Osteoclast-like multinucleated giant cells in sinonasal inflammation of granulomatosis with polyangiitis (Wegener's granulomatosis)

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ABSTRACT

Objective. *To determine whether generation of osteoclast-like multinucleated giant cells (MNG) is a general feature of granulomatosis with polyangiitis (GPA).*

Methods. *MNG phenotype of GPA sinus was examined by immunohistochemistry using antibodies against CD68, and cathepsin K. Tartrate resistant acid phosphatase (TRAP) expression was assessed by enzymatic colour reaction. Effects of bacterial wall components peptidoglycan (PGN) or lipoteichoic acid (LTA) on TRAP+ MNG formation were determined.*

Results. *Tissue infiltrating MNGs in sinus expressed CD68, TRAP, and cathepsin K. They were strikingly less frequent in sinus than in lung lesions (23.1% vs. 70%, p=0.04). PGN and LTA inhibited MNG formation in a dose-dependent manner.*

Conclusions. *While the generation of osteoclast-like MNGs is an intrinsic feature of GPA, MNGs are rare in sinonasal GPA lesions. Inhibition of MNG formation by bacterial cell wall components may occur preferentially in this sinonasal microenvironment, and contribute to these striking regional pathological differences.*

Introduction

A hallmark of granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis, is systemic granulomatous inflammation with geographic necrosis and multinucleated giant cells (MNGs) (1). MNGs or macrophage polykaryons derive from cells of the monocyte-macrophage lineage. In physiologic conditions, bone has distinctive variants of MNGs believed to be specialised in bone remodelling by expressing osteolytic enzymes cathepsin K and tartrate resistant acid phosphatase (TRAP) (2-5). These osteoclasts (OC) arise in the presence of receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor 1 (M-CSF) (6). Recently we reported that lesional MNGs in pulmonary inflammation of GPA express cathepsin K and TRAP and that circulating blood cells in GPA have an increased propensity to generate TRAP+ MNGs as compared to healthy controls (7), prompting us to investigate as to whether the presence of those osteoclast-like cells is not confined to lung granulomata, but is a general feature of the GPA pathology including in upper airway, which is frequently involved as a chronic sinusitis during the disease process (8).

In this study, we show that although OC-like MNGs are present in sinonasal GPA, their frequency is much lower than that found in lung granulomata. Since chronic bacterial colonisation and infection are a frequent feature of GPA, we addressed whether bacterial components could influence the generation of these cells. We found that peptidoglycan (PGN) and lipoteichoic acid (LTA) strikingly inhibit their formation.

Materials and methods

Monocyte isolation

GPA patients who met the American College of Rheumatology 1990 classification criteria for GPA were enrolled. Monocytes were isolated from heparinised blood by density gradient centrifugation using Ficoll-Paque (GE Heathcare, NJ, USA) and magnet bead purification using CD14 bead antibodies (MACS, Miltenyi Biotec, Auburn, CA, USA). Purity of isolated CD14 cells exceeded 90%.

Generation of TRAP+MNGs

In total, 2 x 10^5 monocytes were cultured in OPTI-MEM® I (Gibco/Invitro-

gen, Grand Island, NY, USA), supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin and 1% streptomycin in the presence of 100 ng/ml RANKL (R&D Systems, Minneapolis, MN, USA) and 25 ng/ml M- CSF (R&D Systems, Minneapolis, MN, USA) as well as in increasing concentrations of PGN (Sigma life Science, Buchs, Switzerland) and LTA (Sigma, St Louis, MO, USA) in a 96 well plate in 5% carbon dioxide at 37 C. Media were replenished every 3 days. On day 9, cells were fixed with 3% formaldehyde and stained for TRAP expression per manufacture's instruction (Sigma, St Louis, MO, USA). TRAP+ cells with three or more nuclei per well were counted as TRAP+ MNGs under light microscopy Olympus CKX41.

Immunohistochemistry and TRAP assay

Formalin-fixed and paraffin-embedded GPA lung and sinus tissues were retrieved from the Pathology archive. After tissues were de-paraffinised and rehydrated, they were stained with antibodies directed against cathepsin K (clone 3F9, Abcam, Cambridge, MA, USA) or against CD68 (clone PG-M1, DAKO). TRAP assay was performed per manufacture's instruction (Sigma, St Louis, MO, USA), followed by counterstaining with Meyer's haematoxylin.

Statistical analysis

Two-sided Fisher's exact test for dichotomous variables was performed. $p \leq 0.05$ was considered statistically significant.

