Granulocytic Ehrlichiosis in Mice Deficient in Phagocyte Oxidase or Inducible Nitric Oxide Synthase

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Received 22 February 2000/Returned for modification 24 March 2000/Accepted 28 March 2000

Mice deficient in phox (gp91phox<sup>-/-</sup>) or NOS2 (NOS2<sup>-/-</sup>) were infected with the agent of human granulocytic ehrlichiosis (HGE) to evaluate the importance of these pathways in the eradication of HGE bacteria. NOS2<sup>-/-</sup> mice had delayed clearance of the HGE agent in comparison to control or gp91phox<sup>-/-</sup> mice, suggesting that reactive nitrogen intermediates play a role in the early control of HGE.

Human granulocytic ehrlichiosis (HGE) is a newly recognized vector-borne infectious disease of increasing importance in the United States and Europe (3, 6, 16, 22). Prominent clinical manifestations of disease include fever, headache, and myalgias (23). HGE bacteria primarily infect neutrophils and survive within membrane-bound vacuoles known as morulae (23). A promyelocytic cell line (HL-60) has been used to culture HGE organisms in vitro, and bone marrow precursors have been infected with the HGE agent, stimulating further research (9, 10, 14). Mice can also be infected with HGE bacteria, facilitating in vivo studies of pathogenesis and immunity (2, 11, 21). Immunocompetent mice develop an infection in which the HGE agent is usually detected during the first 10 days of infection and is then generally cleared from the bloodstream (2, 20, 21). Sometimes, however, ehrlichiae can be detected by PCR at later intervals after challenge (2, 20, 21).

Two important microbicidal pathways of phagocytes are the production of reactive oxygen intermediates (ROI) by respiratory burst oxidase (phox) and reactive nitrogen intermediates (RNI) by inducible nitric oxide synthase (NOS2) (4, 7). Mice deficient in phox (gp91<sup>-/-</sup>) or NOS2 (NOS2<sup>-/-</sup>) have also demonstrated the importance of these enzymes in host defense against a variety of pathogens (5, 18, 19). Recent data suggest that HGE bacteria use several strategies to survive within the hostile environment of the neutrophil. Morulae do not fuse with lysosomes, providing one mechanism of persistence (17, 24). HGE bacteria also inhibit the formation of ROI through selective downregulation of the gp91phox component of the NADPH oxidase complex (1). HGE in mice deficient in phox or NOS2 was investigated to understand the role of ROI and RNI in granulocytic ehrlichiosis.

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**FIG. 1.** HGE infection in gp91phox<sup>-/-</sup> mice. At 8 and 12 days, splenocytes from HGE-infected gp91phox<sup>-/-</sup> and control mice (five animals per group) were isolated and pooled, and RT-PCR was performed using HGE-specific primers. One of three studies with similar results is shown.

**FIG. 2.** HGE infection in NOS2<sup>-/-</sup> and control mice. At 8, 12, and 20 days, splenocytes were isolated and pooled. RT-PCR using HGE-specific primers was performed. Five mice were used in each group. One of four experiments with similar results is shown.
of phox. This finding is in agreement with the in vitro observation that HGE bacteria actively inhibit the respiratory burst by downregulating gp91$^{phox}$, thereby developing a local environment that has reduced levels of ROI (1). gp91$^{phox}$-/- and control mice can, however, both clear the HGE bacteria after 12 days, suggesting that alternative mechanisms are responsible for the control of progressive infection. Our studies also demonstrate that NOS2 is important for the control of early infection because HGE can be readily detected at 12 days in NOS2$^{-/-}$ mice. Furthermore, IFN-γ is likely to play a role in the NOS2-mediated clearance of HGE bacteria because NOS2 levels were lower in IFN-γR$^{-/-}$ mice. Studies with Trypanosoma cruzi- and Listeria monocytogenes-infected IFN-γR$^{-/-}$ mice have shown similar reductions in NOS2 expression (8, 12). Nevertheless, in both gp91$^{phox}$-/- and NOS2$^{-/-}$ mice, HGE bacteria were cleared at 12 or 20 days. This demonstrates that neither pathway is necessary for the eradication of persistent infection, perhaps because humoral and cellular responses to HGE can aid bacterial clearance. Indeed, antibodies to HGE bacteria are sufficient to partially protect mice from infection (20). Understanding the host immune response to HGE should enhance our understanding of the pathways that facilitate bacterial clearance and the evolution of HGE infection in mice.

This work was supported by National Institutes of Health grant 51873, the Brown-Coxe Fellowship Program, and a gift from Smith-Kline Beecham Biologicals. E. Fikrig is the recipient of a Clinical-Scientist Award in Translational Research from the Burroughs Wellcome Fund.

We thank C. Nathan (Cornell University Medical College) and J. S. Mudgett (Merck Research Laboratories) for providing us with the NOS2$^{-/-}$ mice and Debbie Beck for technical assistance.

### REFERENCES


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**FIG. 3.** Effect of HGE infection on NOS2 levels in IFN-γR$^{-/-}$ mice. At 12 days splenocytes were analyzed for HGE bacteria and NOS2 induction by RT-PCR. One of three studies with similar results is shown.