Toward Identification of the Genetic Risk Profile for Cryptococcal Disease in HIV-Infected Patients

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ABSTRACT ***Cryptococcus*** **spp. are important fungal pathogens that represent a major cause of morbidity and mortality in both immunocompetent and immunodeficient patients. Although cryptococcal disease is one of the major causes of death in HIV-infected patients, especially in sub-Saharan Africa, not all patients at risk with low CD4 counts develop the disease. It has been thus hypothesized that host genetic variation may represent an important susceptibility risk factor for this infection. In their recent study in *mBio*, Rohatgi et al. [S. Rohatgi et al., mBio 4(5):e00573-13, 2013, doi:10.1128/mBio.00573-13] present an important piece of evidence to support this hypothesis, by demonstrating that the *FCGR3A* 158 F/V polymorphism has an important impact on susceptibility to cryptococcal disease in HIV-infected patients. The authors present both genetic evidence and immunological validation for the hypothesis that humoral immunity in general and *FCGR3A*-mediated uptake and antibody-dependent cellular cytotoxicity (ADCC) in particular play important roles in the pathogenesis of *Cryptococcus* infection. Their discovery that the 158V allele of this polymorphism can increase the risk of *Cryptococcus* infections up to 20-fold in homozygous individuals opens the possibility for risk stratification and personalized treatment of HIV-infected patients.**

Cryptococcus species represent major fungal pathogens in humans. Two major species of *Cryptococcus* previously classified as varieties of the same species have been discerned recently, *Cryptococcus neoformans* and *Cryptococcus gattii*, which share many characteristics but also display differences in their microbiological and epidemiological properties (1). *C. neoformans* can be further subdivided into two varieties: *Cryptococcus neoformans* var. *grubii*, which enjoys a global distribution, and *Cryptococcus neoformans* var. *neoformans*, which is found most often in Europe (2). While cryptococcal infections affect both immunologically competent and compromised individuals, the prominence of *Cryptococcus* in human pathology has been largely due to HIV: indeed, *Cryptococcus* infections have been described from the start of the pandemic as one of the most important complications of AIDS, and they exact a high toll in terms of morbidity and mortality (3). However, despite the clear importance of CD4-mediated immunity for host defense against *Cryptococcus* and the association of CD4 cell loss and susceptibility to cryptococcal infection, not all HIV-infected patients with low levels of CD4 cells will develop the disease. Moreover, CD4-competent individuals also can develop infection with *Cryptococcus*; hence, other host or environmental risk factors must influence susceptibility to the infection as well.

A landmark study performed more than 2 decades ago that assessed mortality due to infectious disease in adult adoptees and their biological parents revealed a very strong genetic component in human susceptibility to various infections (4). Despite the strong possibility that genetic factors impact susceptibility to fungal infections in general and to *Cryptococcus* infections in particular, very few studies have investigated these risks until now. In non-HIV patients, FcγR and mannose-binding lectin (MBL) polymorphisms have been reported to increase susceptibility to cryptococcal infections (5–7). Paradoxically, much less is known about genetic factors that influence susceptibility to cryptococcal disease in HIV-infected patients. The recent study by Rohatgi et al. (8) represents an important first step in that direction by confirming that the *FCGR3A* 158V allele, a susceptibility trait in non-HIV-infected individuals, also increases the risk of cryptococcal disease in HIV patients. Heterozygous individuals have a 2.1-fold-increased risk of contracting cryptococcal disease, while the homozygous individuals display a 21-fold-increased susceptibility to the infection (8). In contrast, no association between the *FCGR2A* 131 H/R polymorphism and cryptococcal disease was observed in this population of HIV-infected individuals, in contrast to earlier studies that identified this association in non-HIV-infected European patients (5). Interestingly, this same polymorphism was not associated with *Cryptococcus* infections in a Chinese cohort (6), suggesting that ethnic differences exist in the genetic susceptibility to cryptococcal infection.

The implications of the study by Rohatgi and colleagues (8) are 2-fold. First, this study confirms the earlier reports that identified the influence of polymorphisms in FcγRs on susceptibility to *Cryptococcus* (5, 6) and establishes the importance of antibody-dependent mechanisms for the pathogenesis of human cryptococcal disease. The authors' previous *in vitro* and experimental studies have suggested a role during the infection for antibody-mediated recognition and phagocytosis of *Cryptococcus* (9–11), and this extends earlier results by other groups (12–14). Now, the authors extend these observations by the study of the genetic risk factors for the human infection. Antibody-mediated immunity is thus demonstrated to play an important, albeit somewhat unexpected, role during cryptococcal infection. Second, the study by Rohatgi et al. (8) takes a first step toward genetic risk stratification in HIV-infected individuals, with homozygous HIV-infected patients displaying a more-than-20-fold-increased risk for infection. This very significant increase in the risk of infection, if confirmed

Published 15 October 2013

Citation Netea MG. 2013. Toward identification of the genetic risk profile for cryptococcal disease in HIV-infected patients. mBio 4(5):e00798-13. doi:10.1128/mBio.00798-13.

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in future validation cohorts, may justify the identification of at-risk individuals and an intensified prophylactic approach to cryptococcal infections in HIV-infected individuals.

