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Mechanisms of immunity to *Rickettsia* and implications for vaccine development

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Abstract

Immunity to spotted fever group Rickettsiae following primary infection prevents reinfection as judged by the absence of documented second infections after recovery. Studies of the mechanisms of immunity to spotted fever group Rickettsiae have been performed to inform us of the immune effectors that may be required to mediate protection by a vaccine.

Innate immunity to Rickettsiae

After entrance into the body, the presence of *Rickettsia* is detected by the inflammasome, a cytoplasmic assembly of proteins that includes a sensor protein, adaptor protein (ASC), and an inactive zymogen protein (pro-caspase-1). Priming and activation of the inflammasome occurs via two separate signals. Toll-like receptor 4 (TLR4) plays a priming role in mononuclear phagocytes, which are the initial target cell in the site of inoculation of Rickettsiae during tick feeding. The rickettsial activation signal is, at least in part, its lipopolysaccharide, and the host sensor pathway includes, at least in part, NLRP3.

Innate immunity triggered by the inflammasome is critically important to protective immunity against Rickettsiae. Mice lacking TLR4 or its adaptor protein MyD88 are more susceptible to fatal spotted fever rickettsioses than gene-intact animals, emphasizing the importance of this inflammasome-priming pathway. Mice defective in the gene for ASC have a higher fatality rate and greater bacterial burdens in their organs than normal ASC —containing mice, further confirming the critical role of the infamous resistance Rickettsiae. Activation of the inflammasome leads to conversion of pro-caspase-1 to active caspase-1, which catalyzes conversion of pro-IL-1β to IL-1β and pro-IL-18 to active IL-18, which are secreted from the macrophage. ASC knockout mice infected with Rickettsiae produce dramatically lower levels of IL-1β, IL-18 and IFN-ɣ. IL-1β has a rickettsiacidal effect that is been demonstrated experimentally in RAW macrophages.IL-18 plays a role in the production of IFN-ɣ by natural killer (NK) cells, and IFN-ɣ plays a key role in the killing of Rickettsiae in their main target, endothelial cells.

Dendritic cells (DC), an important element of innate immunity, are likely prominent among the CD68+ mononuclear phagocytes that engulf Rickettsiae at the portal of entry in the skin. A substantial portion of intra-DC Rickettsiae are retained within phagocytic vacuoles as well as those that escape into the cytosol, enabling antigen presentation via MHCI to CD8 T cells and MHCII to CD4 T cells. Rickettsiae activate DC expression of MHCII and costimulatory molecules. Adoptive transfer of Rickettsiae -infected DC into the footpads of mice that are challenged 24 h later with an ordinarily lethal dose of Rickettsiae control the infection and survive; in contrast mice receiving mock-infected DC do not control the infection and die. The infected DCs

cause expansion of CD4, CD8, and NK cells, which are important host defenses against Rickettsiae. The NK cells are critical for survival of the infection by mechanisms involving cytotoxicity and production of IFN-ɣ, which is also produced by the expanded CD8 T cells, as early anti-Rickettsiae mechanisms.

Adaptive immunity and rickettsiscial effects by endothelial cells

By the time that adaptive immunity is initiated, Rickettsiae have spread via the bloodstream to infect many endothelial cells throughout the body, most critically in the lungs and brain. Indeed, endothelial cells themselves are important effectors of immunity by killing intracellular Rickettsiae by nitric oxide (NO) produced by nitric oxide synthetase 2 (NOS 2) in endothelial cells in association with autophagy and autophagolysosome formation. NO production in endothelial cells is stimulated by the combination of IFN-ɣ, TNF-α, IL-1β, and RANTES, which also activate reactive oxygen species, associated anti-rickettsial activity in endothelium and macrophages, and indoleamine 2, 3-dioxygenase degradation of tryptophan inhibiting the growth of Rickettsiae by limiting tryptophan availability to Rickettsiae and macrophages. The importance of these *in vitro* results regarding IFN-ɣ, TNF-α, and NO were confirmed in infected mice. Infection of gene knockout mice revealed that IFN-ɣ knockout mice are 200 times, perforin knockout mice 1000 times, and MHCI knockout mice 10,000 times more susceptible than gene-intact mice. The latter two results emphasize the critical importance of cytotoxic T lymphocytes in resistance to rickettsial infection.

Study of mRNA expression in human eschars from patients with Mediterranean spotted fever confirms that IFN-ɣ, TNF-α, RANTES, and indoleamine 2, 3-di oxygenase are present at the site of host immune control of *Rickettsia conorii* infection.

Immunosuppressive effects of rickettsial infection

Lethal spotted fever rickettsial infection induces antigen-specific immunosuppression, impairing resistance to rickettsial infection. Splenic T cells from lethally infected mice produce less antigen-specific IL-2 and IFN-ɣ and increased concentrations of IL-10. Lethally infected mice also have greater numbers of induced T regulatory cells (CD4+ CD25+ Foxp3-, CTLA-4^{high}), which suppress IL-2 production and lymphocyte proliferation, possibly by mechanisms involving IL-10 and/or CTLA4.

Immune effectors of anti-rickettsial vaccine

The preceding information explains how the host, which encounters Rickettsiae for the first time, succeeds in clearing the infection or succumbs to it. It is noteworthy that antibodies seem not to play an important role in resistance to primary infection, as they do not appear in experimentally infected mice until after recovery has occurred.

Determining which immune effectors are capable of preventing illness if present prior to exposure to the pathogen is critical to understanding the desired effects of stimulation of the immune system by an effective vaccine. In contrast with its lack of importance in clearing an established infection, polyclonal antibodies from a convalescent animal passively transferred into mice are protective against an ordinarily lethal rickettsial challenge dose. Indeed, even monoclonal antibodies against single epitopes of outer membrane proteins A or B are protective. A monoclonal antibody to spotted fever group rickettsial lipopolysaccharide was not protective. Protection requires the Fc portion of the immunoglobulin molecule. Adoptive transfer experiments have also determined that immune CD4 or CD8 T cells protect animals against an ordinarily lethal challenge, but not at the highest rickettsial doses. Adoptive transfer of immune CD8 T lymphocytes from IFN-ɣ knockout mice exerted an anti-rickettsial effect, suggesting that CD8 T cell cytotoxicity can contribute to vaccine-mediated immunity.

Although cross protection among spotted fever group Rickettsiae and between typhus group Rickettsiae is well-established, it was a surprise that there is cross-protection between spotted fever group and typhus group Rickettsiae, which are not known to stimulate cross-reactive antibodies. Indeed, the cross protection is mediated by T cells. Although immunization or passive transfer of cross-reactive immune T cells does not prevent disease, it does prevent death. These data raise the possibility of a pan-rickettsial protective vaccine. Immunization with attenuated non-pathogenic Rickettsiae expressing cross protective T cell epitopes and both spotted fever rickettsial (e.g. *R. rickettsii*) outer membrane proteins A and B and typhus group rickettsial (e.g. *R. typhi*) outer membrane protein B might be protective against any rickettsial infection.