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Tartrate-Resistant Acid Phosphatase Facilitates Hydroxyl Radical Formation and Colocalizes with Phagocytosed *Staphylococcus aureus* in Alveolar Macrophages

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Tartrate-resistant acid phosphatase (TRAP) is an enzyme expressed specifically in osteoclasts and activated macrophages, two phagocytosing cell types originating from the same hematopoietic stem cells. TRAP contains a binuclear iron centre which has been shown to generate reactive oxygen species (ROS). In this study murine macrophage like cell line RAW-264 overexpressing TRAP was shown to produce elevated levels of hydroxyl radicals compared to parental cells. TRAP transfected cells also had reduced growth rate indicating harmful effects of excessive intracellular ROS levels. Using TRAP specific antibody TRAP protein was shown in alveolar macrophages partially colocalize with late endosomal/lysosomal markers Rab7, Lamp 1 and MHC II molecules that bind antigenic peptides. TRAP also colocalized into compartments where *Staphylococcus aureus* were phagocytosed. These results suggest that TRAP may have an important biological function in the defence mechanism of macrophages by generating intracellular ROS which would be targeted to destroy phagocytosed foreign material.

Key Words: **tartrate-resistant acid phosphatase; ROS; macrophages; antigen presentation.**

Tartrate-resistant acid phosphatase (TRAP EC 3.1.3.2) is an enzyme which is characteristic for osteoclasts (1, 2) and activated macrophages (3). It is also expressed in diverse murine tissues, where it is found in dendritic cells (4). Although the biological function of TRAP is not known, there is evidence that it functions as a protein tyrosine phosphatase (5–7), and that it has a role in iron metabolism. At the transcriptional level the iron is one regulator of TRAP expression (8, 9). It has been also shown, that a binuclear iron centre of TRAP is able to produce hydroxyl radicals from hydrogen peroxide (H_2O_2) via Fenton reaction (10). In osteoclasts these radicals are suggested to participate in destruction of endocytosed bone matrix degradation products (11–13). In resorbing osteoclasts degradation products of bone are transported from the ruffled border through transcytotic route to the cell surface. Ruffled border has indicated to represent late endosomal/lysosomal characteristics (14). An analogous transport route may be involved in macrophages in the antigen presentation process, where antigenic peptides bind to MHC II molecules, and are transported to cell membrane for T-cell recognizition.

After internalization of soluble antigens by endocytosis, MHC II-peptide complexes may be formed in a variety of endosomal or lysosomal compartments, although the majority of this function appears to occur in late endocytic compartments (15–17). Phagosomes themselves have little microbicidal activity, and this activity is delivered to the phagosome through a maturation process that involves a series of complex fusion events with ultimate fusion of terminal lysosomes to form phagolysosome. Phagosome maturation results in strong intravesicular acidification, appearance of lysosomal proteolytic activity, and generation of reactive oxygen radicals. These oxygen species include superoxide ($\bullet O_2^-$, H_2O_2, hydroxyl radicals ($\bullet$OH), singlet oxygen, and nitric oxide and they are believed to play an important role in microbicidal activity of the phagocytic cells.

Conservative structure of the iron centre, wide distribution of TRAP-like enzymes through primitive animals to higher plants and mammals, and a restricted expression of TRAP in macrophage lineage suggest physiological function for it. Evidence of the participation of TRAP in the inflammatory response rises from the studies with knockout mice lacking TRAP. TRAP knockout mice showed delayed clearance of the *Staphylococcus aureus in vivo* (18). As macrophages lacking TRAP were able to phagocytose and kill *S. aureus* normally *in vitro,* TRAP was suggested to influence

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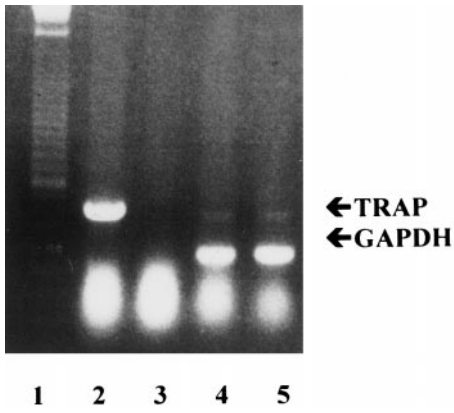

FIG. 1. TRAP gene expression in transfected RAW-264 cells. cDNA from RT-reactions were amplified with human TRAP and mouse GAPDH primers and PCR products were analyzed on an agarose gel. The lanes contained the following: 1, 100 base-pair ladder (Pharmacia); 2 and 4, TRAP cells; 3 and 5, RAW-264 cells.

recruitment of macrophages to sites of microbial invasion.

In this study we have used specific anti-TRAP antibody to analyze the subcellular localization of TRAP protein in alveolar macrophages by immunofluorescence and confocal microscopy, using a series of markers corresponding to known cellular compartments. We also created TRAP overexpressing macrophage cell line which was shown to produce elevated levels of hydroxyl radicals and have reduced growth rate compared to parental cells. Our data presented here support the role of TRAP in the immune defence system.

MATERIALS AND METHODS

Cell culture and transfection. Alveolar macrophages were obtained by bronchoalveolar lavage from patients investigated for respiratory diseases. Cells were collected by centrifucation, suspensed to DMEM medium (Sigma, St. Louis, MO) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (BioClear UK, Wilts, UK). MHC II expression was maintained by the addition of recombinant human interferon-γ (25 ng/ml). Cells were grown on glass coverslips over night before fixation or treatment with bacteria or transferrin.

Staphylococcus aureus (Molecular Probes, Leiden, The Netherlands) stock solution was prepared according to manufacturer's instructions. For working solution, 1:20 dilution to DMEM was used. Macrophages were infected with bacteria for 10 to 60 min before fixation.