Additional studies are warranted to validate the findings of Rohatgi et al. (8). The cohort in this study was relatively small and homogeneous, so it is necessary to assess whether *FCGR3A* 158V is associated with a high risk for *Cryptococcus* infection in other HIV-positive populations as well. Genetic validation in a cohort of patients with both genders is also needed, as the cohort investigated by Rohatgi et al. (8) consisted of males only. On the other hand, the authors partially compensated for the lack of a genetic validation study through functional assays aimed at identifying the biological mechanism behind the effects of the *FCGR3A* polymorphism on susceptibility to the disease. The authors demonstrate that the risk 158V allele is associated with an increased binding of the receptor to *Cryptococcus*-IgG complexes and a potentiation of NK-dependent antibody-dependent cellular cytotoxicity (ADCC). They propose that the increased uptake of *Cryptococcus* contributes to a "Trojan horse"-like spread of the fungus through infected macrophages, while the increased inflammatory reaction may contribute to the damage of the blood-brain barrier and dissemination of the infection to the brain. These are important and persuasive functional insights that contribute to our understanding of the disease. Nevertheless, additional experimental studies are needed to further validate this hypothesis *in vivo*.

The approach of the authors not only to identify a genetic risk factor but also to explain the biological mechanisms behind it is commendable. This is desirable in more genetic studies, in which often only statistical significance is sought, without trying to identify the functional implications of genetic risk factors. The approach to obtain complementary genetic and functional data not only gives the chance to understand better the pathophysiology of the disease but at the same time opens the possibility of creating novel diagnostic and/or therapeutic strategies.

In conclusion, the interesting study by Rohatgi and colleagues (8) represents a first step toward uncovering the genetic susceptibility to *Cryptococcus* infections in HIV-infected individuals. The study identifies the *FCGR3A* 158V allele as an important risk factor for the infection, opening the possibility for risk stratification in patients with advanced HIV infection. As a researcher, I hope that this investigation will embolden the community studying fungal infections to continue and extend these observations. Recruitment of additional and larger cohorts and genetic studies based on both hypothesis-driven and discovery-driven approaches (genome-wide association studies), as well as functional validation studies, are all needed in order to address the major clinical challenge represented by *Cryptococcus* infections.

REFERENCES

1. **Chaturvedi V, Chaturvedi S.** 2011. Cryptococcus gattii: a resurgent fungal pathogen. Trends Microbiol. **19:**564–571.
2. **Franzot SP, Salkin IF, Casadevall A.** 1999. Cryptococcus neoformans var. grubii: separate varietal status for Cryptococcus neoformans serotype A isolates. J. Clin. Microbiol. **37:**838–840.
3. **Chayakulkeeree M, Perfect JR.** 2006. Cryptococcosis. Infect. Dis. Clin. North Am. **20:**507–544.
4. **Sørensen TI, Nielsen GG, Andersen PK, Teasdale TW.** 1988. Genetic and environmental influences on premature death in adult adoptees. N. Engl. J. Med. **318:**727–732.
5. **Meletiadis J, Walsh TJ, Choi EH, Pappas PG, Ennis D, Douglas J, Pankey GA, Larsen RA, Hamill RJ, Chanock S.** 2007. Study of common functional genetic polymorphisms of FCGR2A, 3A and 3B genes and the risk for cryptococcosis in HIV-uninfected patients. Med. Mycol. **45:** 513–518.
6. **Hu XP, Wu JQ, Zhu LP, Wang X, Xu B, Wang RY, Ou XT, Weng XH.** 2012. Association of Fcgamma receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. PLoS One **7:**e42439. doi:10.1371/journal.pone.0042439.
7. **Ou XT, Wu JQ, Zhu LP, Guan M, Xu B, Hu XP, Wang X, Weng XH.** 2011. Genotypes coding for mannose-binding lectin deficiency correlated with cryptococcal meningitis in HIV-uninfected Chinese patients. J. Infect. Dis. **203:**1686–1691.
8. **Rohatgi S, Gohil S, Kuniholm MH, Schultz H, Dufaud C, Armour KL, Badri S, Mailliard RB, Pirofski L.** 2013. Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. mBio **4**(5): e00573-13. doi:10.1128/mBio.00573-13.
9. **Subramaniam K, Metzger B, Hanau LH, Guh A, Rucker L, Badri S, Pirofski LA.** 2009. IgM(+) memory B cell expression predicts HIV-associated cryptococcosis status. J. Infect. Dis. **200:**244–251.
10. **Rohatgi S, Pirofski LA.** 2012. Molecular characterization of the early B cell response to pulmonary Cryptococcus neoformans infection. J. Immunol. **189:**5820–5830.
11. **Szymczak WA, Davis MJ, Lundy SK, Dufaud C, Olszewski M, Pirofski LA.** 2013. X-linked immunodeficient mice exhibit enhanced susceptibility to *Cryptococcus neoformans* infection. mBio **4**(4):e00265-13. doi:10.1128/mBio.00265-13.
12. **Mukherjee J, Scharff MD, Casadevall A.** 1992. Protective murine monoclonal antibodies to Cryptococcus neoformans. Infect. Immun. **60:** 4534–4541.
13. **Mukherjee J, Pirofski LA, Scharff MD, Casadevall A.** 1993. Antibody-mediated protection in mice with lethal intracerebral Cryptococcus neoformans infection. Proc. Natl. Acad. Sci. U. S. A. **90:**3636–3640.
14. **Dromer F, Charreire J, Contrepois A, Carbon C, Yeni P.** 1987. Protection of mice against experimental cryptococcosis by anti-Cryptococcus neoformans monoclonal antibody. Infect. Immun. **55:**749–752.