultured to form osteoclast-like cells in the presence of 10^{-8} M 1,25-$(OH)_2D_3$ showed a 3.5-fold increase in IL-6 levels compared with uninfected cultures. These data suggest that MV infection causes increased IL-6 production in cells in the OCL lineage. Furthermore, addition of a neutralizing antibody to murine IL-6 (anti-IL-6; 100 ng/ml) to MV-infected CD46

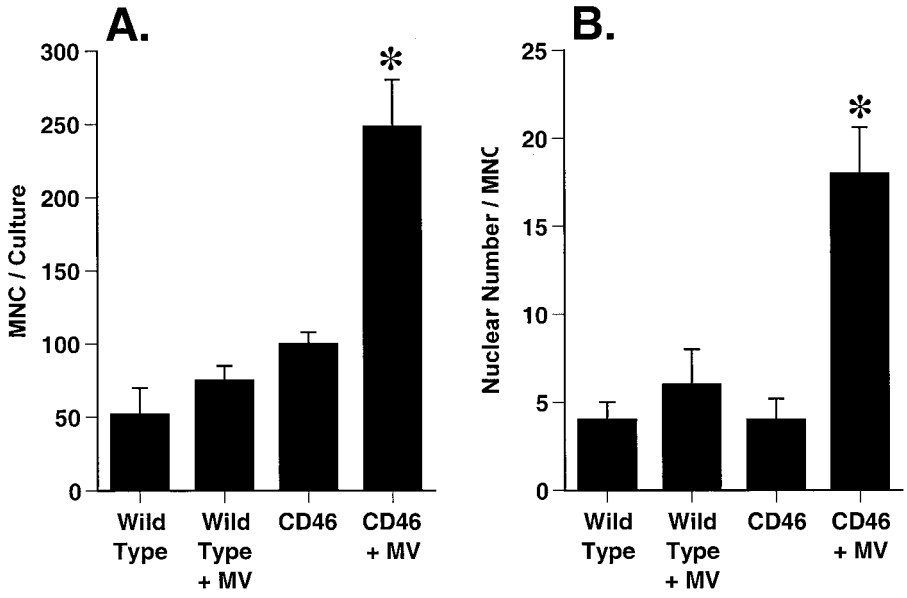

FIG. 6. MV infection increases osteoclast formation in hCD46 transgenic mouse bone marrow cultures. Marrow cells from hCD46 mice or nontransgenic littermates were infected with MV or were mock infected. The cells were cultured with 1,25-$(OH)_2D_3$ (10^{-8} M) to induce osteoclast formation. Results represent the mean ± SEM for quadruplicate cultures from a typical experiment. Similar results were seen in three independent experiments. *, $P < 0.05$. A, Osteoclast formation in hCD46 transgenic mouse bone marrow cells. B, Nuclei number in osteoclasts formed in hCD46 transgenic mouse bone marrow cultures. Results represent the mean ± SEM for quadruplicate determinations for a typical experiment. Similar results were seen in three independent experiments. *, $P < 0.05$.

