THU0056

EFFECTS OF CX3CL1 INHIBITION ON MURINE BLEOMYCIN-INDUCED INTERSTITIAL PNEUMONIA

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Background: Pathological findings of interstitial pneumonia (IP) reveal the accumulation of inflammatory cells and proliferation of fibroblasts in lung tissue. Although a treatment has not yet been established for IP, particularly for IP with collagen diseases, chemokines may play a role in the pathogenesis of IP for inflammatory cell infiltration. We previously reported that chemokine (C-X3-C motif) ligand 1 (CX3CL1, also known as fractalkine) has potential as a therapeutic target for rheumatoid arthritis (RA).^{1,2,3} Humanized anti-human CX3CL1 monoclonal antibody (mAb) is currently undergoing clinical trials for RA.⁴

Objectives: In the present study, we examined the therapeutic effects of CX3CL1 blockade in a murine model of IP.

Methods: Bleomycin (BLM)-induced IP was developed by the intratracheal administration of BLM to C57BL/6 mice. The murine lung was stained with hematoxylin and eosin, and the expression of CX3CL1 and CX3CR1, a receptor for CX3CL1, was analyzed by immunohistochemistry. Mice were treated with anti-CX3CL1 mAb for 2 weeks. Collagen eluted from the lung was quantified using the SircolTM Collagen Assay. The expression of CX3CL1 and CX3CR1 by mouse lung fibroblasts (MLFs) was examined with quantitative RT-PCR and Western blotting, respectively. Cell movement was investigated using the scrape motility assay.

Results: The expression of CX3CL1 and CX3CR1 was upregulated in BLM-induced IP. The treatment with anti-CX3CL1 mAb did not significantly alter inflammatory cell infiltration. However, collagen in the lung was decreased by the treatment with anti-CX3CL1 mAb. Stimulation with CX3CL1 did not alter the *in vitro* production of collagen by MLFs, but significantly enhanced cell movement.

Conclusion: CX3CL1 may be involved in increasing collagen in IP and the cell movement of MLFs. The present results suggest that CX3CL1 plays an important role in fibrosis in IP.

REFERENCES:

- [1] Nanki T, et al. Inhibition of fractalkine ameliorates murine collagen-induced arthritis. *J Immunol*. 2004;173(11):7010-6.
- [2] Nanki T, et al. Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum*. 2002;46(11):2878-83.
- [3] Nanki T, et al. Fractalkine/CX3CL1 in rheumatoid arthritis. *Mod Rheumatol*. 2017 May;27(3):392-397.
- [4] Tanaka Y, et al. Safety, pharmacokinetics, and efficacy of E6011, an anti-fractalkine monoclonal antibody, in a first-in-patient phase 1/2 study on rheumatoid arthritis. *Modern rheumatology*. 2018;28(1):58-65.

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PLASMACYTOID DENDRITIC CELLS IN THE ENTESIS: PHENOTYPING AND FUNCTION INVESTIGATION

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Background: Plasmacytoid dendritic cells (pDCs) play an important role in linking innate and adaptive immune responses and selectively express Toll-like receptor (TLR) 7 and TLR9 that sense RNA and DNA respectively thus regulating the secretion of type I interferons (IFN) and other inflammatory cytokines such as TNF [1]. pDCs have been directly implicated in psoriasis immunopathology and are activated following anti-viral vaccination, with vaccination also linked to psoriatic arthritis onset [2-3]. Inflammation of the entheses (enthesitis) is a cardinal spondyloarthritis associated lesion so we hypothesized the presence of enthesitis resident pDCs

Objectives: To investigate whether the human entheses contains a resident pDC population and to compare the responses of TLR7/9 agonists on enthesal pDCs function relative to peripheral blood derived pDCs.

Methods: Normal interspinous process entheses and matched peripheral blood (PB) were obtained from patients (n=11) undergoing spinal decompression or scoliosis corrective surgery. Cells were isolated from perienthesal bone by mechanical digestion. Cells were stimulated with either TLR7 agonist (imiquimod) or TLR9 agonist (ODN-2216). Flow cytometry was used to phenotype pDCs, and intracellular staining used to measure IFN α and TNF. IFN α from supernatant was also measured by ELISA.

Results: pDCs were identified in the human entheses with a typical phenotype (CD45+HLA-DR+CD123+CD303+CD11c-). By intracellular FACS, following ODN or imiquimod stimulation, IFN α and TNF was detected in enthesal pDCs and also PB. IFN α and TNF induction trended upwards in enthesal pDC when compared with unstimulated pDCs. IFN α was also detected by ELISA following ODN or imiquimod stimulation of enthesal derived pDCs.

Conclusion: The human entheses contains a resident population of pDCs that produce IFN α and TNF following induction with relevant TLR agonists. For the first time, our findings provide a link between viral illness and vaccination and pivotal innate immune cell production of IFN α and TNF.

REFERENCES:

- [1] Melissa Swiecki and Marco Colonna, *The multifaceted biology of plasmacytoid dendritic cells*. *Nat Rev Immunol*. 2015 Aug;15(8):471-85.
- [2] Gunes AT, Fetic E, Akarsu S, et al., Possible Triggering Effect of Influenza Vaccination on Psoriasis. *J Immunol Res*. 2015;2015:258430.
- [3] Nestle FO, Conrad C, Tun-Kyi A, et al., Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med*. 2005 Jul 4;202(1):135-43.

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