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EDITORIAL COMMENTARY

Evaluating the Impact of *LTA4H* Genotype and Immune Status on Survival From Tuberculous Meningitis

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(See the major articles by Thuong et al on pages 1020–8 and Laarhoven et al on pages 1029–39.)

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Emerging evidence suggests that early events in the pathogenesis of tuberculosis set the stage for the clinical outcome of the disease [1]. An early critical measure of antituberculosis host responsiveness is the balance between proinflammatory and anti-inflammatory pathways that are triggered by prostaglandin E2 and lipoxin A4 (LX4), respectively [2, 3]. Mutations in the gene encoding leukotriene A4 hydrolase (*LTA4H*) can lead to preferential LX4 accumulation and increased susceptibility to mycobacterial disease in zebra fish and humans [4]. Unexpectedly, heterozygosity at several *LTA4H* single-nucleotide polymorphisms (SNPs) provided increased protection for pulmonary and meningeal tuberculosis (TBM) [4]. This observation led to the concept that excess LTA4H activity would result in a host-detrimental hyperinflammatory phenotype, whereas lack of LTA4H activity would result in host-detrimental hypoinflammation [4]. Subsequently, the *LTA4H* promoter region SNP rs17525495, occurring in 3 genotypes—TT, CT, and CC—was identified as a likely molecular cause of the genetic susceptibility [5]. Expression levels of *LTA4H* in lymphoblastoid cell lines (LCLs) correlated with SNP genotype, with CC being lowest (hypoinflammation) and TT being the highest (hyperinflammation). In a study of 182 Vietnamese patients with TBM, of whom a subset had received adjunctive dexamethasone treatment, 3 important results emerged. The genotype of the rs17525495 SNP correlated with pretreatment cerebrospinal fluid (CSF) leukocyte counts; in the absence of dexamethasone, heterozygotes at rs17525495 displayed a significant survival advantage; and the main beneficiaries of adjunctive steroid therapy were carriers of the hyperinflammatory TT genotype [5]. Given the suggested clinical relevance of the *LTA4H* polymorphism, additional studies of the role of this genetic variant in TBM are of great interest.

Two studies reported in this issue of *The Journal of Infectious Diseases* investigated the impact of rs17525495 genotypes and inflammatory biomarkers on survival from TBM in the presence of corticosteroid adjunctive therapy. The first study, by Thuong et al, used a cohort of 764 adult Vietnamese patients with TBM, of whom 352 were infected with human immunodeficiency virus (HIV). Using a 9-month serial interval, the authors observed HIV infection as a strong risk factor for decreased survival. British Medical Research Council (BMRC)–defined disease severity grade was a strong risk factor for reduced survival for both HIV-infected and HIV-free patients with TBM. Moreover, the authors studied genotypes of the rs17525495 *LTA4H* polymorphism and markers of CSF inflammation as additional risk factors for death. Owing to pronounced differences in clinical presentation and prognosis of HIV-infected patients with TBM, we will focus only on the HIV-uninfected patients.

Compared to patients who were homozygous for the TT genotype, which is associated with hyperinflammation, HIV-negative carriers of the rs17525495 CC genotype, which is associated with hypoinflammation, had a higher risk of early death, with borderline significance (P = .03). Because of large confidence intervals around the risk estimate, the true impact of homozygosity at this SNP on survival is difficult to evaluate. The *LTA4H* promoter SNP was not associated with CSF leukocyte counts but was associated with 3 pretreatment CSF cytokine levels. However, both low leukocyte counts and global low cytokine levels were strongly associated with reduced survival. Whether the SNP effect on survival is mediated through cytokine levels remains unknown. Disease severity, which was strongly associated with survival, was not associated with leukocyte count, cytokine levels, or *LTA4H* genotype.

A second study, by van Laarhoven et al, enrolled 608 patients with TBM, of whom 93 were HIV infected. Over a 12-month follow-up period, the authors studied clinical and immune characteristics and the *LTA4H* promoter polymorphism as risk factors for reduced survival. As in the Vietnamese cohort, HIV infection and disease severity were strong risk factors

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for reduced survival. In HIV-free patients, motor abnormalities, an increased body temperature, and a low CSF to blood glucose ratio were risk factors for death. In contrast to the Vietnamese study, CSF leucocyte count was not associated with survival. Instead, increased numbers of neutrophils in CSF and blood—2 measures not assessed in the Vietnamese study—were associated with death in Indonesian patients with TBM. Conversely, no information regarding CSF cytokine levels was provided for the Indonesian cohort. Genotypes at the *LTA4H* SNP rs17525495 did not show significant association with any phenotype (ie, outcome) when corrected for multiple testing, including survival times and CSF leukocyte counts.