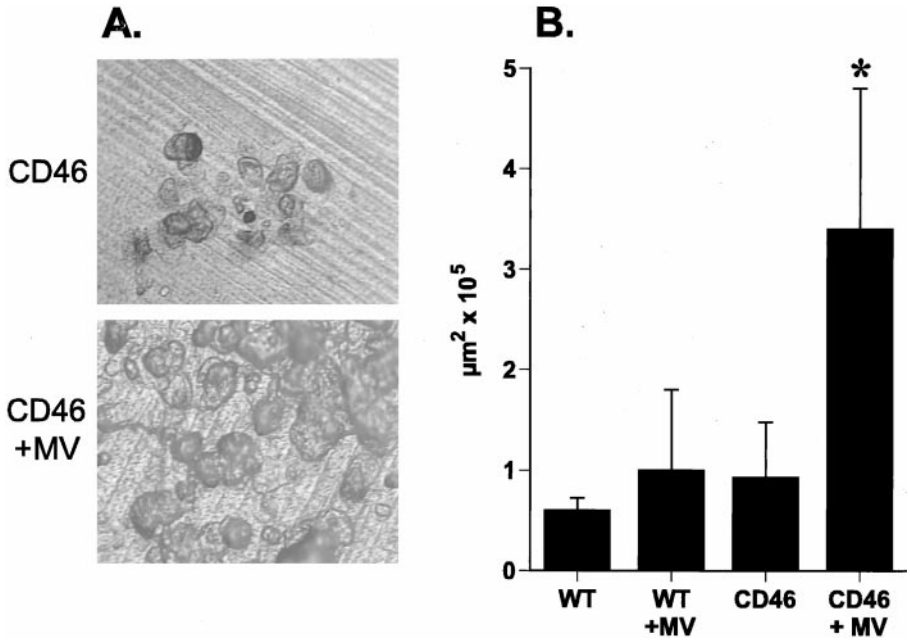

FIG. 7. MV infection increases bone resorption activity of osteoclasts in hCD46 mouse bone marrow cultures. Bone marrow cells from hCD46 mice or nontransgenic littermates (WT) were infected with MV or were mock infected, and then cultured for osteoclast formation. A, Cultures were overlaid with a dentin slice, and at the end of the culture period; B, the percentage of the dentin resorbed was determined, as described in *Materials and Methods*. Results represent the mean ± SEM for quadruplicate determinations for a typical experiment. Similar results were seen in three independent experiments. *, $P < 0.05$; Magnification 200×.

mouse bone marrow cultures significantly decreased OCL formation induced by 1,25-$(OH)_2D_3$. In contrast, anti-IL-6 did not significantly affect OCL formation in wild-type (WT) mouse bone marrow cultures (Fig. 8B).

RT-PCR analysis of 24-hydroxylase mRNA expression

We previously reported that OCL precursors from Paget's patients are hypersensitive to 1,25-$(OH)_2D_3$ (26). Therefore, using RT-PCR we examined 24-hydroxylase mRNA expression in hCD46 transgenic mouse bone marrow cells in response to MV infection. As shown in Fig. 9, RT-PCR analysis of total RNA isolated from MV-infected hCD46 transgenic mouse bone marrow cells in the presence of 10^{-8} M 1,25-$(OH)_2D_3$ demonstrated a 2-fold increase in 24-hydroxylase mRNA expression levels (lane 8), compared with MV-infected bone marrow cells derived from nontransgenic littermates (lane 4). Interestingly, even in the absence of 1,25-$(OH)_2D_3$, MV infection induced 24-hydroxylase mRNA expression in CD46 mouse bone marrow cultures (lane 7). In contrast, MV infection did not induce 24-hydroxylase mRNA expression in the mouse bone marrow cultures from nontransgenic littermates (lane 3). Furthermore, no significant expression of 24-hydroxylase mRNA was detected in bone marrow cells from nontransgenic littermates (lane 1) or hCD46 transgenic mice that were not exposed to 1,25-$(OH)_2D_3$ or MV (lanes 5 and 6). 1,25-$(OH)_2D_3$-induced expression of 24-hydroxylase in bone marrow cells from nontransgenic mice is shown in lane 2, and serves as positive control for these experiments. CD46 transgenic mouse bone marrow cells showed similar response to 1,25-$(OH)_2D_3$ (data