Results

The presence of osteoclast-like cells within sinonasal inflammation of GPA

The lineage and phenotype of lesional MNGs in GPA sinus containing granulomatous inflammation were examined. On haematoxylin and eosin stain, there were intense inflammatory infiltrates including mononuclear cells and polymorphic mononuclear cells. Large MNGs with numerous nuclei were present within the inflammatory infiltrates (Fig. 1A). The MNGs expressed CD68 (brown) (Fig. 1B), indicating their myeloid origin with macrophage differentiation. In addition, MNGs expressed both TRAP (bright purple cytoplasmic staining) and cathepsin K (brown) (Fig. 1 C and E).

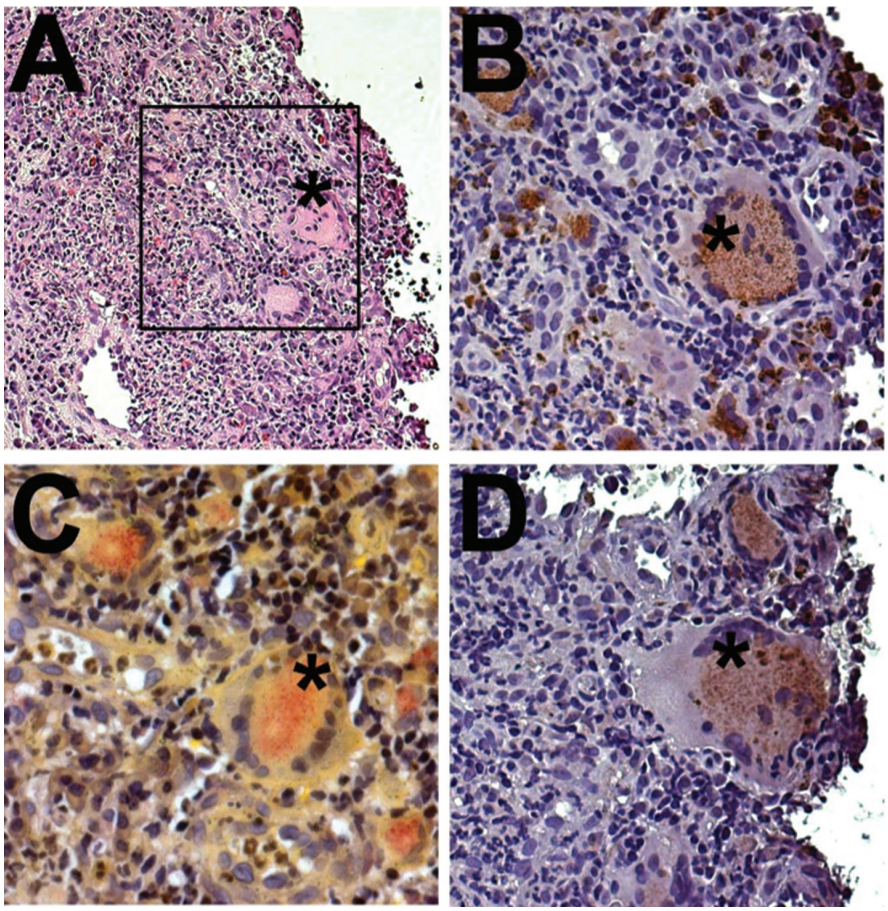


Fig. 1. Presence of osteoclast-like multinucleated giant cells in sinonasal inflammation of GPA. (**A**) Many MNGs in varying sizes were present in the inflammatory infiltrates, comprised of polymorphic nuclear cells and mononuclear cells on HE staining. (**B**) MNGs expressed macrophage marker CD68, tartrate resistant acid phosphatase (TRAP) (**C**) and cathepsin K (**D**). TRAP and cathepsin K were expressed mainly in MNGs.
* MNG. Original magnification 10x for A; 20x for B, C and D.

MNGs are infrequent in sinuses as compared to lungs

We investigated whether MNGs were more prevalent in upper airway since GPA commonly manifests as a chronic sinusitis with frequent sinonasal bone destruction (9). A total of 10 lung and 13 sinonasal biopsies from patients with GPA were examined. Strikingly, 7 (70%) of 10 lung sections contained MNGs as compared to 3 (23.1%) of 13 sinus biopsies (p=0.04). Further, the MNGs in sinuses were smaller in size with less number of incorporated nuclei as compared to MNGs in lung granulomata (data not shown).