For transferrin endocytosis, cells were incubated with transferrin (0.1 mg/ml) in DMEM for 10 min, changed to fresh medium and incubated further for 5–50 min before fixation.

The murine macrophage-like RAW-264 cells were routinely grown in Hepes-buffered DMEM medium (Sigma) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (BioClear UK, Wilts, UK).

The expression plasmid contained human TRAP cDNA and it was a generous gift from Dr. Sakamurai Reddy (University of Texas Health Science Center, San Antonio, TX). Cells were transfected using FuGene-reagent (Roche) and selected by 400 μg/ml geneticin (G418; Calbiochem-Novabiochem, San Diego, CA), replacing the antibiotic containing medium every 3 days over 14 days. Isolated clones were maintained in 200 μg/ml geneticin.

TRAP-staining. Cells were cultured on 35-mm dishes, rinsed with phosphate-buffered saline (PBS), fixed with 3% paraformaldehyde (15 min), and stained for TRAP using a Sigma TRAP kit (No. 386-A, Sigma) according to the manufacturer's instruction.

RNA isolation and RT-PCR. mRNA was isolated from the cells using QuickPrep mRNA isolation kit (Pharmacia, Uppsala, Sweden). Ethanol precipitated RNAs were dissolved in diethyl pyrocarbonate (DEPC) treated water. First-strand cDNA was synthesized in a 20-μl reaction mixture containing 15 μl total RNA, 0.5 mM dNTP (Pharmacia, Uppsala, Sweden), 0.5 μg random hexamer (Promega, Madison, WI), 2 μl of AMV buffer (50 mM Tris–HCl, pH 8.3, 6 mM $MgCl_2$, 40 mM KCl, 4.0 mM DTT) and 20 units AMV reverse transcriptase (Finnzymes, Espoo, Finland). The mixture was incubated at 42°C for 60 min.

Human TRAP sense and antisense primers were used to amplify about 680-nt TRAP-fragment and glyseraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used to control the amount of RNA. PCR was carried out in 50 μl reaction mixture containing 5 μl PCR buffer (10 mM Tris–HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 0.2 mM dNTP, 0.8 μM of sense and antisense primers, 2 μl of RT cDNA, 1 unit DyNAzyme DNA polymerase (Finnzymes Espoo, Finland), and 38.5 μl sterile distilled H_2O. PCR was conducted in a Peltier Thermal Cycler (MJ Research, Watertown, USA) for 27 cycles. After RT-PCR, samples were electrophoresed on 1% agarose gel containing 10 μg/ml ethidiumbromide and viewed under UV-light.

Cellular morphology. Cells were cultured and fixed as mentioned above, stained with toluidin blue, and analysed by image analysis system (MCDI/M2, Imaging Research Inc., Brock University, Ontario, Canada). 50 cells for each assay were analysed.

Measurement of hydroxyl radical production. Hydroxyl radical production of the cells was measured by the method introduced by Halliwell and Gutteridge (19) and modified by Greenwald *et al.* (20). This method is based on the degradation of deoxyribose by reaction with hydroxyl radical to form a colored end product. Briefly, three million cells were incubated for 1 h at 37°C in a 1 ml reaction mixture containing 10 mM deoxyribose, 0.1 mM ascorbate, and 1 mM H_2O_2 in PBS, pH 7.2. EDTA-chelated iron (100 μM) was used as a positive control. Colour was developed by heating for 15 min at 100°C in thiobarbituric acid and trichloroacetic acid. Degradation of deoxyribose was determined by measurement of absorbance at 532 nm.

[^{3}H]thymidine uptake. To measure the rate of [^{3}H]thymidine incorporation into cells, 5×10^3 cells/well were plated onto 96-well plates with DMEM. After 24, 48, and 72 h [^{3}H]thymidine (0.2 μCi/well) was added to the medium and cells were cultured 2 h before measuring the radioactivity incorporated into DNA with MicroBeta Instrument (Wallac, Finland). Also parallel experiments were performed where after 24 h culture, 50 or 100 μM H_2O_2 was added to the cells.

Immunofluorescence staining. Cells were rinsed with PBS, fixed with 3% paraformaldehyde (15 min), permeabilized with 0.2% Triton X-100 (5 min), and blocked with 1% egg white albumin/0.2% gelatine/PBS over night at +4°C. Primary antibodies were diluted with 0.5% BSA/0.2% gelatine/PBS in the following way: anti-TRAP rabbit polyclonal antiserum (21) 1:500, anti-MHC II rat monoclonal (Serotec, Oxford, UK) 1:1000, anti-*S. aureus* mouse monoclonal (QED Bioscience Inc.) 1:500, anti-Lamp 1 and 2 goat polyclonal (Santa Cruz Biotechnology) 1:500, anti-EEA1 mouse monoclonal 1:30 (Santa Cruz Biotechnology), anti-RAB7 goat polyclonal (Santa Cruz Biotechnology) 1:10, and anti-transferrin mouse monoclonal (BioGenex) 1:30.