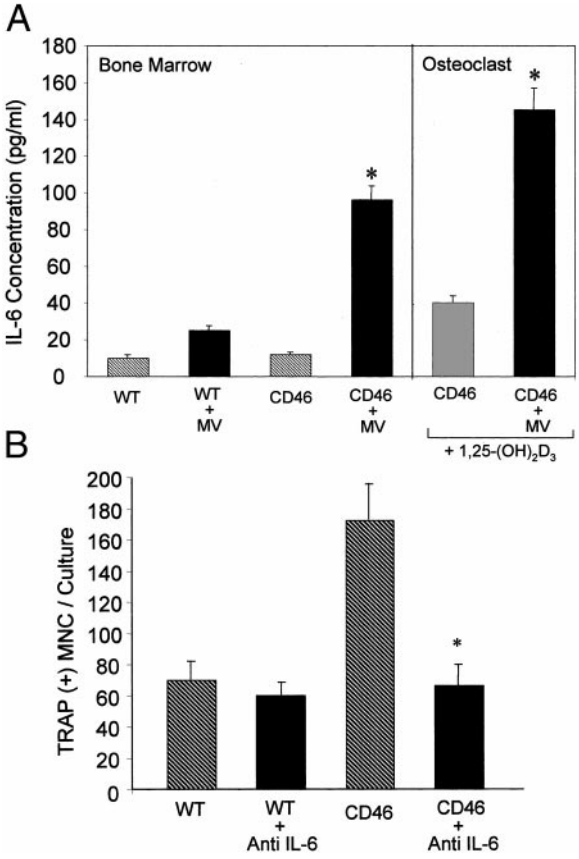

FIG. 8. A, IL-6 levels in MV-infected hCD46 transgenic mouse bone marrow cells and osteoclast cultures. Bone marrow cells obtained from nontransgenic (WT) or CD46 transgenic mice were infected with MV or mock infected, and conditioned media were collected after 48 h of culture. In studies with osteoclasts, bone marrow cells were cultured in the presence of 1,25-$(OH)_2D_3$ to form osteoclasts, and the conditioned media were collected after 7 days of culture when osteoclasts had formed. Results are shown as IL-6 concentration (pg/ml) present in the conditioned media and represent the mean of quadruplicate experiments as measured by ELISA. B, Effects of a mouse IL-6 neutralizing antibody on MV-induced osteoclast formation. Osteoclast cultures and MV infection were performed as described in *Materials and Methods*. In selected cultures, a neutralizing antibody to mouse IL-6 (100 ng/ml) was added to the cultures. *, $P < 0.01$.

not shown). However, the β-actin levels were not significantly altered in all the samples analyzed.

Discussion

Several lines of evidence suggest that the OCLs from patients with Paget's disease express MV or CDV nucleocapsid antigens and their corresponding mRNA transcripts. These abnormal OCLs are increased in size and contain increased numbers of nuclei per OCL. They have increased bone-resorbing capacity and are hypersensitive to 1,25-$(OH)_2D_3$. However, the role that MV infection plays in Paget's disease is unknown. Furthermore, there is no *in vivo* model available to test the potential pathogenetic role of the virus. Therefore, to begin to develop an *in vivo* model of Paget's disease, we targeted expression of hCD46 to the OCL lineage, using the mTRAP gene promoter, to allow testing the effects of MV on OCLs *in vivo* and *in vitro*. We found that OCL formation and

