Commentary

Regulating the T-Cell Immune Response Toward the H99 Strain of *Cryptococcus neoformans*

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Almost 25 years ago, Mosmann et al¹ described murine helper T-cell subsets that differed in cytokine production and function and thus played distinct roles in the immune response. These CD4⁺ T-cell subsets, which were subsequently identified in humans as well, are differentially induced by and also produce a host of inflammatory mediators.²

Two of the most commonly studied CD4⁺ T-cell immune responses are Th1 and Th2, although other cell subsets have been identified and are probably important for cytokine production in immune reactions. The Th1 response is modulated by the production of interleukin (IL)-2, interferon- γ , and lymphotoxin by Th1 helper T cells. Th1 T-cell clones are involved in delayed-type hypersensitivity reactions and cell-mediated immunity and are associated with a proinflammatory response, low antibody titers, and inhibition of B-cell activation. In turn, the Th2 immune response is associated with the production of IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Th2 mediators aid in B-cell antibody production and, in addition, are involved in the pathogenesis of allergic reactions. The Th2 response can inhibit the activity of Th1 cytokines and is also associated with a paucicellular immune reaction.

Both Th1 and Th2 reactions are important in a variety of immune-regulated conditions, including the pathogenesis of autoimmune diseases, tolerance in solid organ transplant recipients, and clearance of infectious pathogens. It is clear that many immune responses involve a balance between the Th1 and Th2 subsets as well as incorporate other defined and possibly not yet defined T-cell subsets.³

The Th1 and Th2 responses toward infectious agents including bacteria, fungi, parasites, and viruses have been widely studied. One particular fungal pathogen with an increasingly interesting immune response is *Cryptococcus neoformans*, which is a dimorphic, often encapsulated, fungus that can cause significant morbidity and mortality in infected hosts. Although localized pulmonary disease is usually the most commonly identified form of infection, the pathogen can disseminate, resulting in meningoencephalitis, which, if untreated, has a 100% mortality rate.

Infection by *C. neoformans* is typically seen in immunosuppressed patients, particularly those with acquired T-cell deficits, and is most severe in those with defects in cell-mediated immunity such as patients with AIDS and those undergoing immunosuppression due to solid organ transplants. The increased incidence of *C. neoformans* infection in patients with T-cell deficiencies indicates that the T-cell arm of the immune system is important in regulating *C. neoformans* infection. However, although *C. neoformans* infection is seen predominantly in individuals with T-cell defects, recent data have demonstrated infections in immunocompetent hosts.⁴ The evidence of increased infectivity in immunocompetent hosts suggests that *C. neoformans* may have developed a means by which to evade immune regulation.

The inflammatory reaction toward *C. neoformans* is believed to be important for regulating this pathogen. The immune response to *C. neoformans* is often driven by Th1 (and the more recently identified Th17) response, in addition to classic activation of macrophages. This response is commonly associated with a marked inflammatory infiltrate that at first is neutrophilic followed by fibrosis and a granulomatous response.⁵

On the other hand, *C. neoformans* infection can also induce a classic Th2 response.⁵ In mouse models, infection with highly virulent strains of *C. neoformans* such as H99 often results initially in severe pneumonitis and subsequently in the development of systemic disease. *C. neoformans* strain H99 has been shown to induce a Th2 response to *C. neoformans*⁶; therefore, it has been postulated that this Th2 response is responsible for the increased virulence of H99.⁶ These data led to the hypothesis that if a Th1-mediated immune reaction to *C. neoformans* strain H99 in the lungs could be achieved, then infected hosts would not develop fatal central nervous system disease.^{7,8} In the manuscript by Zhang et al⁹ in this issue of *American Journal of Pathology*, the authors present data contrary to this belief.

Accepted for publication September 17, 2009.

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Using a murine model, Zhang et al⁹ substantiated their previous findings that a Th2 immune reaction to *C. neoformans* strain H99 is responsible for this organism's virulence. Moreover, they established that mice deficient in two Th2 cytokines, IL-4 and IL-13, which lacked a measurable Th2 response to H99 including absence of pulmonary eosinophilia and elevated IgG, switched to a Th1 (as well as Th17) immune response and switched from alternative to classic macrophage activation. This switch to a Th1 response produced a protective effect in the lung by causing a significant inflammatory reaction, resulting in control of the intrapulmonary fungal growth. However, whereas the Th1 response resulted in a reduction in pulmonary infection, it did not lead to a significant increase in overall survival when compared with that of wild-type mice, as both groups of mice succumbed to fatal meningoencephalitis.

The article of Zhang et al highlights how a nonprotective Th2 immune response to *C. neoformans* can be switched to a Th1 immune response to provide a protective effect in the lung. Such a finding can further our understanding of how fungal infections can be contained and cleared by the infected host. However, it also shows us that despite converting to an intrapulmonary protective immune response in the lung, there is (are) most likely additional mechanism(s) needed to inhibit organism dissemination and subsequent development of fatal meningoencephalitis. Although the Th2 response to H99 is not protective, it is still possible that elements from both Th1 and Th2 immune responses are needed for H99 clearance.^{4,5} The shedding of *C. neoformans* capsular components into the serum may play a role in down-regulating cell-mediated immunity in extrapulmonary sites, which could explain why a strong Th1 response helps with clearing pulmonary infection but may not benefit extrapulmonary growth.⁴ Alternately (or in addition), it has been hypothesized that the lack of IL-6 (a Th2 cytokine) could increase *C. neoformans* virulence.⁴

This monumental study not only furthers our understanding of *C. neoformans* but also leads us to contemplate infection treatment modalities. It indicates that further investigation is necessary to determine other immune mechanisms needed to prevent fungal dissemination and that a simplistic approach to Th1 and Th2 responses will probably not hold true in many immune processes.^{2,3} The manuscript by Zhang et al demonstrates that an immune reaction toward an infectious agent that is not protective can be switched to one that is protective to the host. Although this switch may not entirely regulate the pathogen, it opens up a myriad of ways in which the inflammatory reaction to a variety of immune system-regulated processes can be examined, which allows for the development of means for treating, managing, and, in the future, possibly curing disease processes regulated by immune mediation.

Variable responses for pathogen control have been seen with other infectious agents as well. In murine models, the protozoal pathogen *Leishmania* elicits a different immune response, depending on the murine strain infected.^{10,11} The immune response in these mice is highly dependant on the host environment. Mice that develop a pure Th1 response are able to completely eradicate the infection, whereas

those that induce only a Th2 response show a noncurative response. If the strains with a pure Th2 response are treated with anti-IL-4, the curative Th1 response develops. On the other hand, treating mice that develop strong Th1 responses with antibodies against interferon- γ results in a Th2 response and persistent infection. Moreover, Th1 responses are suppressed during pregnancy, and pregnant mice that normally produce the protective Th1 effect against *Leishmania* take much longer to clear the infection.³

In addition, in mice infected with *Plasmodium falciparum*, Th1 and Th2 responses are important at different stages of the infection.¹² It may therefore be important to determine not only which type of immune response is protective but also during which stage of the disease process the immune response has the most effect. It would not be surprising to find that most infectious pathogens have complex immune regulatory processes involved in their clearance and that the immune response will vary significantly with the host.

The status of the host, the type of immune system, and probably currently unknown mechanisms are required to regulate many immune-mediated processes. We can look forward to additional studies such as that presented by Zhang et al⁹ to further our understanding (and even lack of understanding) of the Th1/Th2 immune responses, especially as it relates to our future management of infectious diseases.

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