Inhibition of TRAP+ MNG formation by bacterial wall components PGN and LTA

Despite being one continuous organ, the lower airway remains relatively sterile, whereas the upper airway is heavily colonised with numerous bacteria (9). Therefore, we examined the effects of bacterial wall components PGN and LTA on MNG formation. Monocytes from 3 GPA patients were cultured in the presence of RANKL, M-CSF and increasing concentrations of PGN or LTA. After 9 days, numerous large TRAP+ MNGs were generated (Fig. 2A). Both PGN and LTA inhibited TRAP+ MNG formation in a dose dependent manner. MNG formation was reduced by 50% approximately at 800 ng/ml PGN or 1000 ng/ml LTA and was completely abolished at 100,000 ng/ml PGN and LTA (Fig. 2 B).

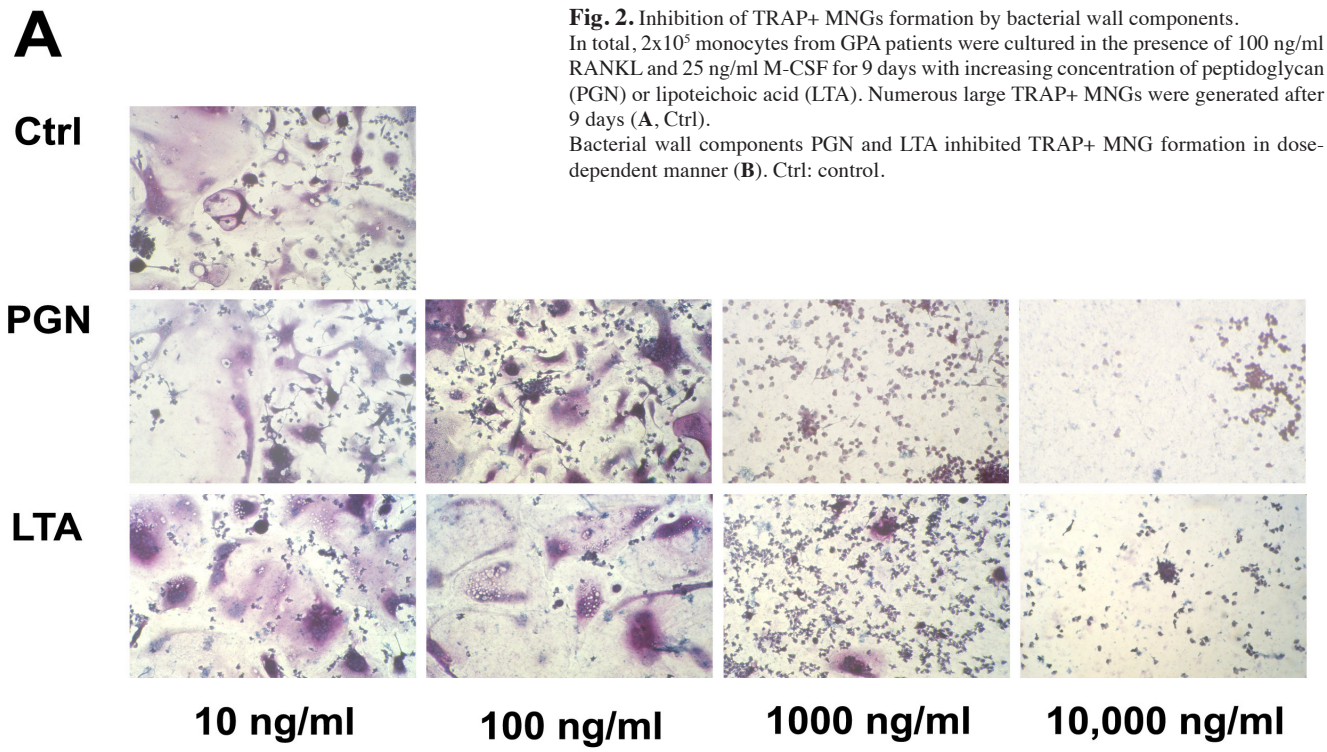
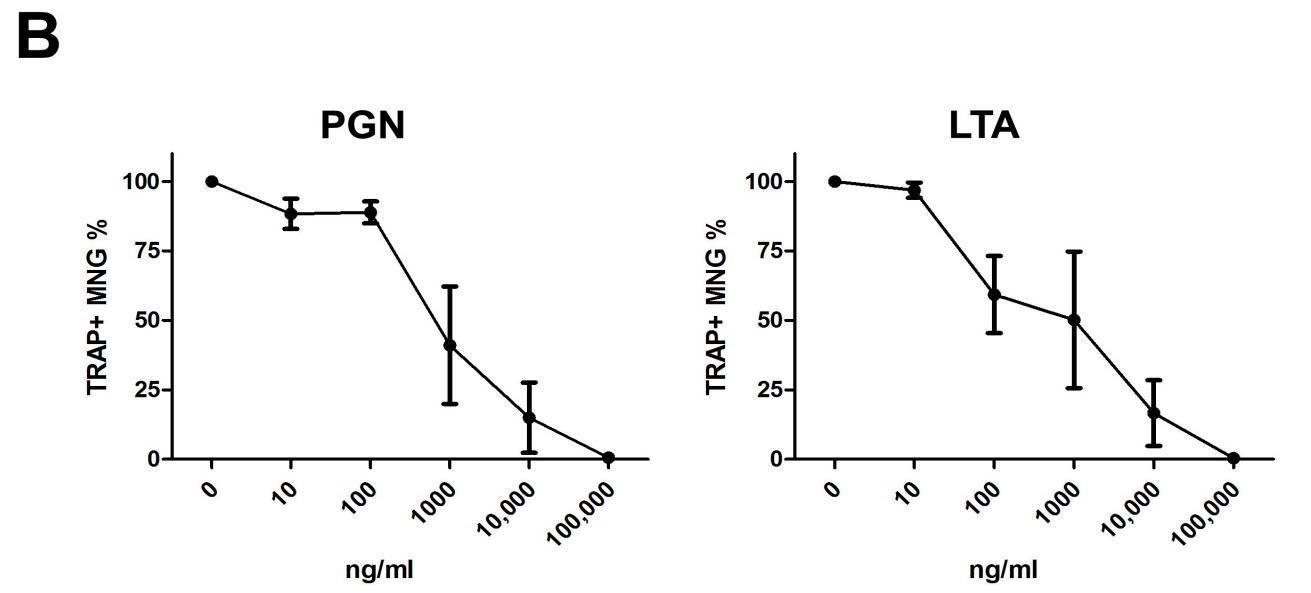


