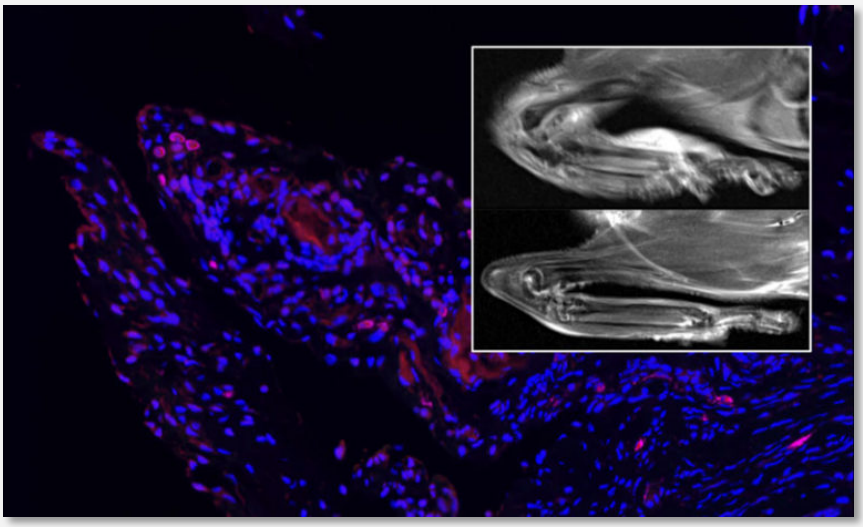
Fra-1 and Arginase-1: Two novel players in macrophage-mediated arthritic inflammation



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Rheumatoid arthritis is a chronic inflammatory disorder of the joints, which affects up to 1 % of the global population. The development is multifactorial and includes genetic as well as environmental factors, inducing an aberrant activation of the immune system; this event manifests in characteristic symptoms of rheumatoid arthritis, such as synovial inflammation in the joints including immune cell infiltration, cartilage and bone destruction. Several immune cells appear to participate in the development of the disease and the initiation of the synovial inflammation. Macrophages in particular have a pivotal role due to their widespread pro-inflammatory, destructive and remodeling capacities that can critically contribute to the disease symptoms.

Macrophages have the capability to initiate an inflammation, as well as to terminate inflammatory processes, maintain and restore tissue homeostasis. They adopt a context dependent phenotype; their precarious responses require adjustment and refinement through a well-orchestrated transcriptional regulatory network, resulting in a broad spectrum of macrophage subsets, each specific in terms of their stimulation and environment. Therefore, their activation constitutes a transcriptional reprogramming, which involves the cooperation of several transcription factors. One of these transcription factors is the activator protein (AP) -1 family.

Due to their sophisticated actions, existing information remains limited and their target genes are not yet completely known. Consequently, the aim of our study (**https://www.jci.org/articles/view/96832**) was to investigate novel AP-1 functions in macrophages, in particular we have focused on the two AP-1 family members Fra-1 and Fra-2. Firstly, we wanted to address if both transcription factors are involved in the regulatory network of macrophages. Gene expression analysis and subsequent gene enrichment analysis demonstrated a very distinct network of Fra-1 and Fra-2 actions in macrophages. It appears that Fra-1 and Fra-2 in myeloid cells have unique and independent functions, whereby each of them has their specific target genes. Our findings are underlined with studies by the Wagner group, who showed that Fra-2 drives macrophage pro-fibrotic functions (INSERT URL of one paper). At the same time, the Reddy group demonstrated an opposite role for Fra-1 during pulmonary fibrosis, where it mediates anti-fibrotic effects (INSERT URL of paper). Together, these data indicate that Fra-1 and Fra-2 transcription factors are involved in the regulatory transcription network, fine tuning macrophage responses depending on their environment and stimulation. It seems that both may act in concert to orchestrate the balance of macrophage functions, avoiding excessive pro-inflammatory or -fibrotic macrophage responses. Moreover, our work extends the functions of Fra-1, showing that Fra-1 skewed macrophage action towards a pro-inflammatory phenotype, suppressing pro-resolving actions through the enzyme arginase-1 (Arg1). We identified Fra-1 as key switch regulator of macrophage function, skewing the macrophage phenotype towards a pro-inflammatory state, thereby regulating macrophage phenotypes and promoting the arthritic joint inflammation. In summary, we could further shed light on the sophisticated macrophage transcription factor network that controls their immune responses.

Our data revealed that enhanced macrophage-derived Arg1 controlled by Fra-1 can ameliorate arthritic joint inflammation. Arg1 is a hallmark of anti-inflammatory macrophages and drives macrophage pro-resolving functions, which initiate the resolution of inflammation and restoration of tissue homeostasis. Therefore, Arg1 is a key enzyme in macrophages, modulating the outcome of their immune responses. In detail, Arg1 hydrolyzes its substrate arginine into ornithine, which is further converted into polyamines and proline. Polyamines support cell proliferation and downregulate pro-inflammatory cytokines production, while proline feeds collagen synthesis and deposition. Type I collagen is the most

abundant bone matrix protein and a major constituent in mineralization of bone. Consequently, it is tempting to speculate that enhanced proline production could restore bone erosion, occurring during arthritis, by rebuilding bone structure through collagen. However, it is also possible that it restores the normal joint tissue architecture.

Indeed, in animal arthritis model we were able to demonstrate that inhibited Arg1 activity worsened arthritic symptoms, including bone destruction. Therefore, we assume that macrophage-derived Arg1 promotes tissue repair, resolution of inflammation, and restoration of tissue homeostasis. These findings lay the foundation to develop novel rheumatoid arthritis therapies. Remarkably, supplementation with the Arg1-substrate arginine in a therapeutical treatment increased Arg1 activity and led to a resolution of inflammation with reduced bone erosion in an arthritis animal model. Therefore, Arg1 may serve as a therapeutical target for bone diseases, where patients suffer from bone and cartilage erosion. Indeed, arginine supplementation is considered as an ergogenic aid for healthy, physically active subjects. These studies mainly focused on muscle strength and performance. However, Arg1-derived products are not only beneficial for tissue regeneration; they also promote vascular fibrosis and thickening, resulting in arterial stiffening, a risk factor for cardiovascular diseases. Arg1 is critically involved in the development of obesity-induced elevation of aortic fibrosis and stiffness (INSERT URL of one paper). Thus, the advantageous and disadvantageous features of Arg1 are highly dependent on its content of activation and local rather than systemic treatment may be appropriate to avoid collateral damage due to excessive Arg1 activation. It would be of great interest to investigate the beneficial effect of arginine supplementation in terms of inflammatory diseases, such as rheumatoid arthritis. Our human data support the role of Arg1 in inflammatory joint diseases. We characterized macrophages in synovium of rheumatoid arthritis patients. We were able to demonstrate that the active disease is characterized by low Arg1, while a milder disease activity is characterized by high level of Arg1 in synovial macrophages. In summary, our findings suggest that Arg1-derived products may contribute to the initiation of a remission phase of rheumatoid arthritic joint inflammation.

Our findings are reported in the article entitled "Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis" in Journal of Clinical Investigation **(https://www.jci.org/articles/view/96832). This work was conducted by Nicole Hannemann, Shan Cao, Daniel Eriksson, Anne Schnelzer, Jutta**

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