

Concise report

Expression of cathepsin K and tartrate-resistant acid phosphatase is not confined to osteoclasts but is a general feature of multinucleated giant cells: systematic analysisJin Kyun Park¹, Antony Rosen², Jeffrey E. Saffitz³, Angeliki Asimaki³, Silvio H. Litovsky⁴, Shannon M. Mackey-Bojack⁵ and Marc K. Halushka⁶**Abstract**

Objective. Cathepsin K and tartrate-resistant acid phosphatase (TRAP) are two proteins expressed in osteoclastic giant cells. Recently we showed that lesional multinucleated giant cells (MNGs) in pulmonary granulomatosis with polyangiitis expressed these proteins. We aimed to clarify whether the expression of these two proteins has any specificity or is a general feature of MNGs associated with multiple types of granulomatous inflammation.

Methods. In total, 7 Crohn's disease (CD), 5 GCA, 5 giant cell myocarditis (GCM), 11 sarcoidosis and 6 tuberculosis cases were examined for expression of cathepsin K and TRAP using immunohistochemistry (IHC). Protein expression was semi-quantitatively classified as none, weak, moderate or strong. In addition, tissue TRAP activity was examined using an enzymatic reaction.

Results. The expression of cathepsin K was robust in >95% of MNGs of all examined disease groups, whereas TRAP expression varied; CD, GCA and tuberculosis showed strong TRAP expression. TRAP expression in sarcoidosis and GCM was weaker (CD vs GCM, $P=0.04$; CD vs sarcoidosis, $P=0.06$). Compared with IHC, TRAP detection using an enzymatic colour reaction had limited sensitivity.

Conclusion. Expression of TRAP and cathepsin K is a general feature of MNGs and their expression might be related to histopathological pattern.

Key words: cathepsin K, tartrate-resistant acid phosphatase, multinucleated giant cells, granulomas, autoimmune, sarcoidosis, myocarditis.

Introduction

Granulomatous inflammation is the result of a chronic inflammatory process that fails to resolve. Deficient removal of foreign materials such as infectious agents and

foreign bodies or a failure to regulate an immune response can sustain inflammation with granuloma formation [1–3]. Granulomatous inflammation has diverse histology, ranging from non-caseating granulomas in sarcoidosis with little to no tissue necrosis to necrotizing or caseating granulomas associated with tuberculosis or granulomatosis with polyangiitis (GPA). Despite the differences in tissue damage pattern and pathogenic pathways, all granulomas are compact collections of macrophages and epithelioid cells that frequently contain multinucleated giant cells (MNGs) [4–6].

MNGs or macrophage polykaryons arise from cell fusion of monocyte-macrophage lineage under the influence of local cytokines [7, 8]. Bone has a distinctive variant of MNG, the osteoclast, believed to be a specialized MNG with bone-resorbing capacity. The osteoclast has been immunohistologically characterized by its

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expression of tartrate-resistant acid phosphatase (TRAP) and cathepsin K. Cathepsin K is a cysteine protease and TRAP is an iron-containing metalloenzyme. Both are highly expressed in lysosomal compartments of osteoclasts and are involved in the degradation of bone matrix throughout homeostatic and pathologic bone remodelling [9, 10] and serve as classic markers of mature osteoclasts. However, emerging evidence shows their expression at low levels in other extraskeletal tissues, including skin, muscle and intestines. Further, these classic markers of osteoclasts have recently been found in MNGs of GPA [11], prompting us to systematically examine MNGs across a spectrum of pathology to define whether those markers are properties of MNGs generally or specific for osteoclasts and GPA.

Our observations indicate that expression of cathepsin K and TRAP is indeed a general feature of MNGs associated with autoimmune, infectious or sarcoid granulomatous inflammation, including tuberculosis, sarcoidosis, Crohn's disease (CD), GCA and giant cell myocarditis (GCM), and their expression pattern might be relevant to tissue destruction in granulomatous inflammation.

