rather comparable between RA and controls by whole blood (mean 1055 (1533) pg/ml vs 1421 (925) pg/ml), as well as by monocytes (285 (593)–6222 (9549) vs 143 (154)–3108 (3635)). Similarly, IL1Ra secretion by whole blood was comparable between RA and controls both when unstimulated and upon NALP3 stimulation (981 (515) pg/ml vs 617 (209) pg/ml).

Conclusions Although basal expression of most of the NLRP3-inflammasome proteins was higher in patients with RA than in controls, they produced comparable IL1b upon NLRP3 activation. Baseline differences may be due to the inflammatory milieu which drives the expression of these inflammatory mediators. Failure to further increase IL1b secretion could be due to cell exhaustion because of chronic activation or LPS tolerance. The authors are currently assessing whether there is a differential activation of NLRP3-inflammasome after cell priming with TLR2/TLR3, and the relative contribution of different caspases in pro-IL1b cleavage.

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A89 RHEUMATOID ARTHRITIS (RA) PATIENTS HAVE VARIABLE BUT COMPARABLE NLRP3-INFLAMMASOME INDUCTION TO CONTROLS

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Background and Objective Data from animal models support an important role for interleukin 1b (IL1b) in the pathogenesis of rheumatoid arthritis (RA).[1 2] NLRP3-inflammasome is one of the main cellular platforms for IL1b production upon activation by external or self danger signals.[3] The authors sought to investigate the expression and function of NLRP3-inflammasome in peripheral blood of patients with RA.

Methods Heparin-anticoagulated whole blood from patients and healthy controls (HC) was used after red blood cell lysis. Monocytes were immunomagnetically isolated from peripheral blood mononuclear cells. TLR4 stimulation with lipopolysaccharide (LPS 250 pg/ml, 2 h) was followed by NLRP3-inflammasome activation with pulse ATP (5 mM, 20 min). Secreted IL1b and TNFα were assessed by ELISA, along with secreted casp-1 and IL1Ra in some cases. Intracellular protein levels of IL1b, caspase-1 and NLRP3 were determined by western blot. Protein densitometry from the western blots was assessed using the TinaScan program.

Results The authors studied 33 patients (3 men), mean age 59.4 (13.7) years and 15 HC (3 men), mean age 33.8 (6.4) years. At baseline, intracellular expression of NLRP3 (0.68 vs 0.21), pro-IL1b (0.5 vs 0.2) and actived IL1b (3.5 vs 0.2) was higher in patients. RA patients had a trend for higher expression of pro-Casp-1 isoform p50 whereas p38 isoform was comparably expressed. Interestingly, intracellular expression of active Casp-1 p20 was reduced in patients (0.02 vs 0.06). ATP plus LPS effectively activated NLRP3-inflammasome and IL1b production ($p<0.001$), but they did not induce the production of NLRP3-independent cytokines (TNFα). Upon NLRP3-inflammasome stimulation, IL1b production was