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Early release of growth factor and cytokines elicited by zymosan in rat tibiotarsal joints - lessons for arthritis therapy

ABSTRACT

Early release of inflammation mediators was investigated in rat tibiotarsal arthritis induced by zymosan. At the 6th hour, proinflammatory cytokines were the most released in the supernatant of joint cavities (IL-1β: 45.3; IL-6: 23.3; TNF-α: 4.0; IL-5: 2.9 fold) *vs.* controls, followed by the chemokines GRO/KC (CXCL1 4.8 fold) and MCP-1 (CXCL2 2.9 fold), and growth factors related to angiogenesis or leukocyte maturation (VEGF: 17.1; GM-CSF: 7.5; M-CSF: 4 fold). The release of anti-inflammatory cytokines (IL-13: 6.7; IL-4: 4.4 fold) suggests a compensatory mechanism. In conclusion, zymosan evokes multiple expressions of acute phase mediators, being the blockage of such targets, particularly VEGF, an interesting therapeutic strategy.

Keywords: inflammation; rheumatoid; mediators; immunoassay

1 INTRODUCTION

Rheumatoid arthritis (RA) is a debilitating inflammatory disease characterized by joint pain, synovial hyperplasia, leukocyte infiltrate, vascular neoformation, and cartilage damage (Yap *et al.*, 2019). The role of proinflammatory cytokines has been demonstrated in RA, and immunotherapies against TNF-α, IL-1, and IL-6 have been approved for clinical use (Noack; Miossec, 2017).

Zymosan-induced arthritis (ZyA) is a useful rodent model to study joint inflammation and to evaluate the efficacy of therapeutic agents. Zymosan activates TLR2/MyD88 signaling, gene expression of TNF-α and IL-1β, acute joint edema, leukocyte infiltration, hypernociception, gait disturbances, and erosive synovitis (Guerrero *et al.*, 2012; Bringel *et al.*, 2020).

Reports on cytokines usually comprise TNF-α, IL-1β, and IL-6 in human disease and ZyA (Noack; Miossec, 2017). Nevertheless, some patients remain refractory to those cytokines blockage or become non-responsive (Paquet *et al.*, 2012), changing the focus on non-conventional mediators. This study aimed to explore the early release of chemokines/cytokines/growth factors from zymosan-inflamed rat joints and to discuss possible therapeutic implications.

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2 MATERIALS AND METHODS

Wistar rats (200-220g) were manipulated under controlled conditions (12h/12h cycle, 22-25 °C, water and food *ad libitum*) following the guidelines of CONCEA/CEUA/UECE (No. 2126961/2015).

Arthritis was induced by zymosan injection (500 µg/25 µL) into the right tibiotarsal joint (Bringel, *et al.*, 2020). Sham animals received 0.9% NaCl (25 µL i.art.). At the 6th hour, animals were euthanized, and joint cavities were washed with 100 µL PBS/EDTA. The supernatant was evaluated by immunoassay (Bio-Plex, #171K1001M) for tumor necrosis factor (TNF-α), macrophage colony-stimulating factors (M-CSF, GM-CSF), vascular endothelial growth factor (VEGF), chemokines (GRO/KC, MCP-1) and interleukins (IL-1β, IL-4, IL-5, IL-6, IL-13). Results (Mean ± SEM; n= 6) were analyzed by Student's t-test.

3 RESULTS AND DISCUSSION

Table 1 presents the mediators in the synovial fluid joints subjected to ZyA. Among proinflammatory cytokines, IL-1β and IL-6 were enhanced more intensively, followed by TNF-α (45, 23, 4-fold). Indeed, TNF-α production is an initial step in the amplified cascade that leads to IL-1β, and IL-6 release (Cunha *et al.*, 2005). Although not for all patients, blockage by antibodies is an effective clinical management of human RA (Noack; Miossec, 2017) and is supported by experimental evidence of cartilage destruction and osteoclastogenesis induced by IL-1β (Van de Loo *et al.*, 1995). IL-6 is overexpressed, showing systemic and local effects on synovial tissues, being its blockage prevented and reduced RA symptoms (Noack; Miossec, 2017). IL-5 is an eosinophilic chemoattractant associated with Freund's adjuvant-induced arthritis (Zheng *et al.*, 2002). In our experiment, its levels were only slighted elevated (2.4-fold).