Materials and methods

Study population

Tissue sections were retrieved from the pathology archives of Johns Hopkins Hospital. The samples were selected based on the clinical and histological evidence of CD ($n=7$), GCA ($n=5$), GCM ($n=5$), sarcoidosis ($n=11$) and tuberculosis ($n=6$). All tuberculosis cases were confirmed by either histologic identification of microorganisms, a positive culture or positive PPD test. Tissues without MNGs were excluded (supplementary Table S1, available at *Rheumatology* Online). Separate informed consent to use these samples was not obtained. The general written informed consent form for surgery at the Johns Hopkins medical institutions contains language that gives permission for excess tissue not needed for diagnosis to be used for research purposes. All samples were provided with limited, non-identifying demographic or clinical information to the authors. The study was approved by the Johns Hopkins University institutional review board.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were deparaffinized with xylenes and rehydrated. Antigen retrieval was performed at 95°C in citrate buffer (DAKO, Carpinteria, CA) and endogenous peroxidase was blocked with hydrogen peroxide (DAKO) for 10 min. After blocking, tissues were incubated with mouse monoclonal antibodies directed at human cathepsin K (clone 3F9; Abcam, Cambridge, MA, USA) or human TRAP (clone 26E5; Abcam) overnight at 4°C. After washing in PBS, HRP-conjugated secondary antibodies were applied for 1 h at 37°C and the staining was visualized with 3,3'-diaminobenzidine (DAKO) for 2 min. Nuclei were counterstained with Mayer's haematoxylin.

TRAP assay

After tissues were de-paraffinized and dehydrated as described above, TRAP expression was performed using the Leukocyte Acid Phosphatase Kit for TRAP per the manufacturer's instructions (Sigma, St Louis, MO, USA), followed by counterstaining with Mayer's haematoxylin. TRAP converts the yellow colour of the substrate into bright pink.

Statistical analysis

The entire histologic sections were thoroughly examined using a conventional light microscope (Olympus BH2, Japan). The average intensity of enzyme expression, taking into account all giant cells in a particular case, was semi-quantitatively graded by two independent investigators as none, weak, moderate and strong or 0, 1, 2 and 3, respectively. Mann-Whitney tests were used to compare the intensities of enzyme expression between disease groups. $P < 0.05$ was considered statistically significant. Statistical analyses were computed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Results

