represent a minor cell population. On the other hand, neutrophils represent 50% of total blood leucocytes and are activated in SLE, especially by chromatin. Toll-like receptor (TLR) 9 recognises certain forms of DNA but its role in SLE is still not elucidated. The authors therefore sought to determine the cellular source of IFN α as well as the natural stimuli in SLE and the impact of TLR9.

Materials and methods Chromatin (mono-nucleosomes) was purified from calf thymus. PBMC and neutrophils were isolated from healthy individuals and SLE patients. Mouse neutrophils were purified from the bone marrow. Cells were activated with different stimuli and IFN α production/secretion was estimated by flow cytometry, ELISA and a bioassay. Neutrophil activation was verified by flow cytometry and ELISA. Gene expression was analysed by qPCR. Neutrophil extracellular trap (NET) induction was estimated by confocal microscopy.

Results Isolated neutrophils produce IFN α upon stimulation with chromatin. IFN α secretion by neutrophils was observed with steady-state neutrophils, and not pro-inflammatory neutrophils, from both healthy donors and SLE patients whereas pDC were less efficient. Neutrophil-derived IFN α was detected in response to free chromatin, and not chromatin-containing immune complexes, as well as TLR9 agonists. Nucleosome-induced IFN α production by neutrophils was associated with IL-8 secretion, CD66b up-regulation, ROS production and NET formation (NETosis). Neutrophil priming is not required. PBMC sustain IFN α secretion by chromatin-activated neutrophils in co-cultures. Importantly, chromatin-induced IFN α secretion occurs independently of TLR9 since neutrophils isolated from both wild-type and TLR9-deficient mice were activated. Finally, chromatin increases gene expression levels of IFN α and several DNA sensors, for example, AIM2 and STING.

Conclusions Neutrophils represent a major source of IFN α . IFN α was detected at the mRNA and protein levels and in an active secreted form. This is the first report showing both that steady-state neutrophils can secrete IFN α and identifying a natural lupus stimulus involved. A key event is thus the presence of increased concentrations of circulating nucleosomes in SLE patients. Chromatin-activated neutrophils (in addition to pDC and low-density granulocytes) may secrete IFN α early during the lupus disease, before immune complexes are produced. The generation of NET and the expression of genes involved in the recognition of DNA may strengthen pDC activation and DNA-mediated activation.

28 TLR9-INDEPENDENT AND IMMUNE COMPLEX-INDEPENDENT INTERFERON α PRODUCTION BY NEUTROPHILS UPON NETOSIS IN RESPONSE TO CIRCULATING CHROMATIN

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Background and objectives Chromatin represents a major autoantigen in systemic lupus erythematosus (SLE). Interferon α (IFN α) plays an important role in lupus development. Although activated plasmacytoid dendritic cells (pDC) are believed to be the main producers of IFN α in SLE, pDC