With much interest we have read the recently published article by Sandanger et al.,\cite{1} where NLRP3 mRNA expression was shown to be increased 3–7 days following permanent occlusion of the left anterior descending artery. The increased expression was observed mainly in fibroblasts. The authors also compared hearts from wild-type (WT) C57Bl/6, NLRP3 \textsuperscript{-/-} mice, using an isolated Langendorff-perfused heart model of acute ischaemia-reperfusion injury. It was reported that deletion of NLRP3 improved cardiac function and reduced infarct size in the isolated heart model. The authors summarized the data and combined the results from the in vivo 3–7 day permanent ischaemia with the results from the ex vivo acute ischaemia-reperfusion model in the title of the article.

However, their main conclusion that NLRP3 mediates ischaemia-reperfusion injury in the isolated heart is complicated for two reasons. First, mid last year we were the first to report on the effects of the NLRP3 inflammasome on acute cardiac ischaemia-reperfusion injury (and ischaemic preconditioning), employing a Langendorff-perfused isolated heart model with hearts from WT, NLRP3 \textsuperscript{-/-}, and ASC \textsuperscript{-/-} mice.\cite{2} The experimental set-up and mice used were similar to the ones described now in the report by Sandanger et al.,\cite{1} yet the results are diametrically opposed. We did not detect any improvement in either cardiac function or cell death when NLRP3 was deleted. Secondly, it can be noted that the reported cardiac function of especially the WT hearts at baseline (thus before I/R) demonstrated instable and poor left ventricular function (LVDP < 60 mmHg). Consequently, the reported baseline WT rate pressure product of 20 000 mmHg/min may be considered rather low for mouse hearts. In addition, the observed infarct size in WT hearts of >50% is much higher than the infarct size of 25% in the model that they refer to.\cite{3} This raises the possibility that their isolated mouse model was not optimal in its physiological performance, which may have skewed the results. Therefore, we believe that their conclusion that the NLRP3 inflammasome mediates (acute) ischaemia-reperfusion in the isolated heart may not be as firmly based as the authors purport. It is more likely that the cardiac NLRP3 inflammasome exerts very little activity within <2 h of an isolated heart experiment due to its low, basal expression in the heart. This is commensurate with the authors own data showing no significant differences in the primary cytokines produced by the NLRP3 inflammasome (IL-1\textbeta and IL-18) between reperfused WT and NLRP3 \textsuperscript{-/-} isolated hearts. This is also commensurate with a recent report by Guarda et al.,\cite{4} showing only significant basal NLRP3 expression in organs densely populated by immune cells (lung and liver) and in cells of the immune system.

In conclusion, the important results reported by Sandanger et al. on increased cardiac expression of NLRP3 following 3–7 days of permanent ligation indicate that the NLRP3 inflammasome, endogenous to the heart itself, will most likely affect the heart’s response to this injury at ≥1 day after infarction.

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References


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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,\cite{5} and also for their valuable comments and concerns regarding our results and interpretations.

As stated by Mezzaroma et al.,\cite{2} 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.,\cite{3} 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.,\cite{2} immunofluorescence images indicate that inflammasome activity in cardiomyocytes in vivo in post-MI and in vitro studies in HL-1 cardiomyocytes supports this finding.\cite{6} However, the study of Kawaguchi et al.,\cite{3} does not find inflammasome activity using neonatal mouse cardiomyocytes. Moreover, investigations in our lab have failed to reveal inflammasome activity in adult mouse cardiomyocytes (unpublished data). Thus, a role for the NLRP3 inflammasome in cardiomyocytes is in our view at present unclear, but an important topic in forthcoming studies.

We acknowledge that the report by Zuurbier et al.,\cite{7} 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 \textsuperscript{-/-} hearts during ex vivo I/R, but revealed highly interesting results on ischaemic preconditioning.\cite{7} Unfortunately, we find no apparent explanations for the discrepancy between the Zuurbier study and our study.\cite{7,5} To respond to the concerns of Jong and Zuurbier, we admit that the pre-ischaemic cardiac function 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

References


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A role for NLRP3 inflammasome in acute myocardial ischaemia-reperfusion injury? Reply
ventricular developed pressure during reperfusion relative to pre-ischaemic values, there were still significant differences between Wt and NLRP3−/− hearts. Moreover, pre-ischaemic coronary flow was similar and within normal range in all hearts, and thus, this cannot explain our reported differences in ischaemic damage and cardiac function. Finally, we would like to stress that all genotypes were strictly randomized and studied using the same system with the same detectors and the same balloons. Nonetheless, the discrepancies between our investigations underscore the need for further studies to elucidate the role for NLRP3 inflammasome within the heart.

The ischaemic damage that occurs in the Langendorff model of I/R tends to vary between different experimental setups, mostly depending on unknown factors. We refer to the study of Martinov et al.,5 where the ischaemic damage was relatively low (30% with 40 min ischaemia). Our results are more in line with the recently published study by Lei et al.6 The ex vivo I/R work in this study was performed at the same time as the NLRP3 study, using the same equipment and achieving a similar degree of ischaemic damage (60% with 40 min ischaemia). Zuurbier et al.7 do not present direct data on ischaemic damage in their study, and we cannot directly compare the degree of damage in our studies.

Basal expression of NLRP3 in the heart is low. Still, Kawaguchi et al.8 found reduced infarct sizes in ASC−/− mice 48 h after I/R in vivo, partly dependent on resident myocardial cells and supporting an ‘early’ effect of inflammasomes. As pointed out, we did not find significant differences between Wt and NLRP3−/− hearts with regard to IL-1β and IL-18 production upon ex vivo I/R, but there were strong trends and significant reduction in ASC−/− hearts.1 The latter may reflect that ASC is central in several other inflammasomes of potential importance in the heart, and that it is not beneficial to inhibit all myocardial inflammasome activity in the ex vivo I/R model. However, it may also be argued that the studies by Zuurbier et al. and Sandanger et al. point towards important inflammasome-independent effects of myocardial NLRP3, as both studies demonstrate a clear difference in phenotypes comparing NLRP3 and ASC-deficient hearts. This is in line with studies performed in renal I/R and which show NLRP3 effects independent of inflammatory cytokine production.7

A central issue in future exploration of NLRP3 in the heart will be characterization of its inflammasome-dependent and inflammasome-independent properties and their relative importance in different forms of cardiac stress and injury. Given recent reports on the association of NLRP3 with the mitochondria,8 studies of NLRP3 in the very mitochondria-dense cardiomyocytes will be of particular relevance.

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References


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