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.