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NLRX1/NOD5 deficiency does not affect MAVS signalling

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Dear Editor,

NLRX1/NOD5 belongs to the NOD clade of the NOD-like receptor (NLR) family. NLRs have emerged as key intracellular sensors for pathogen-derived molecules and endogenous danger signals to activate innate immune responses and inflammation. However, the function of most of these receptors still remains unclear.[1] Beside the NACHT and LRR domains present in most NLRs, NLRX1/NOD5 possesses a *N*-terminal mitochondrial targeting sequence.[2,3] The function of NLRX1/NOD5 is still controversial; one study proposed that NLRX1/NOD5 inhibits the RIG-like receptor (RLR) antiviral pathways by binding the adaptor protein MAVS/IPS-1/Cardif/VISA,[2] whereas another report implicated NLRX1/NOD5 in the generation of reactive oxygen species (ROS) and the amplification of NF-κB and JNK activation triggered by different stimuli such TNF-α, poly (I:C) and Shigella infection.[3] Interestingly, a recent study proposed that NLRX1/NOD5 is imported to the mitochondrial matrix, making NLRX1/NOD5 interaction with the outer mitochondrial membrane protein MAVS difficult to explain.[4]

To clarify the role of NOD5 *in vivo*, we generated NOD5-deficient mice by replacing the first four coding exons with a neomycin selection cassette. Mutant mice are viable and born at the expected Mendelian ratios (data not shown). Considering the proposed role for NOD5 in modulating RLR–MAVS signalling,[2] we tested whether NOD5 deficiency potentiates responses triggered by intracellular poly (I:C). However, when primary NOD5-deficient mouse embryonic fibroblasts (MEFs) were transfected with poly (I:C), we did not observe any major difference in MAVS-dependent IRF3 phosphorylation and IFN-β or IP-10 mRNA induction (Supplementary Figure 1 and data not shown). Moreover, IFN-β and IP-10 mRNA induction upon Sendai virus infection was normal in NOD5-deficient bone marrow-derived macrophages (BMDMs) (Supplementary Figure 1 and data not shown). NOD5-deficient MEFs also produced normal levels of IFN-β and IL-6 upon poly (I:C) stimulation (Supplementary Figure 1 and data not shown). To corroborate these observations *in vivo*, we assessed the effect of NOD5 deficiency on serum levels of IFN-β and IL-6 induced by intravenous injection of poly (I:C), responses largely dependent on MAVS (data not shown and[5]). Importantly, production of these cytokines was not affected by NOD5 deficiency (Supplementary Figure 1 and data not shown). Thus, NOD5 deficiency does not affect MAVS-dependent responses. A second report links NOD5 to ROS production and amplification of NF-κB and JNK signalling upon TNF-α stimulation or Shigella infection.[3] Interestingly, no major differences in TNF-α-induced NF-κB, JNK and p38 activation were observed in NOD5 knockout cells (data not shown). In a complementary approach to define NOD5 function, we carried out a proteomic screen to identify new NOD5 interaction partners. As a significant hit, we found UQCRC2 (ubiquinol-cytochrome-*c* reductase complex core protein 2), a subunit of the complex III of the mitochondrial respiratory chain. UQCRC2 was previously identified as an NOD5-binding partner.[4] In contrast, we failed to detect MAVS in the NOD5 immunoprecipitates. In keeping with this, endogenous UQCRC2, but not MAVS, could be co-immunoprecipitated with NOD5 (Supplementary Figure 1 and data not shown).

Collectively, our data indicate that NOD5 deficiency does not affect RLR signalling *in vitro* and *in vivo*, at least under the conditions tested. The reasons for the discrepancy between our findings and the *in vitro* characterisation of NOD5 as a RLR inhibitor are unclear, but are reminiscent of reports investigating NLRC5/NOD4 function; NLRC5/NOD4 was also proposed as RLR inhibitor based on *in vitro* studies, but no alteration in RLR responses were observed in NLRC5/NOD4-deficient mice.[6,7] Although our findings indicate that NOD5 deficiency does not affect TNF-induced signalling, the confirmation of UQCRC2 as a 'bonafide' interaction partner of NOD5 is consistent with the proposed link between NOD5 and ROS generation.[3,4,8] Mitochondrial ROS has been recently shown to trigger NLRP3 inflammasome activation, and thus modulation of ROS would be an attractive function of NOD5. With the availability of NOD5-deficient mice, this hypothesis is now testable.

Conflict of Interest

The authors declare no conflict of interest.

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