Letters to the Editors

Caspase-1 is active since the early phase of rheumatoid arthritis
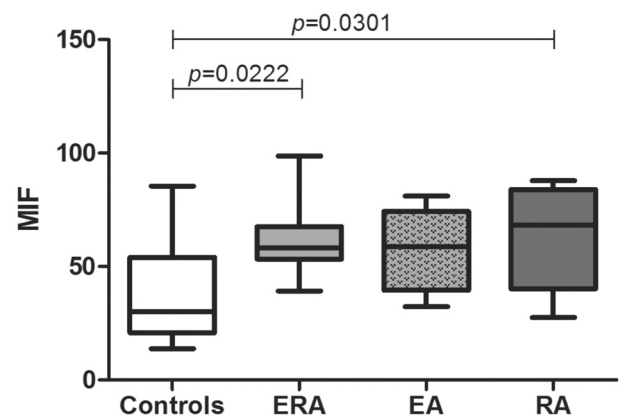
Sirs,

We have recently reported increased interleukin (IL)-1β levels in very recent onset rheumatoid arthritis (RA) (1). Together with IL-6, which we also found to be increased in these patients, IL-1β can promote the polarization of Th17 cells, which have an essential role in the pathogenesis of autoimmune diseases (2), such as RA. It has also been reported that RA synovial tissue contains higher concentrations of cytokines including IL-1β and shows activation of the nuclear transcription factor NF-κB (3), suggesting that IL-1β/IL1 receptor signalling may contribute to arthritis onset and progression (4).

Caspase-1 is activated by multiprotein complexes known as inflammasomes. One of the best characterised examples is the NLRP3 inflammasome (5), which is able to process immature proinflammatory cytokines, such as pro-IL-1β and pro-IL-18 (6). Interestingly, it has been reported that polymorphisms in the *nlrp3* gene are associated with increased susceptibility and a worse prognosis in RA (7, 8). Therefore, we hypothesised that the inflammasome could be an active contributor to the inflammatory response in RA and set out to evaluate the activation of caspase-1 in early and established RA patients.

To study the early phase of RA, we collected blood samples from 10 untreated early RA (ERA) and 9 untreated early arthritis patients who have not evolved to RA (EA) with less than 12 months of disease duration. ERA patients had a mean age of 48±9 years and DAS28 of 5.0±1.8. Among ERA patients, 90% were female, 60% were rheumatoid factor (RF) positive and 40% were anti-cyclic citrullinated peptide (anti-CCP) positive. EA patients had mean age of 60±17 years and DAS28 of 5.5±2.3. In the EA group, 67% were female, 22% were anti-CCP positive and all patients were RF negative. To study the chronic phase of RA, we collected blood samples from 11 established RA patients. Established RA patients had a mean age of 54±10 years and DAS28 of 4.5±1.3, 70% were RF positive and 36% were anti-CCP positive, and 80% of them were females. This group of patients had a mean disease duration of 13±8 years and all of them were under methotrexate (MTX) treatment with a mean dose of 14±5 mg/week and a low dose of prednisolone (less than 10 mg). We also collected blood samples from 10 age- and sex-matched healthy donors for comparison.

We measured caspase-1 activity using the Carboxyfluorescein FLICA Detection kit (Immunochemistry Technologies, LLC, USA) following the reagent instructions. Samples were acquired using a FACS Calibur (BD biosciences, USA) and analysed using FlowJo software (Tree Star Inc). Statistical differences were determined with parametric unpaired *t*-test and Bonferroni's multiple comparison test using GraphPad Prism (GraphPad, San Diego, CA), and differences were considered statistically significant for $p<0.05$.

We found that ERA patients have significantly higher levels of basal active caspase-1 than in healthy controls (Fig. 1, $p=0.0222$). Also established RA patients have higher basal levels of active caspase-1 as compared to healthy controls (Fig. 1, $p=0.0301$). In addition, no differences were found when comparing EA with healthy controls or between ERA and EA patients, neither between ERA and EA with established RA patients. Of note, we previously did not find any significant differences in the percentages of leukocyte populations between patients and healthy controls (9, 10).

These results show that the level of active caspase-1 is increased in circulating leukocytes of early and established RA patients, supporting our hypothesis that the caspase-1 pathway is already activated since the early phase of RA, plays a role in this disease and may be a promising treatment target in early RA. Given the relative inefficacy of blocking IL-1β in established RA we thus suggest that this treatment strategy should be re-evaluated in early RA patients.

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Fig. 1. Early and established RA patients have a persistent activation of caspase-1. Notice that, in contrast to EA patients, both in early and established RA patients there are higher active caspase-1 levels. (Unpaired t-test and Bonferroni's multiple comparison test).

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