Advancing Translational Immunology in HIV-Associated Cryptococcal Meningitis

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(See the major article by Jarvis et al on pages 1817–28.)

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Cryptococcal meningitis is a disease that afflicts approximately 1 million human immunodeficiency virus (HIV)–infected individuals annually, with >600 000 deaths, predominantly in the developing world, where antiretroviral therapy is less available [1]. Therapy of cryptococcal meningitis has been poorly effective in resource-limited settings, where 10-week mortality rates have averaged 25%–30%, even when treatment conditions were optimized under experimental protocols [2, 3], and >50% in routine practice, where access to diagnostic tests and medications is difficult [4]. A rational approach to improving these outcomes has sought to understand microbial and host factors that distinguish favorable from adverse outcomes. Recent work on the microbial side of the equation has successfully demonstrated that rates of fungal clearance from the cerebrospinal fluid are an important prognostic marker in cryptococcal meningitis [5, 6]. However, understanding host factors associated with successful outcomes has been more problematic. The majority of our understanding of the host response to *Cryptococcus neoformans* comes from animal data [7]. Such studies have laid important foundations, such as the role of T-helper 1 (Th1)–type T cell responses [8, 9] and the associated cytokines interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) [10] in the successful activation of macrophages and microbial killing. In contrast, T-helper 2 (Th2) mechanisms, associated with interleukin 4 and interleukin 10, are detrimental [11]. However, translation of these immunological principals into efficacious treatments for human cryptococcal meningitis infections has been difficult, as exemplified by the lack of mortality benefit to immunotherapy with the "protective" cytokine interferon gamma [12], as well as by the "Cryptococcal Optimal ART Timing" (COAT) study, which sought to improve effective immune responses to the fungus but was stopped prematurely because of excess deaths in the early antiviral treatment arm [13]. The outcome of the COAT study was particularly vexing as recent studies have shown benefit of early antiretroviral therapy in other opportunistic infections, including pneumocystis pneumonia [14] and tuberculosis [15–17].

Thus, to explore the human host response in HIV-associated cryptococcal meningitis, Jarvis et al [18], as reported in this issue of the *Journal*, undertook a detailed study of cryptococcal-specific peripheral CD4 T-cell responses and selected cerebrospinal fluid cytokines in 44 HIV-infected patients with cryptococcal meningitis. Samples were collected at baseline and during follow-up from patients in the trial of interferon gamma therapy, cited above, that was performed in Cape Town, South Africa, between 2007 and 2010 [12]. A partially purified mixture of T-cell–activating cryptococcal mannoproteins was used as the stimulant of peripheral blood cells. Cryptococcal mannoproteins are extensively O-mannosylated and facilitate recognition by mannose receptors on antigen-presenting cells, particularly dendritic cells, resulting in efficient antigen uptake and presentation to T cells [19]. These *C. neoformans* mannoprotein–induced responses were compared with responses to *Mycobacterium tuberculosis* or cytomegalovirus (CMV), using specific antigens, since over half of the patients were either being treated for tuberculosis or had a history of tuberculosis; CMV exposure is widespread among individuals in Africa, as it is in most regions of the world [20].

These studies demonstrated that *C. neoformans*–specific CD4 T-cell responses were characterized by the production of CCL3/macrophage inflammatory protein 1α (MIP-1α), IFN-γ, and TNF-α. Interestingly, using a newly developed

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statistical platform for comparing measurement distributions among groups of multicomponent data samples [21], the investigators showed an overall difference in patterns of T-cell response between patients who survived 2 weeks versus those who died during this period. Although it was difficult to attribute a specific cytokine response to these patterns because of insufficient statistical power, a trend appeared to separate outcomes into 2 groups, one with high IFN-γ/TNF-α production, better survival, and lower fungal burdens and another with higher MIP-1α responses and poor outcomes. Such a relationship is biologically plausible, as IFN-γ and TNF-α are protective against cryptococcal infections in mice and could also have been responsible for increased CSF cell counts in the surviving arm, owing to more effective cell recruitment. This is an important hypothesis to develop for future validation, as it may help to target subgroups more precisely for immune-based therapies. For example, there was also a positive trend in response to adjuvant interferon gamma therapy in patients who had low production of IFN-γ/TNF-α by CD4 T cells versus those expressing high levels of these protective cytokines.

Despite a lack of statistical power in comparing such extensive multiple comparisons by means of modestly sized cohorts, it is important to report such discovery studies prior to their complete validation because they can help generate hypotheses. They can also help refine future studies and conserve valuable resources that might otherwise have been used in less promising investigations. For example, future studies assessing immunological modifiers may profit from inclusion of a select subgroup of markers from these studies that may help to both validate the markers and provide secondary end points for outcome analysis. The challenge, of course, is to translate such sophisticated immunologic analyses into resource-appropriate allocation algorithms.

An additional benefit of such studies is the generation of models of infection that reflect conditions in the human host, compared with those derived from mouse studies alone. Mouse models have limitations; for example, mice elicit a significant pulmonary neutrophilic response to *C. neoformans*, and some mouse strains, such as C57BL/6J, produce prominent pulmonary eosinophilia, neither of which is typical of human infections, which tend to elicit histiocytic responses with a spectrum of giant cell formation, depending on the degree of retained cellular immunity in the infected patient [22, 23]. Human data concerning the host response to *C. neoformans* is not as extensive. Epidemiologic data have defined a role in HIV-infected individuals for CD4 T-cell depletion in the acquisition of cryptococcal meningitis, and ex vivo stimulation studies of cells isolated from these individuals have demonstrated reduced *C. neoformans* killing by macrophages [24] with retained stimulation of β-chemokines such as MIP-1α/CCL3 [25]. Thus, the present, more-detailed studies provide additional data regarding human HIV-associated cryptococcal meningitis and suggest differences with data from prior mouse studies. For example, in the present study, higher mannoprotein-dependent MIP-1α/CCL3 CD4 T-cell responses were prominent among the group with higher mortality and higher fungal burdens. In mice, MIP-1α/CCL3 is protective, and depletion results in reduced cellular recruitment and higher fungal burdens [26] with markedly reduced survival [27]. This difference may be due to species-related responses or to confounding effects from coinfections in the human cohort. MIP-1α has been implicated in the induction of lymphotropic HIV that typically accompanies late-stage infections [25], but viral loads were equivalent between the 2 outcome groups. Antibodies to *C. neoformans* were not measured in the 2 groups, which have been shown to have a variety of protective and nonprotective effects in cryptococcal meningitis [28] and have been associated with β-chemokine induction, including MIP-1α /CCL3 [29, 30]. A surprising finding was the lack of an observable Th2 bias in patients with poor clinical responses, which was anticipated by mouse studies showing that poor outcomes were associated with an elevated Th2 response. The lack of a Th2 bias in human T-cell responses could have been due either to a Th1-bias in the mannoprotein stimulant [19] or to an overall Th1 bias in the human response to *C. neoformans* infections versus that of the mouse. While such studies will clearly require further validation of T-cell response profiles and specific cytokines, the study by Jarvis et al provides an important first look into detailed immunophenotyping of this important disease. As Wolff suggested in an editorial about clinical immunology almost 40 years ago in this journal [31], "major challenges await the clinical investigator who can turn his attention to the problems of developing countries." Despite such challenges, these efforts offer the promise to push back the global burden of infectious diseases.

Notes

Disclaimer. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the US government.

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