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Differential HLA Allele Frequency in *M. africanum* vs. *M. tuberculosis* in Mali

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Abstract

Tuberculosis is caused by *Mycobacterium tuberculosis* complex (MTBC), however, the distribution and frequency of MTBC lineages and sublineages vary in different parts of the globe. *Mycobacterium africanum*, a member of MTBC is responsible for a large percentage of TB cases in West Africa, however, it is rarely identified outside of this part of the World. Whether or not differential HLA polymorphism (an important host factor) is contributing to the geographic restriction of *M. africanum* to West Africa is unknown. Here, we conducted a cohort study in Mali of newly diagnosed individuals with active pulmonary TB and normal healthy controls. The MTBC isolates were spoligotyped to determine the TB study groups (*M. tuberculosis* sensu stricto LAM10 and *M. africanum*), and HLA typing was performed on peripheral blood. Unlike previous reports on other populations, we found that HLA Class-I alleles were significantly associated with active TB disease in this population. HLA-B alleles (B*07:02, B*08:01, B*14:02, B*15:03, B*15:10, B*18:01, B*42:01, B*42:02, B*51:01 and B*81:01) were significantly associated with *M. africanum* (40–45%) and *M. tuberculosis* (75%) compared to healthy controls. Many HLA-A alleles (A*02:05, A*34:02, A*66:01 and A*68:02) were also associated with both TB groups (65–70%). However, many Class II HLA-DR variants were found to be associated with *M. tuberculosis* but not *M. africanum* with the exception of the DRB1*03:01, which was associated with both groups. The differential HLA distribution observed in this study might be at least partially responsible for the geographical restriction of *M. africanum* infections to West Africa.

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CONFLICT OF INTEREST

There are no conflicts of interest

Keywords

Tuberculosis; M. africanum; HLA; Mali; West Africa

INTRODUCTION

Despite recent advances, tuberculosis (TB) remains a major cause of morbidity, mortality and economic burden in Mali and other West African countries. In 2017, the WHO estimated the prevalence, incidence and mortality of TB in Mali to be 66, 10 and 10.1 per 100,000 population, respectively.[1]

The phylogeny of *Mycobacterium tuberculosis* complex (MTBC) contains seven known lineages of TB, of which two lineages (5 and 6) are collectively referred to as *M. africanum,* whereas the other five lineages (1–4 and 7) are referred to as *M. tuberculosis* sensu stricto. First isolated in Senegal in 1968, *M. africanum* is geographically restricted to West Africa and accounts for up to half of all pulmonary TB cases in this region.[2–4] In Mali *M. africanum* accounts for 28% of TB disease, whereas *M. tuberculosis* sensu stricto accounts for 71%.[4] Interestingly, *M. africanum* is rarely identified outside of Western Africa, and when it is diagnosed in other parts of the world, it appears to have a predilection for individuals of West African descent.[5] Relative to *M. tuberculosis* sensu stricto, *M. africanum* appears to be less likely to progress from latent infection to active tuberculosis disease, less likely to be multidrug resistant, and more likely to cause active disease in older individuals who are malnourished or infected with HIV.[6,7] A prior investigation of whole genome sequencing of *M. africanum* clinical isolates from TB patients in Mali identified some lineage-specific mutations that may explain some of these phenotypes that are unique to *M. africanum*.[8] The apparent specificity of *M. africanum* for West Africans provides a unique opportunity to study host-pathogen interactions and to evaluate differential human susceptibility to TB.