Primary antibody incubations (2 h) were followed by anti-mouse, anti-rabbit, anti-goat or anti-rat secondary antibodies conjugated to rhodamine or FITC (Jackson ImmunocResearch) in 1:100 dilution (30 min). Samples were embedded in 80% glyserol/PBS, and analyzed with Leica TCS-SP confocal laser scanning microscope

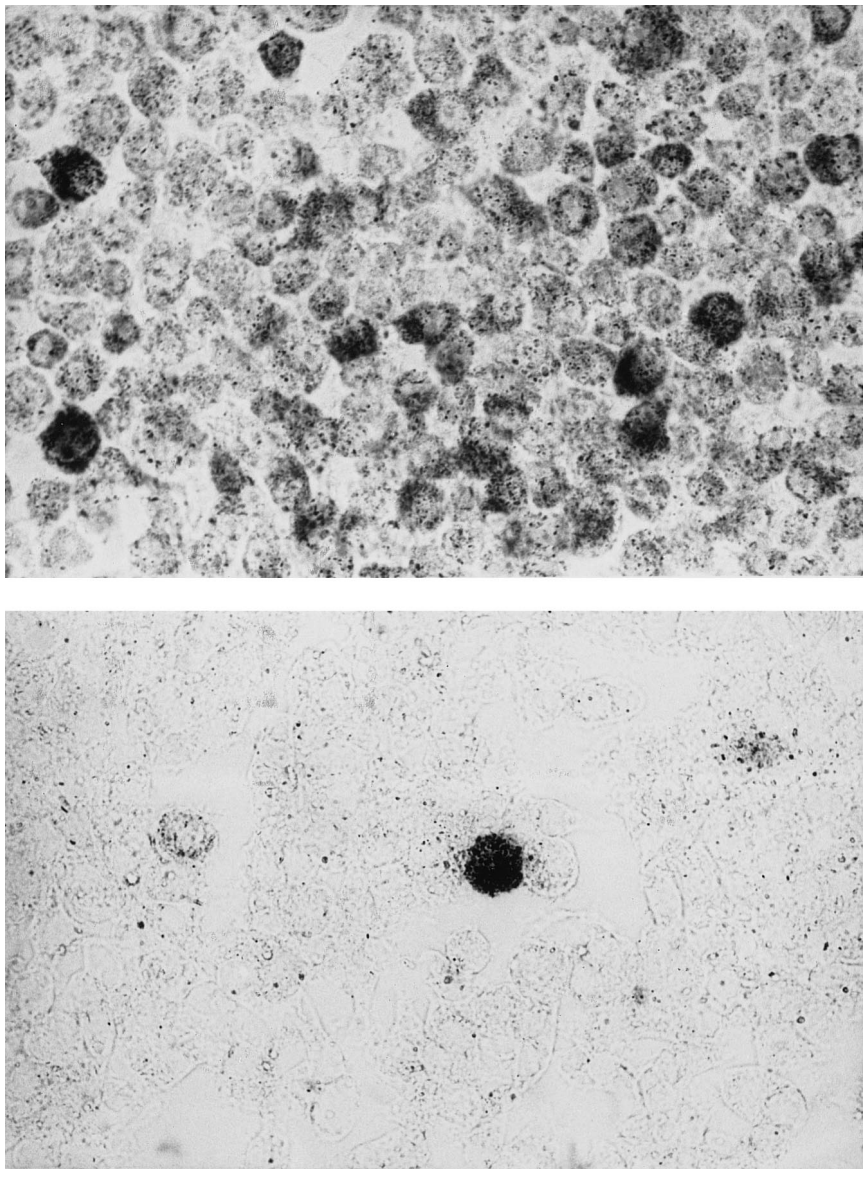

FIG. 2. TRAP-stained RAW-264 cells. (A) Transfected cells, (B) parental cells.

equipped with Argon-Krypton laser (Leica Microsystems Heidelconfocal laser scanning microscope (Leica Microsystems Heidelberg GmbH, Heidelberg, Germany).

RESULTS

Overexpression of TRAP in RAW cells. Human TRAP cDNA was transfected into murine macrophage-like RAW-264 cells in order to get stable TRAP overexpressing cell line. After selection four geneticin-resistant clones were obtained, and the clone with the highest level of TRAP protein was chosen for further experiments.

RT-PCR was used to detect TRAP mRNA expression in cells. The TRAP specific primers produced a 680-nt fragment in TRAP cells while no product was seen in RAW cells, which suggests that endogenous TRAP expression in parental cells was too low to be detected (Fig. 1).

Histochemical staining showed quite intense TRAP-activity in transfected cells whereas only some positive cells were detected in parental cell line (Fig. 2).

In accordance to histochemical staining, immunofluorescence staining with polyclonal human TRAP antiserum showed that TRAP-protein was expressed in high levels in transfected cells (Fig. 3).

Cellular morphology. Because TRAP-overexpressing cells were observed to have altered phenotype compared to the parental cells, morphological analysis with image analysis system was performed. Four different parame-

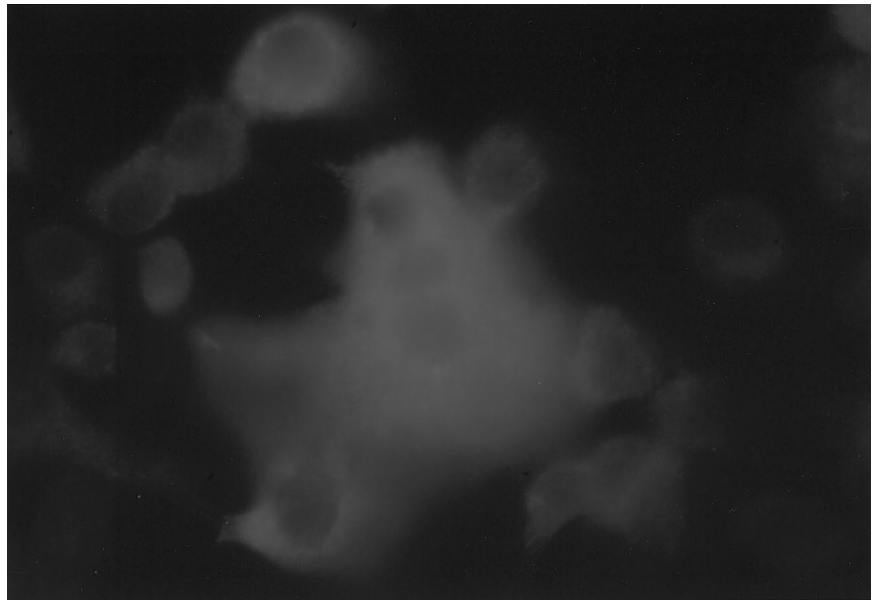

FIG. 3. Immunofluorescence staining for detection of TRAP protein in transfected RAW-264 cells. Fixed cells were stained with rabbit anti-human TRAP antiserum followed by rhodamine-conjugated anti-rabbit Ig.

ters were measured, in which three were altered in transfected cells (Fig. 4). TRAP-cells had less projections than parental cells, which was indicated in decreased form factor (form factor value 1 represents round form). Missing projections apparently caused the decreased area and average perimeter also detected in TRAP-cells. Average diameter in transfected cells was not statistically different compared to parental cells (data not shown).