Fig. 2. Inhibition of TRAP+ MNGs formation by bacterial wall components.
In total, $2x10^5$ monocytes from GPA patients were cultured in the presence of 100 ng/ml RANKL and 25 ng/ml M-CSF for 9 days with increasing concentration of peptidoglycan (PGN) or lipoteichoic acid (LTA). Numerous large TRAP+ MNGs were generated after 9 days (**A**, Ctrl).
Bacterial wall components PGN and LTA inhibited TRAP+ MNG formation in dose-dependent manner (**B**). Ctrl: control.



Discussion

In this study, we demonstrated that osteoclast-like MNGs expressing TRAP and cathepsin K are present in sinonasal inflammation of GPA. Thus, the presence of those cells is not only confined to lung granulomata but their generation may represent a general immunopathologic feature of GPA. Interestingly, MNGs were much less frequent in sinuses compared to lungs, although sinusitis is present in up to 85% of GPA during the course of disease process and often precedes lung involvement (8). Here, the frequency of MNGs was 23.3% in sinuses and 70.0% in lungs. Although the presence of MNG as a cellular hallmark of granulomatous inflammation is not required for its diagnosis, the observed frequencies in our cohort were strikingly consistent with the previously reported frequency of granulomatous inflammation in sinuses at 20–33% and lungs at 60% at the time of GPA diagnosis (1, 10). Therefore, the scarcity of the MNGs is not from sampling biopsy but suggests the presence or absence of unique micro-environmental factors in the upper airway. Of note, RANKL, the key cytokine of TRAP+ MNG formation, and RANK expressing cells were abundantly present in infiltratory lesions of both lungs and sinuses (data not shown), suggesting that additional inhibitory signalling may play an important role. In this context, heavy bacterial

colonisation in sinus is remarkable (9). Indeed, bacterial components PGN and LTA efficiently inhibited TRAP+ MNG formation, consistent with the prior observations that toll-like receptor (TLR) signalling inhibits osteoclastogenesis (11). Beside its possible role in MNG formation, bacterial colonisation could contribute to sinonasal disease activity (12, 13); TLR signalling induces proteinase 3 expression on neutrophils and those neutrophils can be further activated by circulating anti-PR3 antibodies (14).

An important limitation of our study is that sinus and lung biopsies were not obtained from the same patients. Patients with early, localised disease would be favourably subject to nasal biopsy, whereas patients with more generalised involvement underwent lung biopsy, potentially leading to a selection bias. Our previous observation showed that peripheral blood of generalised GPA patients has a higher propensity to generate TRAP+ MNG compared with that of localised GPA and of healthy controls (7). As such, the propensity of MNG generation in conjunction with unique microenvironments including bacterial colonisation may determine the presence and extent of granulomatous inflammation in GPA.

In conclusion, we propose that generation of osteoclast-like MNGs is an important immunopathologic feature of GPA and those cells are present in multiple environments. Therefore, understanding the pathophysiologic microenvironment promoting MNG formation might offer novel insights in GPA pathogenesis.

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