In addition to bacterial factors, host factors are critically important in mediating the susceptibility and severity of TB disease. The innate and adaptive immune responses to the disease are unique and complex and are influenced by host factors including the human leukocyte antigen (HLA) genes.[9,10] HLA (the major histocompatibility complex) plays an important role in cellular immune response and its modulation,[11,12] and is known to be involved in self-antigen tolerance and its resulting cellular immune responses to tumours and pathogens. HLA is characterized by an important genetic diversity with more than 2,155 alleles identified on HLA Class I loci (A, B and C types).[13] Class I HLA-A, -B, and -C loci are well described to be essential for both innate and adaptive cellular immune responses. Their crucial interaction with T-cell receptors on cytotoxic T-lymphocytes (CTLs) mediates adaptive immune responses against microbial agents. Class II HLA molecules are involved in adaptive immune responses through antigen presentations to immune cells. The role of HLA in tuberculosis disease has been demonstrated,[9] mainly a strong association with the HLA class II alleles in strain-specific manner.[10] For example, a study in Thailand showed that HLA-DRB1*09:01 and HLA-DQB1*03:03 alleles are highly involved in the susceptibility to modern strains of TB.[10] A recent study in Uganda suggested an important

role of HLA-DQB1*03:03 allele in the progression to TB disease.[14] Several other studies in South-East Asia including India, Indonesia, Iran, and Thailand have shown that HLA II alleles are important in terms of susceptibility to TB disease.[15–20]

Whether or not differential HLA allele frequency contributes to the geographic restriction of *M. africanum* in West Africa is unknown. In this study, we investigated the frequency of HLA Class I and Class II alleles among individuals with a new diagnosis of active pulmonary TB due to *M. tuberculosis* sensu stricto LAM10 and *M. africanum* in comparison to healthy controls in Mali. The goal of this investigation was thus to characterise the HLA Class I and II alleles in TB disease in an area of West Africa where *M. africanum* is responsible for a substantial number of TB cases.

METHODS

Ethical approval

The study protocol was approved by the ethics committee of the Faculty of Medicine and Odontostomatology of the University of Sciences, Technics and Technologies of Bamako, Mali and the Institutional Review Board (IRB) of Northwestern University in Chicago, USA. Written informed consent was obtained from all study participants or their legal representatives before enrolment.

Subjects

Adult individuals (≥18 years of age) with active pulmonary tuberculosis (defined by positive sputum culture) and healthy volunteers were prospectively recruited from the HIV/TB Research Project, SEREFO at the University of Bamako, in Mali, from June, 2014 to June, 2017. To meet criteria for study inclusion, healthy controls had to be asymptomatic have a negative QuantiFERON-T Gold test (no latent TB infection). Study participants were not related to each other.

Study design and procedures

This cohort study compared HLA typing from individuals newly diagnosed with active TB due to *M. tuberculosis* sensu stricto LAM10, active TB due to *M. africanum*, and normal healthy controls (first 20 participants in each study group). Individuals newly diagnosed with active TB were recruited prior to initiation of TB treatment. In a standardized interview and medical examination, demographic information, symptom screening and details regarding prior TB and/or HIV treatment were recorded. Stored blood samples for HLA typing, and early morning sputum samples for mycobacterial culture and speciation were collected and processed prospectively. The infecting mycobacterial strains were isolated and spoligotyped in order to retrospectively classify participants into the two active TB study groups. To eliminate strain-specific bias, individuals harboring the most prevalent spoligotype of *M. tuberculosis* sensu stricto, LAM-10, were selected to serve as the *M. tuberculosis* group.

Mycobacterial cultures and identifications

Sputum specimens were digested and decontaminated using the standard N-Acetyl-L-Cysteine/4% NaOH method, before inoculating both liquid (*Mycobacterium* Growth

Incubator Tube [BBL™ MGIT™ Becton Dickinson, Sparks MD, USA]), and solid (Middlebrook 7H11 Agar and Selective 7H11 Agar) media. Simultaneously, an aliquot of concentrated specimen was prepared for indirect commercial Auramine/Rhodamine staining (BBL™ Becton Dickinson, Sparks MD, USA). Positive cultures were confirmed to belong to the MTBC by acid-fast bacilli and nucleic acid probe (AccuProbe® GenProbe, San Diego, CA, USA). As previously described [21], spoligotyping was performed using a commercially available kit (Isogen Life Science, Netherlands) to identify *M. tuberculosis* and *M. africanum* species and subspecies.