Although the 2 studies agree on poor prognosis for HIV infection and more-severe disease (high BMRC grade or low Glasgow Coma Scale score), they differ on the impacts of a CSF hypoinflammatory phenotype and the *LTA4H* promoter genotype on the risk of death. This raises the question why genetic effect modifiers are detected in some but not other studies. Both studies only genotyped a single SNP, based on the experimentally supported assumption that the SNP was causally linked to survival times and CSF leukocyte counts [5]. Still, from a purely genetic view, this is not an ideal approach since it is not possible to correct for genome background effects. Single SNP analysis in different ethnicities also raises the possibility of differences in linkage disequilibrium (LD). Alleles at different genomic locations that are in strong LD are difficult to distinguish by association studies. Hence, it is possible to erroneously implicate a tested SNP allele as the cause of a phenotype when, in truth, the phenotype is caused by a second unknown SNP with alleles strongly correlated with the tested one. Such correlation patterns (ie, LD) often differ between ethnic groups.

While we do know the *LTA4H* LD pattern in Vietnamese individuals (KHV; available at: http://www.internationalgenome.org), the LD across *LTA4H* in the Indonesian sample can only be estimated. In Vietnamese individuals, there is strong LD across much of the *LTA4H* gene, opening a possible involvement of *LTA4H* alleles distal to the promoter region. If the causative SNP was, indeed, in a more downstream region of the gene and if LD was shorter in the Indonesian patients, this might explain the lack of significant association in the Indonesian patients. However, an in silico scan of SNPs across *LTA4H* in ensemble [6], haploreg [7], and the UCSC Genome Browser [8], coupled with a cursory overview of rare variant clusters [9, 10], did not reveal additional candidate risk SNPs toward the 3' region of *LTA4H*. Indeed, the strongest evidence for an effect on *LTA4H* gene expression is provided by SNPs in the 5' gene region, and this region shows strong conservation of LD across highly divergent ethnic groups. However, the direction of the SNP allele effect on gene expression displays unexpected plasticity, as the high-expressor genotype in LCL is the low-expressor genotype in whole blood (available at: http://www.gtexportal.org) [11]. This observation suggested that genetic control of *LTA4H* expression was sensitive to cell type and, possibly, to environmental factors. Environmental factors have a strong impact on gene expression levels, and the genetic control of gene expression can, itself, be modulated by environmental factors [12–14]. Hence, it is possible that the *LTA4H* rs17525495 SNP is the cause of varying levels of gene expression in Vietnam but not in Java.

Perhaps more pertinent are differences in patient characteristics between the 2 cohorts. Any genotype-phenotype association that is mainly carried by a specific patient subgroup might be lost if proportions of patient subgroups are different between studies. The most striking divergence between both studies are differences in mortality (40.7% in Indonesia vs 18.9% in Vietnam; HIV-free patients). Indeed, half of all deaths occurred within 6 days of enrollment in Indonesia, while the median time to death appeared to be substantially longer in Vietnam. In addition, culture-confirmed diagnosis was somewhat higher in Indonesia (55.3% vs 42.8% in Vietnam), a substantially larger proportion of patients were classified as having BMRC grade I severity in Vietnam (37% vs 11% in Indonesia), and the median age of patients was higher in Vietnam (41 years vs 29 years in Indonesia). Older age was a strong risk factor for Vietnamese patients but only was of borderline significance among Indonesians, suggesting the importance of late-onset risk factors. Age-correlated risk factors can be important modulators of disease pathogenesis, and genetic phenotype associations can be age dependent [15, 16]. However, adjustment on age in the Vietnamese sample did not have impact on the association between *LTA4H* and survival, and earlier studies in Vietnam enrolled younger patients [5] and still detected significant *LTA4H* association with time to death. This argues against an age effect on the association of *LTA4H* with time to death, although the impact of age on the inflammatory phenotype remains unknown.

Severity of disease is a strong predictor of time to death, and a larger proportion of patients with more severe illness were enrolled in Indonesia. Is it possible that the *LTA4H* effect is primarily seen in less severe cases? In both studies, the distribution of *LTA4H* genotypes across grade of disease severity was not different. However, this does not rule out the possibility that the impact of a given genotype on time to death is different for each grade. Adjustment by disease grade did not affect the evidence for an association in the Vietnamese study. Yet, depending on the specifics of the model used, this does not rule out the possibility that the effect of the polymorphism is clustered in the lowest grade, with little impact on survival in higher grades. To evaluate the impact of disease severity, a stratified analysis may be the best approach. Stratified analysis in the Indonesian sample found a strong ratio of the hazard for genotype TT to the hazard for genotypes CC and CT combined (hazard ratio, 0.37) for patients classified as having less severe disease. However, owing to small numbers in this stratum in the Indonesian

sample, the result was not significant. If the effect of the *LTA4H* polymorphism is only detectable in less severe cases, this would imply that the mechanisms underlying early death impacted by LTA4H and severe disease are different and, hence, might explain why the immune phenotypes in both studies were also divergent.

Some of the scenarios mentioned can be tested easily (eg, the interaction of severity with *LTA4H* association), whereas others will be more involved (eg, the expression quantitative trait loci effect on *LTA4H* expression in CSF cells). Similarly, associations of exposure variables with outcome are always susceptible to confounding, and establishing causality will be a critical research task. Perhaps more importantly, the 2 studies argue for a good integration and, as far as possible, harmonization of clinical protocols in the investigation of complex clinical outcomes.

Notes

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