

2 h. One group of animals received one single dose (0.25 mg) of prednisolone intravenously before in vivo imaging. Afterwards, the animals were killed and cryo-, and paraffin sections were prepared for immunofluorescence and histomorphological analysis, respectively.

Results GMC were barely detectable in healthy animals but were abundant in the synovial tissue as soon as clinical arthritis was apparent. GMC were motile and migrated randomly through the synovial tissue with a reduced mean velocity ($2.75 \pm 1.17 \mu\text{m}/\text{min}$) of as compared to healthy controls ($3.11 \pm 1.51 \mu\text{m}/\text{min}$; $p < 0.001$). In CIA subjects, the frequent formation of dynamic cell clusters was observed that consisted of both EGFP^{high} neutrophilic granulocytes and EGFP^{low} monocytes. In addition EGFP^{low} F4/80⁺ TRAP⁺ osteoclast precursor cells were occasionally observed at the synovial-bone junction and areas of bone erosions. Prednisolone treatment reduced the mean velocity of cell migration ($2.19 \pm 1.06 \mu\text{m}/\text{min}$; $p < 0.001$) and significantly diminished the immigration of GMC into the synovial tissue, but did not affect GMC allocation within cell clusters or throughout the entire tissue.

Conclusion The combined application of real time in vivo microscopy together with elaborate static postmortem analysis of GMC enabled the description of dynamic migratory characteristics of GMC together with their precise allocation in a complex anatomical environment. Moreover, this approach was found sensitive enough to detect subtle therapeutic effects within a very short period of time.

3 REAL TIME IN VIVO ANALYSIS OF GRANULOMONOCYTIC CELL MIGRATION IN THE COLLAGEN INDUCED ARTHRITIS MODEL

Ruth Byrne,¹ Eva Rath,¹ Anastasiya Hladik,¹ Birgit Niederreiter,¹ Michael Bonelli,¹ Sophie Frantal,² Michael Klimas,¹ Josef S Smolen,¹ Clemens Scheinecker¹ ¹Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Vienna, Austria; ²Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Vienna, Austria

10.1136/annrheumdis-2011-201238.3

Background Granulomonocytic cells (GMC) drive the inflammatory process at the earliest stages of rheumatoid arthritis (RA). The migratory behavior and functional properties of GMC within the synovial tissue are, however, only incompletely understood. This tempted us to study GMC in the murine collagen induced arthritis (CIA) model of RA with the help of multi-photon real time in vivo microscopy together with the subsequent and sequential ex vivo analysis of GMC on tissue sections.

Methods CIA was induced in LysM-EGFP C57BL/6 transgenic animals that carry the EGFP fluorescence protein under the lysozyme promoter. Individual joints were prepared by surgical microscopy in healthy control and in CIA subjects and EGFP⁺ GMC were analysed by 2-photon laser microscopy over