Letters to the Editor

We read with interest the article by Sandanger et al. on the role of NLRP3 (also known as cryopyrin or NALP3) in acute myocardial infarction (AMI). We want to congratulate the authors for confirming the central role of NLRP3 in the myocardial response to ischaemic injury (as earlier reported by our group in 2011) while expanding the findings to explore the differences between the NLRP3 KO and the mouse with deletion of the gene for the Apoptosis-associated Speck-like protein containing a C-terminal Caspase-recruitment domain (ASC KO), which had been characterized by Kawaguchi also in 2011.

We fully agree that NLRP3 (cryopyrin) is central in AMI: targeted deletion of NLRP3 using a genetic KO model as done in the current study or with silencing RNA as done in our earlier study reduces apoptosis, infarct size, and cardiac dysfunction during AMI. We disagree, however, on the emphasis placed on the role of the fibroblast when compared with the cardiomyocyte. The authors state that the fibroblast is the most commonly encountered cell type in the heart in terms of number of cells, we may reply by saying that the cardiomyocyte is the most commonly encountered cell type in terms of cell volumes and are the cells that actually contract in the heart. The determinants of infarct size during myocardial ischaemia is generally considered to be the loss of the contractile mass (also shown with elevated plasma levels of components of the sarcomere), therefore if NLRP3 deletion reduces infarct size, it is implied that cardiomyocytes are spared. In our previous report, we show that the ASC/NLRP3 inflammasome is formed also in cardiomyocytes in vivo during AMI and in vitro upon stimulation with LPS/ATP, associated with increased cell death. Accordingly, the authors show up-regulation of NLRP3 during AMI in both non-cardiomyocytes (approximately three-fold) and in cardiomyocytes (∼25-fold), and although in the cardiomyocyte the expression is lower (when normalized to a common housekeeping gene), the relative induction is more impressive in the cardiomyocyte than in the non-cardiomyocyte.

Moreover, the current study highlights the differences between NLRP3 KO and ASC KO in this model of ex vivo ischaemia, without circulating blood cells, and with the ASC KO in the model of in vivo ischaemia.

While there is no clear interpretation of why such differences exist, it is plausible that the inflammasome, as a platform to activate caspase-1, is functionally active and important in different cell types: the fibroblast as resident cell with powerful synthetic function and paracrine effects, the leucocytes as infiltrating cells providing the largest source of pro-inflammatory mediators, and the cardiomyocytes, which are rather the targets of these effects and determine the overall contractile function of the heart (Figure 1).

**Conflict of interest:** none declared.

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Role of the NLRP3 inflammasome in the leucocyte, fibroblast, and cardiomyocyte.
A role for NLRP3 inflammasome in acute myocardial ischaemia-reperfusion injury? Reply

We thank Mezzaroma et al. and Jong and Zuurbier for their interest in our article, and also for their valuable comments and concerns regarding our results and interpretations.

As stated by Mezzaroma et al., several studies have now addressed the role of inflammasomes in the heart. We fully agree with the authors that in order to understand the role of NLRP3 in the myocardium, identification of the cell types involved in NLRP3-mediated responses is of great importance. We also share the authors’ claim that cardiomyocytes are the most prominent cell type in the heart. Likewise, loss of contractile tissue is also the most important consequence of a myocardial infarction. However, this does not necessarily imply that the NLRP3 inflammasome needs to be functional in the cardiomyocytes. In fact, while we clearly do not exclude a functional role of NLRP3 inflammasome in cardiomyocytes, we, in line with the study of Kawaguchi et al., suggest that myocardial fibroblasts, being a potent inflammatory cell, could contribute to NLRP3-mediated inflammation during ischaemia-reperfusion (I/R), and thereby also affecting cardiomyocytes.

In the study by Mezzaroma et al., immunofluorescence images indicate that inflammasome activity in cardiomyocytes in vivo in post-MI and in vitro studies in HL-1 cardiomyocytes supports this finding. However, the study of Kawaguchi et al. does not find inflammasome activity using neonatal mouse cardiomyocytes. Moreover, investigations in our lab have failed to reveal inflammasome activity in adult mouse cardiomyocytes. Furthermore, we acknowledge that the report by Zuurbier et al., in PLoS One last year should have been considered and referred to in our study. This article did not find any difference in cardiac function and cell death comparing WT and NLRP3−/− hearts during ex vivo I/R, but revealed highly interesting results on ischaemic preconditioning. Unfortunately, we find no apparent explanations for the discrepancy between the Zuurbier study and our study. To respond to the concerns of Jong and Zuurbier, we admit that the pre-ischaemic preconditioning of the hearts in our study was unstable and that this is not ideal. However, we are confident that this has not had a major impact on our results. Importantly, when calculating left