HLA typing

DNA was extracted from stored blood samples using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to Manufacture's procedures. Extracted DNA was quantified by spectrophotometry using the NanoDrop One C (Thermo Scientific, Massachusetts, USA).

HLA-I typing: 900–980 bp fragments encompassing exons 2 through 3 of HLA-A, -B, or -C were PCR amplified in three separate reactions using locus-specific primers targeting conserved regions of each respective HLA genes, as previously described.[22–24] Briefly, the first-round PCR contained Amplitaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA), 200 nM of each of the published primers,[22–24] and genomic DNA (20–100 ng) in a final volume of 50 uL. Thermocycling conditions were: 10 min at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 45 seconds at 72°C; followed by 7 min at 72°C. First-round PCR products were used in subsequent PCR- SSP-based genotyping reactions for the targeted variants in HLA-A, -B, and -C, respectively.

HLA-II typing: was performed using extracted DNA that was PCR-SSP amplified for HLA-DR typing using specific published primers.[25]

All amplicons from HLA-I and HLA-II typing PCR were separated with 1% Agarose gel electrophoresis in TAE (Tris-Acetate EDTA) 0.5X buffer and DNA stained by GelRed for visualization under UV light.

Sample size calculation and statistical data analysis

Sample size calculations were conducted using the Fleiss Method. We estimated that 20 participants would be needed in each group in order to compare each group with another with a precise 95% confidence level, 80% power and two-sided significant level (α) of 0.05. We assumed, based on previous studies,[4,26] that 42% of study participants are exposed to the outcome (*M. africanum* infections), with an Odds Ratio of 14, a risk/prevalence ratio of 8.5 and a risk/prevalence difference of 37.

The HLA allele frequencies in each group were compared with the other groups, using a Chi-square test. Results were considered significant for a p-value less than 0.05. Analyses were performed using IBM SPSS Statistics version 20.

RESULTS

Demographic and clinical characteristics

Our analysis included 20 individuals who were retrospectively classified by spoligotyping as having active TB due to *M. tuberculosis* LAM10, and 20 individuals with active TB due to *M. africanum*, in addition to 20 healthy controls. Demographic and clinical characteristics for each study group are described in Table 1. All study participants were from Mali and were HIV negative. The study groups were similar in all demographic and clinical parameters, with the exception of over-representation of younger individuals among the healthy controls ($p = 0.047$)

Association of HLA-I allele frequency with pulmonary TB and *M. africanum* in Mali

Genotyping of HLA-I and HLA-DR was performed to assess the frequency of HLA alleles among individuals with *M. tuberculosis* LAM10, with *M. africanum*, and normal healthy controls. We observed consistent strain-specific differential expression patterns of HLA types and subtypes among individuals with active *M. tuberculosis* and *M. africanum* in comparison to healthy controls. In particular, some HLA-A alleles were observed to occur at a significantly higher frequency in TB infected participants (both *M. tuberculosis* and *M. africanum*) relative to healthy controls, such as A*02:05, A*34:02, A*66:01 and A*68:02 alleles (Table 2 and 3). However, *M. tuberculosis* and *M. africanum* had statically similar proportions for these alleles (Table 2). HLA-B alleles were more frequently observed in *M. tuberculosis* than *M. africanum* infected participants (Table 2). The HLA-Cw alleles did not appear to have significantly differential allele frequency among the three groups. In contrast to other regions,[10,15,17,27] the Class I of HLA (Type A and B) appeared to be associated with the susceptibility to pulmonary TB infections Mali. This finding may help elucidate the mechanisms behind the specificity of TB infections in this population, in particular the geographical restriction of *M. africanum* to West Africa and West Africans.

