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Response to Gerlic et al.

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Gerlic et al. contacted us a few months ago regarding the concerns expressed in the accompanying letter. We have communicated with them about their observations relative to conclusions from our original paper (Im et al., 2011) and for the field moving forward. Based on their data, we performed a separate DNA sequence analysis of the Nlrp1a locus in our SREBP-1a-deficient (SREBP-1aDF) mice and confirmed that it is derived from the 129 strain. However, the issues raised by this observation are almost certainly more complicated than the tight linkage between the Srebf1 and Nlrp1a loci and the extent of backcrossing. In fact, similar complications could affect observations regarding Nlrp1a function from another recent report from Gerlic and colleagues (Masters et al., 2012). In this study, the *Nlrp1a* locus from the C57BL/6 strain, a line where Nlrp1a and Nlrp1c are expressed in bone marrow-derived macrophages (BMDMs), was inserted into the BALB/c strain where Nlrp1a/Nlrp1c also appear to be silent in BMDMs (similar to the 129 strain).

In addition to this study, there are numerous publications that suggest that strain differences at the murine Nrlp1 locus significantly influence responses to pathogens and inflammatory stimuli. However, a major relevant issue that has not been solved is why the *Nlrp1a* locus in the 129 strain, and several others, including BALB/c, seems to be silenced at least in BMDMs cultured in vitro. To begin to address this issue, we compared the Nlrp1a DNA sequence and putative mRNA coding regions from the C57BL/6 and 129 strains (Figure S1). The alignment predicts almost complete identity at the protein level between the two strains, with the exception of only two amino acid differences, both of which correspond to residues that display variations between different mouse strains (Sastalla et al., 2013). Interestingly, the Nlrp1a 5′-flanking sequences from the two strains are even more highly conserved. The extensive conservation strongly suggests that the Nlrp1a coding sequence is intact in both strains and that major structural alterations surrounding the *Nlrp1a* gene are an unlikely explanation for the absence of *Nlrp1a* expression in 129 BMDM. This is important because it suggests the Nlrp1a locus is under evolutionary pressure to maintain the coding integrity and thus predicts that it is expressed under the appropriate circumstances in 129 mice, we would argue in response to SREBP-1a activation. Also, it should be noted that the absence of 129-derived *Nlrp1a* transcripts in cultured macrophages is not reflective of *Nlrp1a* expression in an in vivo context where the SREBP-1aDF mice exhibit a profound inflammatory phenotype (Im et al., 2011).

The data in Figures 5 and 6 of our original paper demonstrate that Nlrp1a is directly activated by SREBP-1a because reintroduction of SREBP-1a into the SREBP-1aDF macrophages through either adenovirus vector delivery or plasmid transfection activates Nlrp1a mRNA expression and restores LPS-dependent IL-1β secretion to wild-type levels (Im et al., 2011). We recently repeated the activation experiment with identical results. These observations demonstrate that the Nlrp1a locus in the SREBP-1aDF strain can be expressed in isolated macrophages when SREBP-1a is reintroduced. Why *Nlrp1a* is not expressed in 129 BMDMs as well as from several other strains is an intriguing issue, one that deserves more investigation. Similarly, why further backcrossing to the C57BL/6 strain (which would alter many loci on chromosome 11 and elsewhere throughout the genome) would restore *Nlrp1a* expression deserves more study as well.

With regard to a second issue, IL-1β secretion, data in our original paper

showed that the SREBP-1aDF macrophages secreted reduced levels of IL-1β following LPS challenge relative to wild-type control mice (Im et al., 2011). This defect was not observed by Gerlic et al. We cannot explain this inconsistency, but it could relate to differences in the basal inflammatory state of the mice/macrophages in different mouse facilities. The defect in IL-1β secretion observed from the SREBP-1aDF macrophages was reproduced in two different laboratories that contributed to the original study and in response to both LPS and *Salmonella* Typhimurium challenge. These results were consistent over the course of several years as our project developed. In addition, our results with cultured macrophages are also consistent with our in vivo experiments where the SREBP-1aDF mice challenged either with LPS or subjected to cecal ligation and puncture (CLP) had reduced serum levels of IL-1β. Additionally, using siRNA knockdown, we also showed that Nlrp1a and Nlrp3 both contribute to stimulation of maximal IL-1β secretion following LPS challenge in C57BL/6 macrophages. Importantly, these responses are also consistent with results from the Nlrp3 and Asc knockout mice as discussed in our original paper (Im et al., 2011).

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.02.016.

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