Hydroxyl radical production. To test if the expressed TRAP-protein was able to produce hydroxyl radicals from H_2O_2, the deoxyribose-method was used to compare the hydroxyl radical production in transfected and parental cells. As shown in Fig. 5, TRAP-overexpressing cells produced significantly more hydroxyl radicals than parental cell line.

Cell growth kinetics. Since ROS are known to be involved in the control of cell proliferation, the growth rate of TRAP overexpressing cells and parental cells was compared by measuring the uptake of [^{3}H]thymidine. TRAP cells grew significantly slower than control cells in all three time points as shown in Fig. 6. Cell growth was compared also after addition of H_2O_2 which can serve as a substrate for hydroxyl radical production by TRAP. Addition of 50 μM H_2O_2 did not affect the growth of parental cells whereas in TRAP-cells growth was reduced at time point 48 h and 72 h by 8.5% and 21%, respectively. A 100 μM concentration of H_2O_2 caused growth reduction also in parental cells which was detected at 48 h time point but this reduction was overcome at time point 72 h. In TRAP-cells higher concentration of H_2O_2 reduced the growth rate more than in parental cells, and this effect was not reversed at time point 72 h.

Subcellular localization of TRAP in alveolar macrophages. To characterize the TRAP containing subcellular compartments in macrophages, colocalization studies with an anti-TRAP antiserum, and a number of antibodies directed against known cellular structures were performed. These experiments were done in alveolar macrophages in order to exclude possible mistargeting of TRAP due to overexpression.

Alveolar macrophages showed intensive vesicular TRAP staining. This staining pattern was distinct from the staining for EEA 1 (early endosomal autoantigen 1) (Fig. 7A). On the other hand, TRAP staining shared clear but not compete colocalization with small GTP binding protein Rab7, which is considered as a late endosomal marker (Fig. 7B). Furthermore, TRAP was colocalized in some large compartments with late endosomal/early lysosomal marker Lamp 1 (Fig. 7C). Double staining of TRAP and MHC II revealed that they colocalized in some rather large vesicles but most of the MHC II positive vesicles did not contain TRAP (Fig. 7D). Endocytosed transferrin colocalized with TRAP in small vesicles and also in some large roundish compartments (Fig. 7E). By confocal microscopy it was not clear whether these structures were single cell compartments or gatherings of small vesicles. When macrophages were infected with heat-killed *S. aureus,* the phagocytosed bacteria were localized partially to the same compartments with TRAP within 15 min, and this colocalization was continued during the following