The Class II HLA-DR allele frequency in associations with TB in Mali

As it has been previously reported in other regions of the world, [17,28] a couple of HLA-DR loci showed significant association with *M. tuberculosis* infections compared to healthy controls including DRB1*03:01, DRB1*11:01, DRB1*16:01, DRB1*16:02, DRB3*01:01 alleles. However, only DRB1*03:01 loci of the HLA-DRs tested was significantly associated with *M. africanum* infections (Table 2).

DISCUSSION

While TB is a global problem, human disease due to *M. africanum* (*M. tuberculosis* Lineages 5 and 6) appears to be geographically restricted to West Africa and West Africans, for reasons that are not well understood. In this study, we found that certain HLA-B alleles appeared to confer greater risk of active TB due to *M. tuberculosis* sensu stricto LAM10 (75%) rather than *M. africanum* (40–45%) (Table 2). This suggests that HLA allele frequency may be important in the control of tuberculosis in strain-specific manner. Our data also show that HLA-A alleles (A*02:05, A*34:02, A*66:01 and A*68:02) and many HLA-B

of MHC Class I are consistently associated with host susceptibility to active TB disease in our study population (Table 3), unlike previous reports from other populations of the globe.

While the association between HLA allele frequency and active TB has been previously investigated,[10,15,17,27] to the best of our knowledge, this is the first report of a very high correlation between active TB disease with HLA-A and B alleles of MHC Class I. These data may help explain one mechanism behind the apparent geographical restriction of *M. africanum* to West African populations and their descendants.

The role of HLA in the susceptibility to tuberculosis is population and strain dependent.

HLA is known to be implicated in many diseases including tuberculosis, although the magnitude of implication and polymorphism involved are very diverse in different pathological conditions. Moreover, HLA-DRB1 alleles (MHC class II) have been the most reported to be associated with tuberculosis in many different populations and areas where the disease is endemic including India, China, South Africa, Iran, Portugal and others [28]. This underscores the role and implications of host and environmental factors in the susceptibly and resistance to the disease, on top of the bacterial factors and strain diversity [28]. HLA genes have been determined to be relevant biomarkers for disease outcomes of many infectious diseases, because of its important role in the host immune response.[28] Our analysis in this particular Malian population showed a significant correlation of HLA-A and B alleles (MHC class I) with tuberculosis infection when compared with healthy participants (Table 3). However, HLA-DRB1 (MHC class II) was at some extend associated with *M. tuberculosis* infections but not *M. africanum.* Only HLA-DRB1*03:01 was associated with both *M. tuberculosis* and *M. africanum* infections.

The role of HLA in the geographical restriction of *M. africanum*.

It is now becoming evident that specific lineages and/or strains of *M. tuberculosis* complex (including *M. tuberculosis* and *M. africanum*) are more likely to be associated with active TB in one group of people than another.[29] This includes the epidemiological evidence that *M. africanum* is common in distinct regions of West Africa and in West Africans [5] and is rare in other regions or in individuals from other regions.[30] It also includes the results of studies of multiple lineages of *M. tuberculosis* and their hosts in San Francisco and in Switzerland,[31] that have yielded evidence for a higher frequency of clinical TB that is ‘sympatric’ (the infecting bacterial strain is from a lineage whose geographic origin matches that of the ethnicity of the infected patient) rather than ‘allopatric’ (the infecting strain and ethnicity of the infected patient do not have the same geographic association).[29] As highlighted earlier, the profile of tuberculosis infection in West Africa is dominated by the MHC class I unlike many other places and could explain this specific host-pathogen relationship in this region. It will be interesting to deeply study the mechanisms underlying these complex interactions of host HLA diversity and tuberculosis disease susceptibility and outcomes in this population. These new data, therefore offer the opportunity for new models to further investigations around this question. Our findings have implications for other endemic diseases in this region as well, such as malaria and HIV. Similarly, to tuberculosis, it has been reported that West African children have a higher frequency of a HLA class I

(HLA-A and HLA-B) (rare in other parts of the World), which is also known to play a role in malaria disease severity.[32,33]

Difference in HLA profiles in populations other than West Africans.

