A131 **ANALYSIS OF POLYMORPHONUCLEAR NEUTROPHIL (PMN) PHENOTYPE AND FUNCTION AT THE ONSET OF COLLAGEN-INDUCED ARTHRITIS**

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Background Polymorphonuclear neutrophils (PMNs) participate in the initiation of rheumatoid arthritis (RA), but their precise role at the earliest stages of the synovial inflammatory response is only partially understood. We therefore analysed the initial steps of synovial inflammation in the mouse model of collagen-induced arthritis (CIA) with regard to the role of PMN.

Materials and Methods CIA was induced in C57/BL6 mice. Mice were tested for anti-CII serum antibodies by ELISA. Mice were killed on days 10 and 20 after the first immunisation before clinical signs of arthritis occurred and at various time points after the onset of arthritis. Hind and front paws were collected and non-decalcifying cryostat sections as well as conventional paraffin sections were prepared. Joint sections were analysed after immunohistochemistry (haematoxylin and eosin, tartrate-resistant acid phosphatase (TRAP),

toluidine blue, Neu7/4-granulocyte marker) and immunofluorescence double staining (Neu7/4, complement component - C3a, MHC class II, CD11c) by confocal laser scanning microscopy. In addition, synovial biopsies were taken at various time points after the onset of arthritis. Single cell suspensions were prepared by collagenase digestion, stained for Neu7/4, F4/80, GR1, MHC class II, CD11c, CD80, CD86, and analysed by flow cytometry (FACS).

Results Mice developed clinical signs of arthritis 38 ± 7 TS: Insert thin space> days after the first immunisation with type II collagen. Severe periarticular and intra-articular neutrophilic infiltrates together with first signs of cartilage destruction were detectable already on the first day of clinical arthritis. In addition, strong staining of complement factor C3a was detectable in areas of inflammation and in the bone marrow of diseased animals. The extent of infiltrates did not substantially increase over time but correlated with the degree of joint swelling. No histological signs of inflammation were detectable before the development of clinical signs of arthritis. In addition, no C3a deposits were detectable before disease onset, suggesting that complement activation concurs with the influx of PMN. Double stainings revealed an upregulation of MHC class II on Neu7/4 positive neutrophils in areas of inflammation.

Conclusions Periarticular neutrophilic infiltrates are prominent in CIA and are detectable already at the earliest stages of the disease. No histological signs of inflammation, especially complement activation, preceded the clinical signs of arthritis. Neutrophils in inflammatory infiltrates upregulate MHC class II expression which might contribute to their functional capacities. Ongoing experiments have been designed to further determine the kinetics and mechanism(s) that control the migratory behaviour and function of PMN in CIA as well as strategies to target PMN in therapeutic settings.