Broeren et al .:

A Three-Dimensional Model to Study Human Synovial Pathology

Supplementary Data

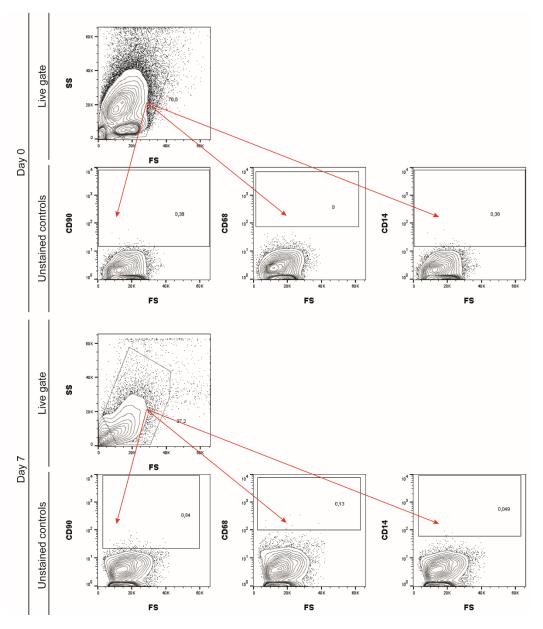


Fig. S1: Gating strategy and unstained controls for flow cytometry

A RA synovial biopsy was digested using Liberase[™]. Cells were cultured for < 24 h or mixed with Matrigel and cultured for 7 d, after which the Matrigel was re-liquified on ice. Unstained controls are shown. Similar profiles were obtained in at least 3 experiments with unique donors. FS, forward scatter; SS, side scatter. Data of stained samples are shown in Figure 1.

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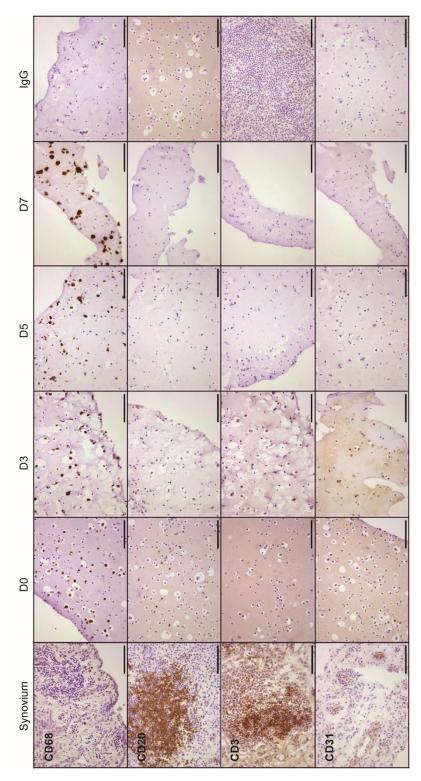


Fig. S2: Cell type composition during micromass formation RA synovial biopsies were divided into several pieces. One piece (synovium) was processed and sections were stained for macrophage marker CD68, B cell marker CD20, T cell marker CD3, and endothelial cell marker CD31. The remaining pieces were digested and used for micromass formation of complete cell suspensions. At day 0, day 3, day 5, and day 7 micromasses were fixed and embedded in paraffin. 7 µm sections were stained for cell markers. Pictures were taken at 200x magnification; black scale bars represent 100 µm. IgG, isotype IgG negative control.

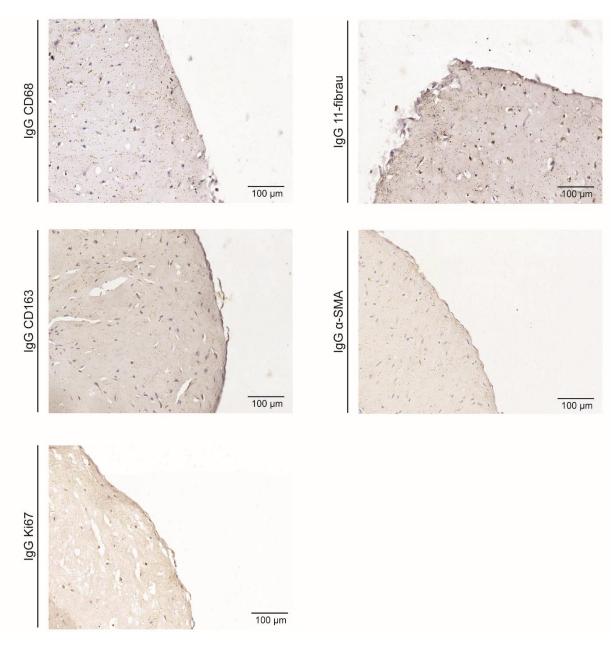


Fig. S3: Histological analysis of synovial micromasses Micromasses were generated from primary RA FLS and CD14⁺ PBMCs and were fixed and embedded in paraffin. 7 μ m sections were stained with anti-CD68, antibodies against fibroblast marker 11-Fibrau, anti-CD163, anti- α -SMA, and anti-Ki67, or their IgG controls. Representative pictures of isotype IgG negative controls are shown. Pictures were taken at 200x magnification. Stainings are shown in Figures 1, 2, 4, and 5.