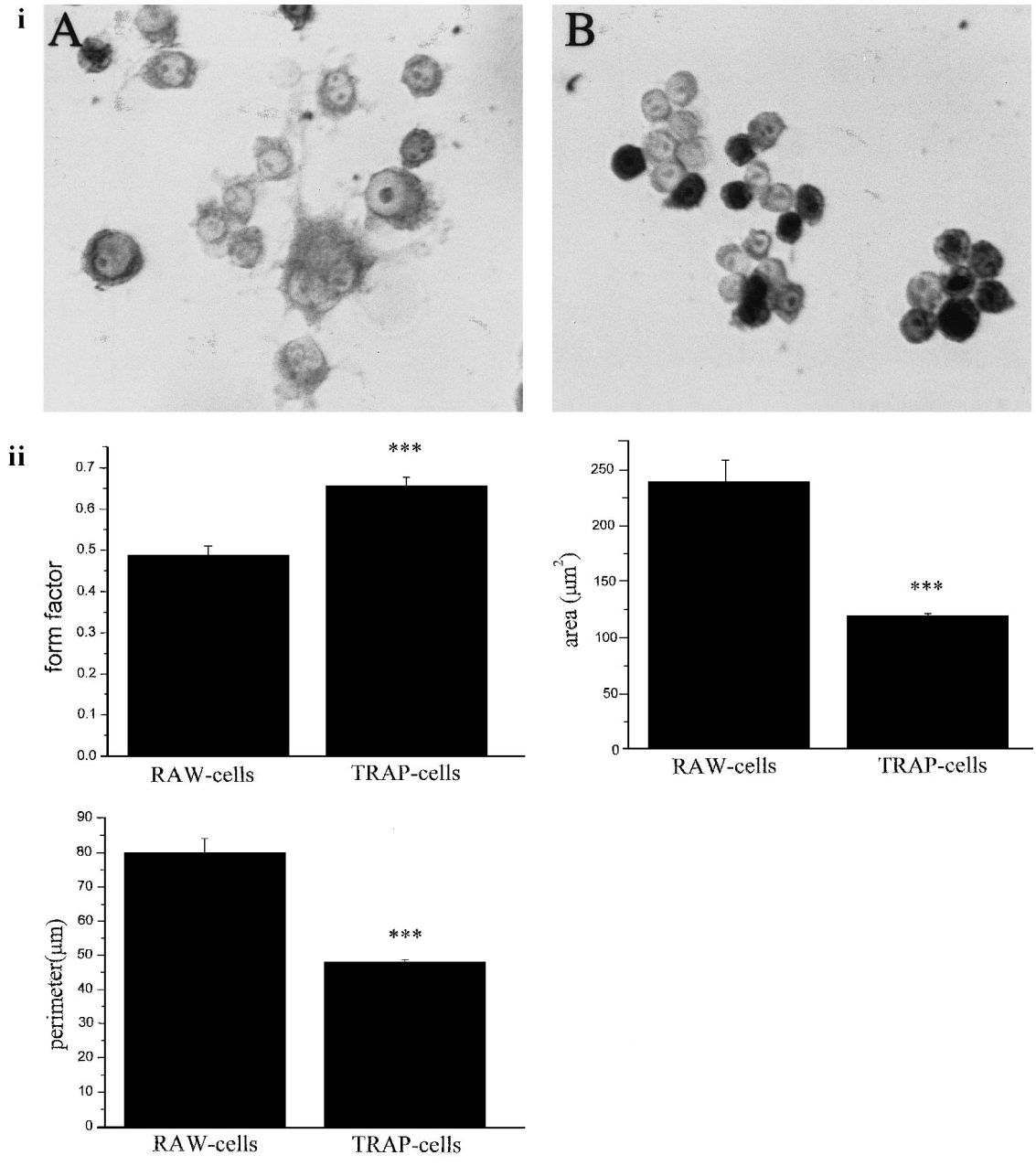

FIG. 4. Cellular morphology of TRAP overexpressing cells. (i) Toluidin blue stained parental cells (A) and TRAP overexpressing cells (B). (ii) Morphological parameters of the cells assayed with image analysis system as described under Materials and Methods. Data are expressed as means ± SD, $n = 50$, *** $P < 0.001$.

60 min period (Fig. 7F). Similar large structures as with transferrin staining were observed.

DISCUSSION

TRAP is widely used histochemical marker for osteoclasts and activated macrophages, particularly alveolar macrophages. Alveolar macrophages, which are continuously exposed to noxious and infectious materials are known to express TRAP also in normal physiological conditions (22). Pathologically increased TRAP expression occurs in cells of hairy cell leukemia (23, 24) and Gauchers's disease (25). In human monocyte-derived macrophages TRAP has been localized to lysosomes as shown by colocalization with lysosomal membrane marker Lamp1 (26). In this study we wanted to define the localization of TRAP in macrophages, particularly its possible presence in the phagosomes. Our immunohistochemical data indicate that TRAP is localized in a heterogenous group of intracel-

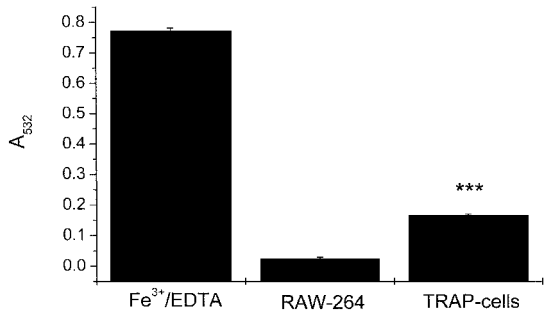

FIG. 5. Hydroxyl radical production of TRAP transfected cells. •OH production was detected by measuring the formation of colored end product (A_{532}) from deoxyribose fragments. Data are expressed as means ± SD, $n = 6$, $^{***}P < 0.001$.

lular vesicles. Double staining experiments revealed that many of these vesicles also express late endosomal/lysosomal characteristics. More importantly, we observed that endocytosed *S. aureus* entered into large TRAP containing phagosomes.

RAW-264 cells transfected with TRAP cDNA expressed TRAP mRNA and protein, but this expression was lost in a few months. In a previous study TRAP was transiently expressed in hamster cells, and Nuthmann *et al.* reported failure in stable expression of TRAP (27). They suggested that the inability to establish stably transfected cell line could be caused by negative effects of TRAP expression in the host cells. We were able to maintain expression few months, so probably TRAP expression itself was not deleterious to the cells. We found out that CMV promoter we had in our expression vector switches off quite quickly in RAW-264 cells (28) explaining the transient expression. The changes in the cell morphology of TRAP-overexpressing RAW-264 cells could be specific to TRAP protein or they might be only side effects of transfection. Nuthmann *et al.* did not detect any alterations in cell morphology of TRAP-overexpressing BHK-21 cells as observed by phase-contrast microscopy.

TRAP-overexpressing cells showed increased hydroxyl radical production compared to parental cells, as expected according to previous studies. Because TRAP in its native reduced form has an Fe(III)-Fe(II) active site, the ferrous ion is able to react with hydrogen peroxide to produce a ferric ion and a hydroxyl radical. The ferric ion formed can further react with hydroxen peroxide (29) to form a superoxide anion and a ferrous ion. Thus a sequence of reactions generating both hydroxyl radicals and superoxide anion may occur as long as hydroxen peroxide is available. The potential activity of TRAP as a Fenton catalyst was first recognized in studies of porcine enzyme (30). Later Hayman *et al.* demonstrated that recombinant human TRAP is active in the catalysis of luminol peroxidation indicating that iron centre is accessible for ferroxidation (31). Furthermore, in previous studies we have shown that recombinant rat bone TRAP and iron-chelated EDTA were approximately equally potent in facilitating hydroxyl radical formation from H_2O_2 (13). In macrophages these hydroxyl radicals may participate in de-