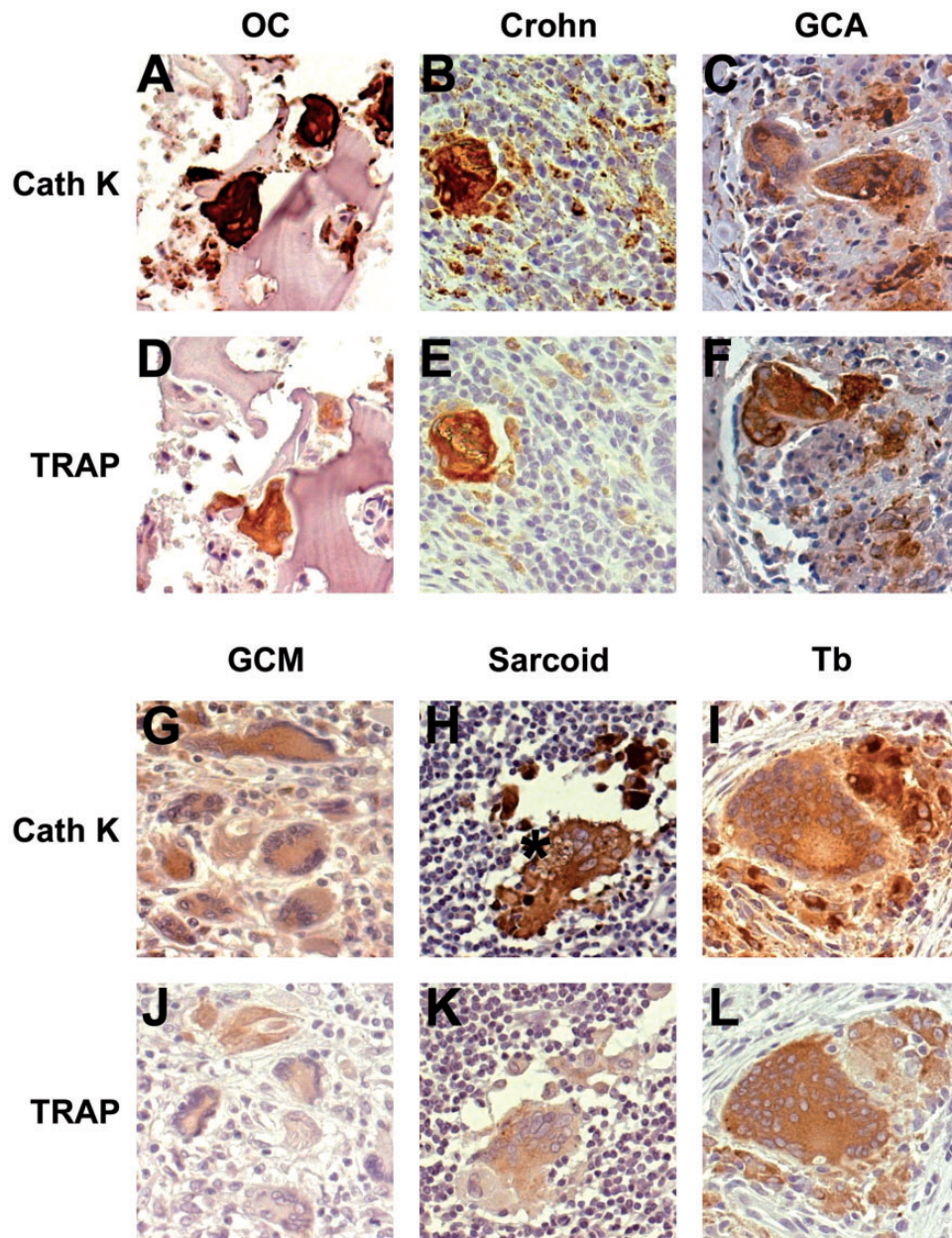
Morphology of MNGs

Within bone, osteoclasts were relatively small with few nuclei. MNGs in CD, GCA and GCM were relatively scarce and had little cytoplasm with few nuclei. MNGs associated with sarcoidosis and tuberculosis were abundant, with generally increased cytoplasm and nuclei relative to other MNG types (Fig. 1). Asteroid bodies were only observed in sarcoid MNGs.

Expression of cathepsin K and TRAP in MNGs

Osteoclasts at the bone surface strongly expressed cathepsin K and TRAP. MNGs in all examined disease groups showed overall robust expression of cathepsin K (Figs. 1 and 2A). In rare cases, small percentages of individual MNGs (<5% of all examined MNGs) lacked cathepsin K expression (supplementary Fig. S1A, arrow, available at *Rheumatology* Online). Interestingly, those rare MNGs were surrounded by the cathepsin K expressing MNGs on the same tissue. TRAP was present in most MNGs as well (>75%), but the intensity significantly varied between disease groups (supplementary Fig. S1B, available at *Rheumatology* Online). MNGs associated with CD expressed a high level of TRAP, whereas MNGs in sarcoidosis and GCM had relatively weak expression (GCM vs Crohn's, $P=0.04$; sarcoidosis vs Crohn's, $P=0.06$) (Fig. 2B).

A significant discrepancy between IHC and enzymatic detection of TRAP was observed (supplementary Fig. S2, available at *Rheumatology* Online). Alveolar macrophages, which served as controls, had detectable TRAP expression per both enzymatic reaction (activity) and IHC (supplementary Fig. S2A and B, available at *Rheumatology* Online). However, most MNGs (>95%) were negative for TRAP activity even with strong TRAP IHC (supplementary

Fig. 1 Expression of cathepsin K and TRAP in MNGs.

Osteoclasts expressed both cathepsin K and TRAP strongly (**A, D**). MNGs in CD, GCA, GCM, sarcoidosis and tuberculosis (Tb) showed robust cathepsin K expression (**B, C, G, H** and **L**), whereas TRAP expression varied between disease groups; low expression was observed especially in GCM with TRAP-negative MNGs (**J**) and sarcoid MNGs (**K**). Sarcoid MNGs showed an asteroid body that was not stained (*). Original magnification $\times 20$.