Besides the extensive expression of proinflammatory cytokines, there were significantly augmented levels of IL-13 (6.7) and IL-4 (4.4-fold) in ZyA. These anti-inflammatory cytokines are known to protect cartilage in murine collagen-induced arthritis (Joosten *et al.*, 1997) so that a compensatory mechanism may be in course in order to refrain the inflammation evoked by zymosan, being supported by the zymosan-intensified joint incapacitation after systemic administration of serum containing antibodies against these cytokines (Vale *et al.*, 2003).

The chemokines GRO/KC (CXCL1) and MCP-1 (CXCL2) had their levels mildly elevated in ZyA (4.8 and 2.9-fold). GRO/KC production was previously described for mice receiving zymosan (Guerrero *et al.*, 2012) but in a magnitude greater than that on rats. A discrepant proportion between the amount of inductor and joint volume of each species may account for this difference, given that a similar pattern was also observed for TNF-α. Besides, MCP-1 is chemotactic for monocytes in ankle RA, enhancing TNF-α and IL-β, the proliferation of fibroblast-like synoviocytes, and angiogenesis markers via PI3K/P38 (Tong *et al.*, 2020).

Acute release of growth factors would constitute an interesting investigation, especially if massive leukocyte recruitment is considered. In our study, zymosan increased GM-CSF (7.5) and M-CSF (4.0-fold), modulators of development and function of monocyte/macrophage in RA, as well osteoclastogenesis (Noack; Miossec, 2017). M-CSF blockade inhibits pain, cell infiltration, synovial hyperplasia, and proteoglycan loss in the mice model of ZyA, preventing but not attenuating the disease onset (Saleh *et al.*, 2018). Besides, GM-CSF is critical in inflammatory pain via CCL17 and TNF-α pathways (Achuthan *et al.*, 2016). In a clinical trial, administering a monoclonal antibody against the GM-CSF receptor-α significantly decreased RA severity (Burmester *et al.*, 2017), suggesting its relevance at the disease onset.

A special mention is deserved to VEGF, which was increased by 17-fold by zymosan. VEGF is a potent angiogenic in invasive synovial hyperplasia (rheumatoid "pannus"), and its inhibition is a sound anti-rheumatic strategy (Sakalyte *et al.*, 2022); being its production previously described in ZyA of knee mice at hour 48 (Ruth *et al.*, 2010). However, ZyA in the tibiotarsal joint indicates that VEGF release may occur early (6h).

In conclusion, zymosan evokes multiple expressions of cytokines, chemokines, and growth factors at an acute phase in rat tibiotarsal joints. Future research may focus on growth factors in the ZyA pathophysiology as targets for novel therapeutical approaches.

Table 1 - Mediators in synovial fluid of rat tibiotarsal joint. *p<0.05 *vs.* sham (Student t-test)

Mediator (pg/mL)	**Sham** (25 µL 0.9% NaCl, i.art)	**ZyA** (500 µg/25 µL, i.art)
IL-1β	1363 ± 935	61760 ± 10720*
IL-4	0.3 ± 0.10	1.5 ± 0.35*
IL-5	28.7 ± 5.21	69.1 ± 14.24*
IL-6	556 ± 244	12980 ± 4310*
IL-13	3.3 ± 1.65	22.1 ± 7.12*
TNF-α	73.1 ± 16.0	290.6 ± 29.7*
GRO/KC	57.8 ± 41.2	278.1 ± 103.1
MCP-1	743 ± 372	2156 ± 445*
GM-CSF	2.8 ± 0.80	21.1 ± 7.31*
M-CSF	48.7 ± 20.4	195.8 ± 37.0*
VEGF	4.5 ± 2.13	77.2 ± 40.74*

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REFERENCES

ACHUTHAN, A. *et al.* Granulocyte macrophage colony-stimulating factor induces CCL17 production via IRF4 to mediate inflammation. **The Journal of Clinical Investigation**, v. 126, n. 9, p. 3453-3466, 2016.