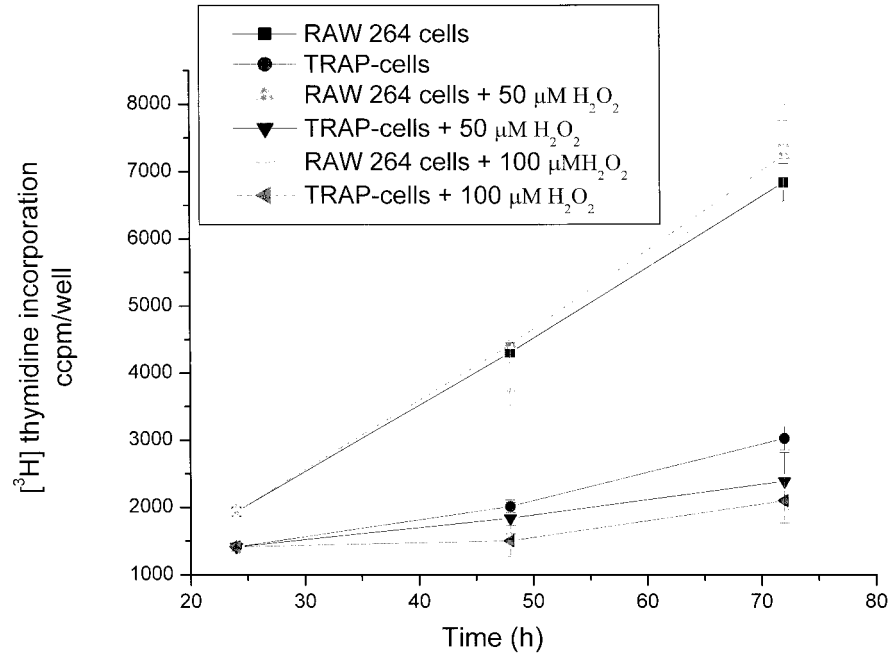

FIG. 6. Growth curves of TRAP overexpressing and parental cells. [^{3}H] thymidine incorporation into cells growing on 96-well plates was measured over 72 h period. In parallel experiments 50 or 100 μM H_2O_2 was added to the cells after 24 h culture.

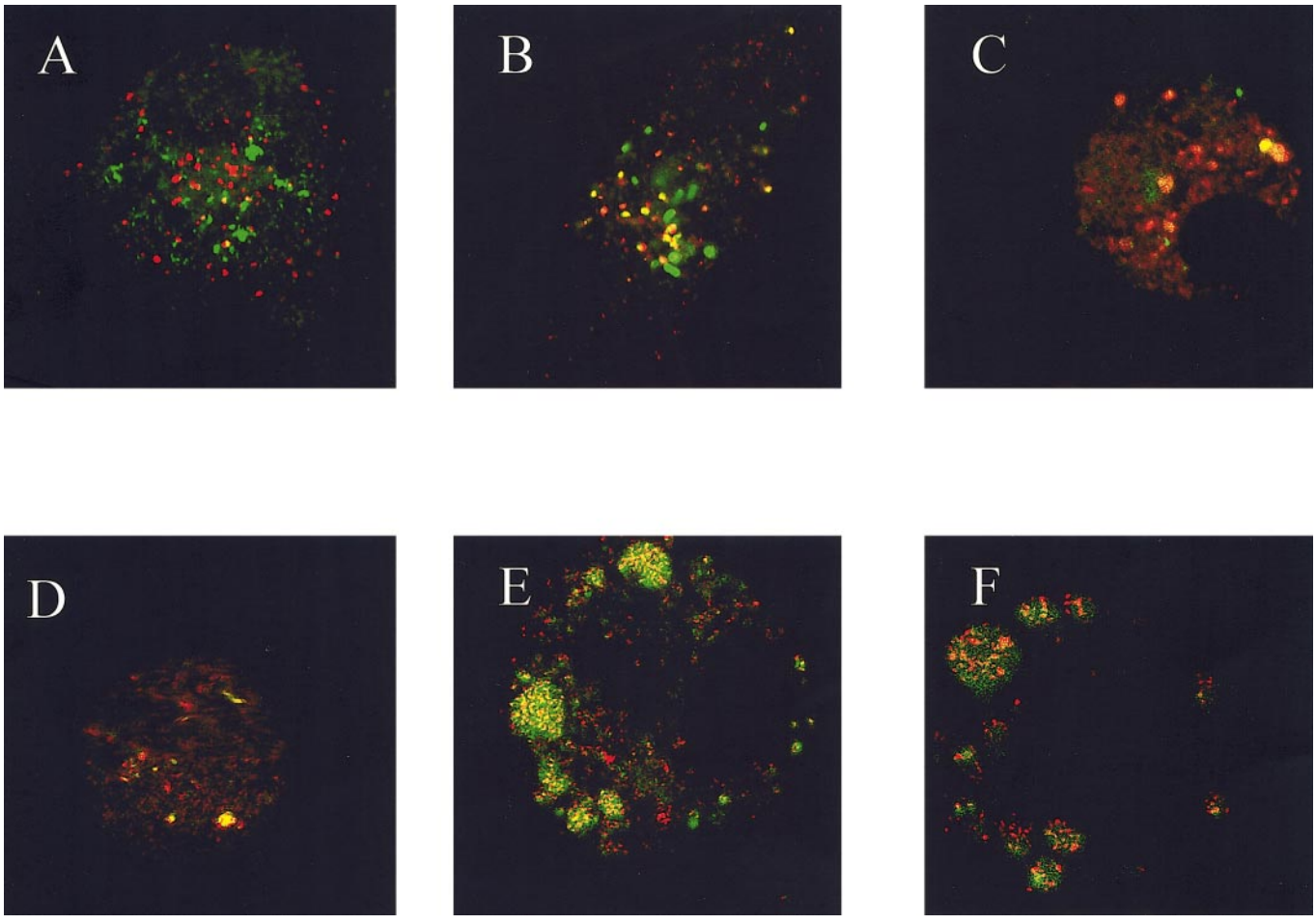

FIG. 7. Subcellular localization of TRAP in alveolar macrophages. Double-staining with TRAP protein and markers of known cellular compartments. (A) TRAP (green) and EEA1 (red), (B), TRAP (red) and Rab7 (green), (C) TRAP (red) and Lamp1 (green), (D) TRAP (green) and MHC II (red), (E) TRAP (green) and endocytosed transferrin (red), (F) TRAP (green) and phagocytosed *S. aureus* (red). FITC (green) and rhodamine (red) images were overlaid to identify colocalization (yellow).

