NOD2 MEDIATES HOST RESISTANCE TO *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* INFECTION

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The similarities between Paratuberculosis and Crohn’s disease have stimulated efforts to investigate whether Crohn’s susceptibility genes mediate control of *M. avium paratuberculosis* (MAP) infection. To test this hypothesis in the specific case of NOD2, we have obtained *Nod2*+/+ and *Nod2*-/− mice, to assay bacteriologic and immunologic outcomes during *ex vivo* and *in vivo* infection. Following stimulation of peritoneal macrophages with heat-killed MAP, we observed impaired innate recognition of the pathogen with *Nod2* disruption, characterized by decreased TNF-α production. We also observed NOD2-dependent TNF-α responses after infection with live MAP, at a time when the bacterial burden in macrophages was not affected by NOD2 disruption (24 h after infection). When extending the *ex vivo* infection to day 5, we observed a ~0.3 log relative increase in bacteria in *Nod2*-/− cells. To test whether these findings translated into differences *in vivo*, we infected mice by the intraperitoneal route; 4 weeks after infection, there was no effect of *Nod2* status on MAP burden in the livers, spleens and mesenteric lymph nodes, indicating that NOD2-independent processes exist to control early MAP infection. However, NOD2 disruption was associated with diminished antigen-specific interferon-γ responses (enumerated by ELISpot). Furthermore, splenocytes from MAP-infected *Nod2*+/+ animals when co-cultured with MAP-infected macrophages resulted in a decrease in bacterial numbers; in contrast, splenocytes from uninfected animals or from infected *Nod2*-/− animals provided no anti-mycobacterial activity when placed in co-culture with infected macrophages. In summary, disruption of NOD2 was associated with diminished killing of MAP by macrophages, reduced innate and adaptive immunity in the host and impaired immune responses required for control of intracellular mycobacterial infection.