The pro-inflammatory role of platelet signaling in rheumatoid arthritis

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Background

Rheumatoid arthritis (RA) is an autoimmune disease that destroys the cartilage and the bone in the joint causing disability. It also contributes to pulmonary vasculitis, athrosclerosis, and other cardiovascular diseases. Approximately 1.2% of Canadians live with this condition. Genetic predisposition, epigenetics aberration and environmental factors contribute to the formation of RA. In RA, abnormally citrullinated proteins induce the production of anti-citrullinated protein antibodies (ACPA). Then, ACPA stimulates the release of a second antibody, rheumatoid factor (RF). RF-ACPA complex activates complement proteins, resulting in intense inflammation and subsequent cartilage and bone damage.

The involvement of FLS in rheumatoid arthritis

Normally, fibroblast-like synoviocytes (FLSs) line the intimal lining of the joint. These cells supply nutrients and extracellular matrix components of the cartilage under normal condition. However, in RA conditions, they interact with macrophages, T cells, and B cells to promote inflammation. Moreover, they proliferate and invade nearby cartilage. The matrix metalloproteinases (MMPs) they secrete degrade joint cartilage. This information is summarized in **Figure 1**.

The involvement of platelets in rheumatoid arthritis

Platelets are disc shaped blood cells that function in hemostasis. Addition to their hemostatic functions, they also drive inflammation. In RA patients, elevated circulating cytokines cause platelets to degranulate and release many cytokines including a major alpha-granule component, PF4. Platelets also release interleukin-1 (IL-1) containing microparticles. These microparticles travel to the synovial fluid and activate fibroblast like synoviocyte (FLS). This information is summarized in **Fig. 1**. Unpublished data suggest that PF4-knock out (PF4-KO) mice have reduced joint inflammation when induced with K/BxN serum, suggesting that PF4 is an essential mediator in the pathogenesis of RA. However, it is unknown whether (and how) PF4 directly activates FLS.

Hypothesis and objective

HYPOTHESIS: Platelet factor 4 (PF4) promotes a tissue-degrading phenotype in fibroblast-like synoviocytes (FLS).

The objective of this project is to determine the mechanism(s) by which PF4 signals to fibroblast-like synoviocytes (FLSs). The focus will be on the gene regulation and secretion of cytokines and MMPs from FLS.

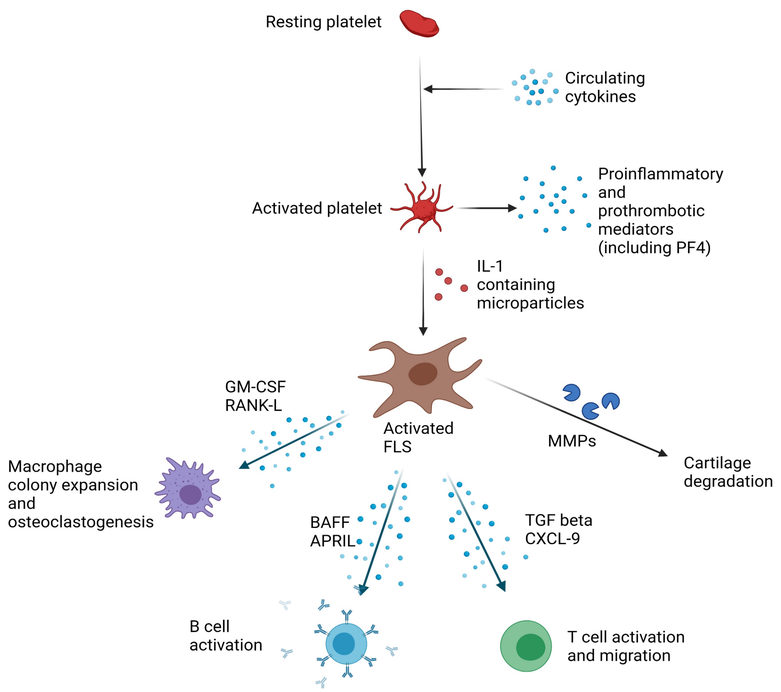

Figure 1. The activation of Platelets and FLSs. Platelets are activated by circulating cytokines. Activated platelets subsequently activate FLSs, which interact with other immune cells and degrade cartilage of the joint. This figure was created with BioRender.com.

Cell line selection

Previous studies have employed the SW982 synovial sarcoma cell line to study FLS. However, since the physiology of cancerous cell lines is different from that of normal cells, we will instead study the K4IM cell line, which is obtained by immortalizing primary cells from healthy synovium using SV40 T antigen.

Proposed project

To study the effect of PF4 on synovial fibroblasts, recombinant PF4 will be added to cultured K4IM cells, at varying concentrations and for differing times. Eight (8) different markers will be studied: interleukin-1beta (IL-1beta), matrix metalloproteinase-1 (MMP-1), MMP-3, granulocyte monocyte-colony stimulating factor (GM-CSF), receptor activator of nuclear factor kappa B (RANK-L), B-cell activating factor (BAFF), a proliferation inducing ligand (APRIL), Transforming growth factor-beta (TGF-beta), and CXCL-9 will be compared between vehicle control- and PF4-treated cells. Both the mRNA transcription and the amounts of secreted protein will be measured using qPCR and ELISA, respectively. Moreover, the rate of proliferation of FLS will be measured using EdU assay. The workflow is summarized in **Figure 2**.

The rationale for measuring the levels of IL-1beta is that it serves as a general marker for inflammation. In addition, the following secreted proteins from activated FLSs related to RA pathogenesis: MMP-1 and MMP-3 (cartilage degradation); GM-CSF and RANK-L (macrophage activation); BAFF and APRIL (B-cell activation); TGF-beta and CXCL-9 (T-cell activation). Both qPCR and ELISA will be used to measure both gene expression and protein production. The EdU assay measures DNA synthesis and will serve as an indicator of cell proliferation.

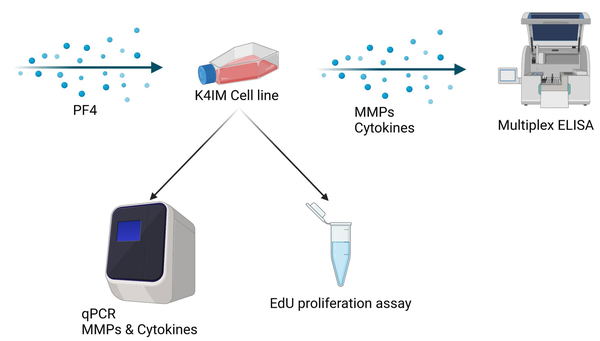

Figure 2. Schematic of the proposed workflow. K4IM cells will be cultured in the presence or absence of recombinant PF4. The levels of MMPs and cytokines will be measured by multiplex ELISA and qPCR. The rate of proliferation will be measured using EdU assay. This figure was created with BioRender.com.

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