struction of phagocytosed foreign material similarly as they function in bone matrix degradation. Interestingly, Schindelmeiser *et al.* (32) reported TRAP expression together with nitric oxide synthase I (NOS-I) in the superficial cells of the human ureter. They suggested that nitric oxide generated by NOS-I and superoxide anion produced by TRAP form peroxynitrite, which is strong oxidant and may be directed against pathogenic microorganisms of the urine. The importance of hydroxyl radicals produced by Fenton type reaction in microbicidal activity of macrophages is supported by the recent findings, where Nramp 1 (natural resistance-associated macrophage protein 1) was found to be recruited to the membrane of phagosome upon *Mycobacterium* phagocytosis, and transport iron into the bacterium containing phagosome. This iron was suggested to catalyse the hydroxyl radical production by Fenton/Haber–Weiss reaction, and account for the increased capacity of the cells to limit mycobacterial growth (33). In TRAP-deficient mice after stimulus increased superoxide and nitrite production was observed (18). However, TRAP-deficient mice showed reduced capacity to clear bacterial pathogens from the peritoneal cavity *in vivo*. This suggests that the enhancement of other ROS production could not overcome the defect in immune system in TRAP-lacking mice.

TRAP-overexpressing cells showed reduced growth rate compared to parental cells. This might be only secondary effect due to exogenous DNA insertion to the genome. On the other hand, also transiently TRAP expressing BHK-21 cells grew at a 50% lower rate than the control cells (27). Furthermore, ROS are known to participate to the control of cell proliferation; they stimulate or inhibit cell growth depending on the intracellular redox state. A certain ROS concentration is physiological, and oxygen radicals are used as second messengers to activate many transcription factors. However, an excess of ROS is harmful to cells as they damage DNA, RNA, protein and lipids. Cells can coun-

teract oxidative stress by antioxidant enzymes and molecules. In our previous study we overexpressed carbonic anhydrase III in NIH/3T3 cells, and noticed increased growth rate compared to parental cells. In these cells also lowered level of free radicals was detected indicating antioxidant capacity of carbonic anhydrase III (34). Relatively high concentration of H_2O_2 is available at the sites of inflammations. Thus TRAP has substrate for hydroxyl radical production, which can have direct destructive function, and can also have a role in cellular signalling.

In summary, colocalization of *S. aureus* and TRAP in large phagosomes indicates that ROS production catalysed by TRAP could be used to enhance cells' ability to destroy phagocytosed bacteria and other material. It remains to be studied if the presence of TRAP in the antigen presentation route also indicates a more defined role for it in the processing and presentation of antigens.