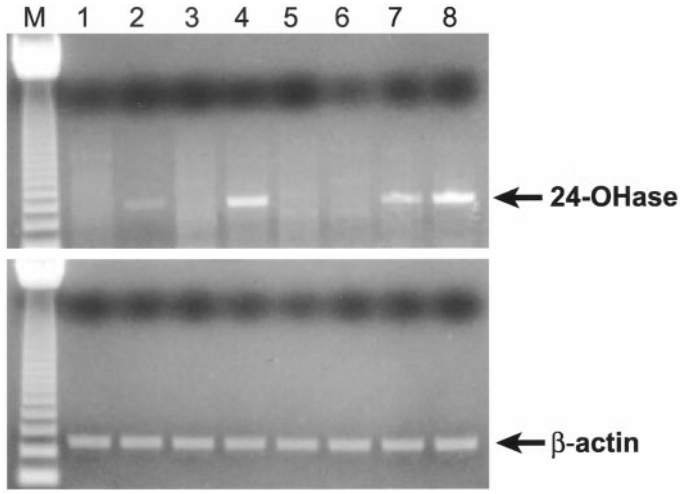

FIG. 9. RT-PCR analysis for 24-hydroxylase (24-OHase) mRNA expression in MV-infected bone marrow cells from hCD46 transgenic mice. Mouse 24-OHase mRNA was RT-PCR amplified using a sense primer (5′ AAG GAC ACA GAG GAA GAA GCC 3′) and an antisense primer (5′ GAA TGG CAC ACT TGG GGT AAG 3′), following the conditions as described in *Materials and Methods*. 24-OHase mRNA expression is shown in nontransgenic mouse bone marrow cells not treated with 1,25-$(OH)_2D_3$ (lane 1), cultured in the presence of 1,25-$(OH)_2D_3$ (lane 2), infected with MV (lane 3), or treated with a combination of 1,25-$(OH)_2D_3$ and MV (lane 4). Similarly, 24-OHase mRNA expression is shown in hCD46 mouse bone marrow cells not treated with 1,25-$(OH)_2D_3$ (lanes 5 and 6), infected with MV (lane 7), or treated with a combination of MV and 1,25-$(OH)_2D_3$ (lane 8). β-actin transcripts amplified from all the samples analyzed are shown in the *bottom panel*. M, DNA size marker.

bone resorption were significantly increased by hCD46 mouse marrow cells infected with MV, and that the OCLs were increased in number and size. In addition, we demonstrated the presence of MV nucleocapsids in these OCLs. These are all features of Paget's disease and support a potential pathogenetic role for MV in the abnormal OCL activity in Paget's disease. Similarly, Mee and co-workers (27) have previously shown that *in vitro* infection of canine marrow cells with CDV produced a dose-dependent increase in the number and size of OCL-like cells. Furthermore, they have also shown that CDV infection of canine bone marrow cells *in vitro* induces IL-6 and c-*fos* gene expression similar to that seen in pagetic OCLs.

We have previously shown that IL-6 is produced at high levels by pagetic OCLs and is an autocrine/paracrine factor that increases OCL formation in patients with Paget's disease (28). In the present study, MV infection enhanced IL-6 production by hCD46 OCL precursors, and a neutralizing antibody to IL-6 blocked the enhanced OCL formation seen in hCD46 marrow cultures infected by MV. These data demonstrate that increased production of IL-6 by MV infection is in part responsible for increased OCL formation/activity induced by MV in these studies. In agreement with the studies of Tamura *et al.* (29), IL-6 did not enhance OCL formation in marrow cultures from nontransgenic littermates.

Further support for a pathogenetic role for MV in Paget's disease is provided by our recent studies which showed that normal human OCL precursors (CFU-GM) transduced with retroviral vectors expressing the MV nucleocapsid gene, dis-

play a pagetic phenotype (20). Normal OCL precursors expressing the MV nucleocapsid gene formed OCLs more rapidly; formed large OCLs which contained many more nuclei than normal OCLs; were hypersensitive to 1,25-$(OH)_2D_3$; and had an increased bone-resorbing capacity compared with normal OCLs. In contrast, normal OCL precursors transduced with the MV matrix gene did not express an abnormal phenotype. In the present study, MV infection of bone marrow cells from hCD46 transgenic mice demonstrated increased levels of 24-hydroxylase mRNA expression in the absence of 1,25-$(OH)_2D_3$. These data further support the hypersensitivity of OCL precursors to 1,25-$(OH)_2D_3$ and a potential role for MV infection in this process.

Other cell culture models have also been used for studies of MV infections. MV infection of human macrophage-like cell line U937 resulted in prominent giant cell formation indicating that these cells are susceptible to viral-induced fusion (30). Recently, Korte-Sarfaty *et al.* (31) have shown that MV infection of the mouse macrophage cell line RAW264.7 expressing hCD46 resulted in cytopathologic infection with formation of extensive multinucleated cells. Furthermore, MV infection of human monocytes results in immunosuppression and cytokine responses that include a decrease in IL-12, IL-2, γ-interferon and an increase in production of IL-6 (32).

Our results suggest that the hCD46 transgenic mouse may be a useful model for examining the effects of MV infection on OCL formation and activity *in vivo*. Transgenic expression of MV receptor on neurons and infection with MV have been used to develop a mouse model for subacute sclerosing panencephalitis, a neurodegenerative disease (33).

Furthermore, macrophages in CD46 transgenic mice that also lack the α/β interferon receptor appear to be responsible for lymphatic dissemination of virus (34). Future studies will have to determine whether deletion of the α/β interferon receptor is required for *in vivo* MV infection of OCL precursors in our mouse model, because high levels of CD46 expression are sufficient for disseminated viral infection of CD46 transgenic mice (33).

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