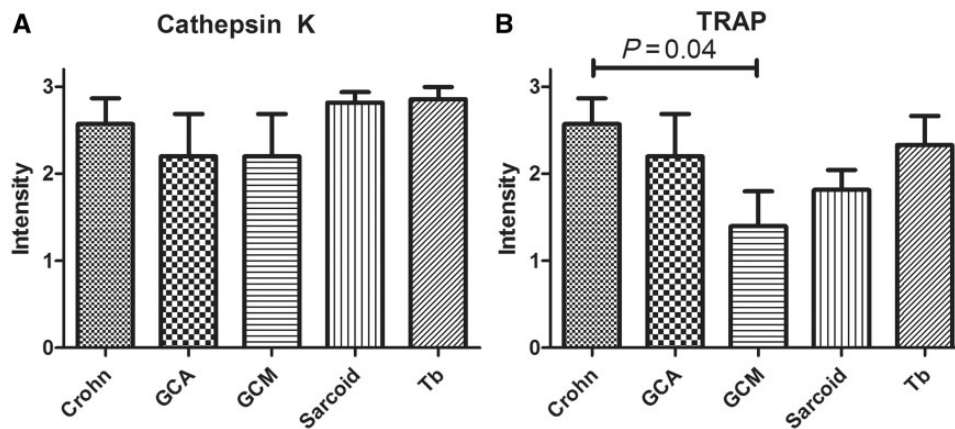
Fig. S2C–F, available at *Rheumatology* Online). Only in cases where the IHC for TRAP was particularly robust did MNGs show TRAP activity (supplementary Fig. S2G and H, available at *Rheumatology* Online).

Discussion

Our study clearly demonstrates that the expression of cathepsin K and TRAP is not confined to the

bone-resident osteoclasts or GPA, but is a general feature of MNGs associated with autoimmune and infectious granulomatous inflammation.

Cathepsin K was previously reported in MNGs associated with sarcoidosis, tuberculosis, foreign body reaction, hypersensitivity pneumonitis, sarcoidosis, GPA, berylliosis and tuberculosis [12, 13]. Here we expand this observation that cathepsin K is also a feature of MNGs associated with autoimmune diseases such as CD, GCA

Fig. 2 Semi-quantitative expression of cathepsin K and TRAP in MNGs.

While cathepsin K expression is robust in all disease groups (A), TRAP expression in GCM and sarcoidosis was lower (B). Numbers are presented as mean \pm S.E.M. Tb: tuberculosis.

and GCM, a disease thought to be of autoimmune aetiology [14]. There was a small percentage (<5%) of MNGs that did not stain for cathepsin K, which we interpret as an indication of variable functional status of MNGs, possibly influenced by local cytokines. Cathepsin K expression is therefore a reliable marker for identifying MNGs in granulomatous inflammation.

In contrast to the robust expression of cathepsin K across a spectrum of pathology, TRAP expression intensity varied markedly (Fig. 2). In bone, both cathepsin K and TRAP are involved in degradation of the extracellular organic matrix during physiologic and pathologic bone remodelling [9, 10] and similar roles are anticipated within granulomatous inflammation contributing to tissue damage. Therefore this differential expression pattern might be relevant to disease-specific pathology. In line with this, strong TRAP expression of MNGs in CD and tuberculosis is striking since both diseases are associated with considerable tissue damage, including fistula formation in Crohn's enteritis and massive lung destruction in tuberculosis. Further, TRAP expression in MNGs of sarcoidosis was quite low. Sarcoidosis histology is characterized by the absence of considerable cell-mediated tissue necrosis as the prototype of a non-caseating granulomatous disease. The low TRAP staining in GCM, a destructive process, is counter to this idea. However, MNG production in GCM may be IL-4 mediated, and IL-4 is known to inhibit TRAP expression [15, 16]. Thus the higher expression of TRAP might lead to or result from an inflammatory environment associated with substantial cell-mediated tissue damage. Indeed, TRAP-expressing dendritic cells promote the generation of pro-inflammatory Th1 [17] and MNGs are reported to function as effective antigen-presenting cells [18].

Of note, we made an important observation that TRAP detection using an enzymatic assay has limited sensitivity compared with IHC. TRAP may lose its activity with time, while anti-TRAP antibody could still detect remaining and

preserved epitopes of enzymes, especially in bone tissue that requires decalcification for tissue preparation with subsequent enzyme destruction. Another possible explanation for this discrepancy in TRAP staining between IHC and enzymatic activity may be a differential functional status of TRAP. It is possible that the TRAP protein may be present in an inactive form in most MNGs and becomes activated only in a specific inflammatory microenvironment.

In conclusion, we found that two markers, cathepsin K and TRAP, historically assigned to osteoclasts, are actually common in MNGs found in multiple pathogenic states, and we believe these shared markers suggest shared mechanisms of giant cell formation across diseases.

Rheumatology key messages

- Expression of TRAP and cathepsin K is a general feature of multinucleated giant cells.
- TRAP expression is variable between multinucleated giant cells within the same lesion.
- TRAP enzymatic activity assay to identify expression is inferior to immunohistochemical methods of detection.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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