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REFERENCES

1. Minkin, C. (1982) Bone acid phosphatase: Tartrate-resistant acid phosphatase as a marker of osteoclast function. *Calcif. Tissue Int.* **34,** 285–290.
2. Yaziji, H., Janckila, A. J., Lear, S. C., Martin, A. W., and Yam, L. T. (1995) Immunohistochemical detection of tartrate-resistant acid phosphatase in non-hematopoietic human tissues. *Am. J. Clin. Pathol.* **104,** 397–402.
3. Efstratiadis, T., and Moss, D. W. (1985) Tartrate-resistant acid phosphatase in human alveolar macrophages. *Enzyme* **34,** 140–143.
4. Hayman, A. R., Bune, A. J., Bradley, J. R., Rashbass, J., and Cox, T. M. (2000) Osteoclastic tratrate-resistant acid phosphatase (Acp5): Its localization to dentritic cells and diverse murine tissues. *J. Histochem. Cytochem.* **48,** 219–227.
5. Lau, K. H. W., Farley, J. R., and Baylink, D. J. (1985) Phosphotyrosyl specific protein phosphatase activity of a bovine skeletal acid phosphatase isoenzyme. *J. Biol. Chem.* **260,** 4653–4660.
6. Janckila, A. J., Woodford, T. A., Lam, K. W., Li, C. Y., and Yam, L. T. (1992) Protein-tyrosine acid phosphatase activity of hairy cell tartrate-resistant acid phosphatase. *Leukemia* **6,** 199–203.
7. Ek-Rylander, B., Flores, M., Wendel, M., Heinegård, D., and Andersson, G. (1994) Dephosphorylation of osteopontin and bone sialoprotein by osteoclastic tartate-resistant acid phosphatase. Modulation of osteoclastic adhesion in vitro. *J. Biol. Chem.* **269,** 14853–14856.
8. Alcantara, O., Reddy, S., Roodman, G., and Boldt, D. (1994) Transcriptional regulation of tartrate-resistant acid phosphatase gene (TRAP) by iron. *Biochem. J.* **298,** 421–425.
9. Reddy, S., Hundley, J., Windle, J. J., Alcantara, O., Linn, R., Leach, R., Boldt, D. H., and Roodman, G. D. (1995) Characterization of the mouse tartrate-resistant acid phosphatase (TRAP) gene promoter. *J. Bone Miner. Res.* **10,** 601–606.
10. Sibille, J.-C., Doi, K., and Aisen, P. (1987) Hydroxyl radical formation and iron binding proteins. *J. Biol. Chem.* **262,** 59–62.
11. Salo, J. J., Lehenkari, P., Mulari, M., Metsikkö, K., and Väänänen, H. K. (1997) Removal of osteoclast bone resorption products by transcytosis. *Science* **276,** 270–273.
12. Nesbitt, S. A., and Horton, M. A. (1997) Trafficking of matrix collagens through bone-resorbing osteoclasts. *Science* **276,** 266–269.
13. Halleen, J. M., Räisänen, S., Salo, J. J., Reddy, S., Roodman, G. D., Hentunen, T. A., Lehenkari, P. P., Kaija, H., Vihko, P., and Väänänen, H. K. (1999) Intracellular fragmentation of bone resorption products by reactive oxygen species generated by osteoclastic tartrate-resistant acid phosphatase in osteoclasts during bone resorption. *J. Biol. Chem.* **247,** 22907–22910.
14. Palokangas, H., Mulari, M., and Väänänen, H. K. (1997) Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. *J. Cell Sci.* **110,** 1767–1780.
15. Peters, P. J., Neefjes, J. J., Oorschot, V., Ploegh, H. I., and Geuze, H. J. (1991) Segregation of MHC class II molecules from MHC class I molecules in Golgi complex for transport to lysosomal compartments. *Nature* **349,** 669–676.
16. Germain, R. N., and Margulies, D. H. (1993) The biochemistry and cell biology of antigen processing and presentation. *Annu. Rev. Immunol.* **11,** 403–450.
17. Bakke, O., and Nordeng, T. (1999) Intracellular traffic to compartments for MHC class II peptide loading: Signals for endosomal and polarized sorting. *Immunol. Rev.* **172,** 171–186.
18. Bune, A. J., Hayman, A. R., Evans, M. J., and Cox, T. M. (2001) Mice lacking tartrate-resistant acid phosphatase (Acp 5) have disordered macrophage inflammatory responses and reduced clearance of the pathogen, *Staphylococcus aureus*. *Immunology* **102,** 103–113.
19. Halliwell, B., Gutteridge, J., and Aruoma, O. (1987) The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* **165,** 215–219.
20. Greenwald, R., Rush, S., Moak, S., and Weitz, Z. (1989) Conversion of superoxide generated by polymorphonuclear leucocytes to hydroxyl radical: A direct spectrophotometric detection system based on degradation of deoxyribose. *Free Rad. Biol. Med.* **6,** 385–392.
21. Halleen, J., Hentunen, T., Hellman, J., and Väänänen, H. K. (1996) Tartrate-Resistant Acid Phosphatase from human bone: Purification and development of an immunoassay. *J. Bone Miner. Res.* **11,** 1444–1452.
22. Schindelmeiser, J., Schewe, P., Zonka, T., and Müstermann, D. (1989) Histochemical and immunological demonstration of purple acid phosphatase in human and bovine alveolar macrophages. *Histochemistry* **92,** 81–85.
23. Yam, L. T., Li, C. Y., and Lam, K. W. (1971) Tartrate-resistant acid phosphatase isoenzyme in the reticulum cells of leucemic reticuloendotheliosis. *N. Engl. J. Med.* **284,** 357–360.
24. Ketcham, C.M., Baumbach, G. A., Bazer, F. W., and Roberts, R. M. (1985) The type 5 acid phosphatase from spleen of humans with hairy cell leukemia. *J. Biol. Chem.* **260,** 5768–5776.
25. Robinson, D. B., and Glew, R. H. (1980) A tartrate-resistant acid phosphatase from Gaucher spleen. *J. Biol. Chem.* **255,** 5864–5870.
26. Hayman, A. R., Bune, A. J., Bradley, J. R., Rashbass, J., and Cox, T. M. (2000) Osteoclastic tartrate-resistant acid phosphatase (Acp5): Its localization to dendritic cells and diverse murine tissues. *J. Histochem. Cytochem.* **48,** 219–227.
27. Nuthmann, V., Dirks, W., and Drexler, H. (1993) Transient

expression of the tartrate-resistant acid phosphatase (TRAP) gene in hamster cells: A pilot study. *Leukemia* **12,** 1960–1964.

28. Costelloe, E., Stacey, K., Antalis, T., and Hume, D. A. (1999) Regulation of the plasminogen activator inhibitor-2 (PAI-2) gene in murine macrophages. Demonstration of a novel pattern of responsiveness to bacterial endotoxin. *J. Leukoc. Biol.* **66,** 172–182.
29. Keough, D. T., Dionysius, D. A., DeJerse, J., and Zerner, B. (1980) Iron-containing acid phosphatases: Characterization of the metal ion binding site of the enzyme from pig allantoic fluid. *Biochem. Biophys. Res. Commun.* **94,** 600–605.
30. Sibille, J. C., Doi, K., and Aisen, P. (1987) Hydroxyl radical formation and ironbinding proteins. Stimulation by the purple acid phosphatases. *J. Biol. Chem.* **262,** 59–62.
31. Hayman, A. R., and Cox, T. M. (1994) Purple acid phosphatase of the human macrophage and osteoclast. Characterization, molecular properties, and crystallization of the recombinant di-iron-oxo protein secreted by baculovirus-infected insect cells. *J. Biol. Chem.* **269,** 1294–1300.
32. Schindelmeiser, J., Münstermann, D., Mayer, B., Holstein, A. F., and Davidoff, M. S. (1997) Occurrence of enzymes of free radical metabolism suggests the possible cytotoxic capacity of transitional epithelium of the human ureter. *Cell Tissue Res.* **287,** 351–356.
33. Kuhn, D., Baker, B., Lafuse, W., and Zwilling, B. D. (1999) Differential iron transport into phagosomes isolated from the RAW264.7 macrophage cell lines transfected with Nramp1^{Gly169} or Nramp1^{Asp169}. *J. Leukocyte Biol.* **66,** 113–119.
34. Räisänen, S., Lehenkari, P., Tasanen, M., Rahkila, P., Härkönen, P., and Väänänen, H. K. (1999) Carbonic anhydrase III protects cells from hydrogxen peroxide-induced apoptosis. *FASEB J.* **13,** 513–522.