BRINGEL, P. H. S. F. *et al.* The lectin isolated from the alga *Hypnea cervicornis* promotes antinociception in rats subjected to zymosan-induced arthritis: involvement of cGMP signalization and cytokine expression. **Inflammation**, v. 43, n. 4, p. 1446-1454, 2020.

BURMESTER, G. R. *et al.* A randomised phase IIb study of mavrilimumab, a novel GM-CSF receptor alpha monoclonal antibody, in the treatment of rheumatoid arthritis. **Annals of the Rheumatic Diseases**, v. 76, n. 6, p. 1020-1030, 2017.

CUNHA, T. M. *et al.* A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. **Proceedings of the National Academy of Sciences of the United States of America**, v. 102, n. 5, p. 1755-1760, 2005.

GUERRERO, A. T. G. *et al.* Toll-like receptor 2/MyD88 signaling mediates zymosan-induced joint hypernociception in mice: participation of TNF-α, IL-1β and CXCL1/KC. **European Journal of Pharmacology**, v. 674, n. 1, p. 51-57, 2012.

JOOSTEN, L. A. *et al.* Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. **Arthritis and Rheumastism**, v. 40, n. 2, p. 249-260, 1997.

NOACK, M.; MIOSSEC, P. Selected cytokine pathways in rheumatoid arthritis. **Semin Immunopathol**, v. 39, n. 4, p. 365-383, 2017.

PAQUET, J. *et al.* Cytokines profiling by multiplex analysis in experimental arthritis: which pathophysiological relevance for articular versus systemic mediators? **Arthritis Research & Therapy**, v. 14, n. 2, 2012.

RUTH, J. H. *et al.* Interleukin-18 as an in vivo mediator of monocyte recruitment in rodent models of rheumatoid arthritis. **Arthritis Research Therapy**, v. 12, n. 3, R118, 2010.

SAKALYTE, R. *et al.* VEGF Profile in Early Undifferentiated Arthritis Cohort. **Medicina**, v. 58, n. 6, p. 833, 2022.

SALEH, R. *et al.* CSF-1 in inflammatory and arthritic pain development. **Journal of Immunology**, v. 201, n. 7, p. 2042-2053, 2018.

TONG, X. *et al.* Monocyte chemoattractant protein-1 promotes the proliferation, migration and differentiation potential of fibroblast-like synoviocytes via the PI3K/P38 cellular signaling pathway. **Molecular Medicine Reports**, v. 21, n. 3, p. 1623-1632, 2020.

VALE, M. L. *et al.* Antinociceptive effects of interleukin-4, -10, and -13 on the writhing response in mice and zymosan-induced knee joint incapacitation in rats. **The Journal of Pharmacology and Experimental Therapeutics**, v. 304, n. 1, p. 102-108, 2003.

VAN DE LOO, F. A. *et al.* Role of interleukin-1, tumor necrosis factor alpha, and interleukin-6 in cartilage proteoglycan metabolism and destruction. Effect of in situ blocking in murine antigen- and zymosan-induced arthritis. **Arthritis and Rheumatism**, v. 38, n. 2, p. 164-172. 1995.

YAP, H. Y. *et al.* Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development. **Cells**, v. 7, n. 10, p. 161, 2018.

ZHENG, C. L. *et al.* Complete Freund's adjuvant suppresses the development and progression of pristane-induced arthritis in rats. **Clinical Immunology**, v. 103, n. 2, p. 204-209, 2002.