The antigens are well known to vary amongst different ethnic and racial groups even in healthy populations.[34] Our study showed a high frequency of different MHC class I alleles in participants with TB disease compared to healthy participants. These high proportions suggest an important contribution of these alleles in infection acquisition and TB pathogenesis rather than a high frequency of the alleles in our study population. The proportions of the A and B subtypes reported in other populations (such as United Arab Emirates, Arabian Gulf Peninsula, South Mediterranean and North African) are higher than what we have seen in our healthy control participants, but significantly lower or similar than the proportions in our TB infected groups.[34] It has to be noted that susceptibility to TB infection and disease progression may involve other host and microbial factors.

HLA diversity and T-cell recognition of epitopes via MHC class I and II.

There is strong evidence that the adaptive immune system represented by CD4+ and CD8+ T lymphocytes is an important mechanism for host recognition and control of tuberculosis infection.[35] Recognition of foreign antigens by T cells depends on binding of short peptide fragments (termed epitopes), derived by proteolysis of foreign proteins, to the major MHC in humans-HLA proteins, on the surfaces of macrophages and dendritic cells. CD4+ T cells recognize peptide epitopes bound to MHC class II; CD8+ T cells recognize peptide epitopes bound to MHC class I. Again, it would be interesting to understand the immunological mechanisms by which the type A and B HLA alleles involved in TB infections in our population, influences the host T-cell response to *M. tuberculosis* and *M. africanum* infections.

It's possible that MHC class I profiles of those infected with TB changes the dynamics of CD8+T cells, which could ultimately render these individuals more susceptible to TB progression in general and to even lesser degree for a less virulent strain such as *M. africanum*.

Limitations of the study.

While some of the results discussed here are statistically significant, a larger confirmatory study is necessary. This pilot study included 60 participants with 52 individual alleles typed for each for a total of 3,120 typings. Furthermore, this study only focused on HLA-A, HLA-B, HLA-Cw and HLA-DR. Future studies will expand the analysis to HLA-DQ.

In conclusion, in a Malian population, we observed differential MHC Class I HLA-B allele frequency in individuals with active TB due to *M. tuberculosis* sensu stricto LAM10 in comparison to active TB due to *M. africanum*. Specific HLA-A and HLA-B alleles were identified at higher frequency in individuals with active tuberculosis disease (all strains) compared to controls. Varying HLA subtypes may be partially responsible for the geographical restriction of *M. africanum* to West Africa, however, larger confirmatory studies are necessary.

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REFERENCES

1. Organization WH. Global tuberculosis report 2016 2016.
2. Castets M, Boisvert H, Grumbach F, Brunel M, Rist N. [Tuberculosis bacilli of the African type: preliminary note]. Rev Tuberc Pneumol (Paris) 1968;32(2):179–184. [PubMed: 4985104]
3. Kallenius G, Koivula T, Ghebremichael S, et al. Evolution and clonal traits of Mycobacterium tuberculosis complex in Guinea-Bissau. J Clin Microbiol 1999;37(12):3872–3878. [PubMed: 10565899]
4. Traore B, Diarra B, Dembele BP, et al. Molecular strain typing of Mycobacterium tuberculosis complex in Bamako, Mali. Int J Tuberc Lung Dis 2012;16(7):911–916. [PubMed: 22508197]
5. de Jong BC, Antonio M, Gagneux S. Mycobacterium africanum--review of an important cause of human tuberculosis in West Africa. PLoS Negl Trop Dis 2010;4(9):e744. [PubMed: 20927191]
6. de Jong BC, Adetifa I, Walther B, et al. Differences between tuberculosis cases infected with Mycobacterium africanum, West African type 2, relative to Euro-American Mycobacterium tuberculosis: an update. FEMS Immunol Med Microbiol 2010;58(1):102–105. [PubMed: 20002176]
7. de Jong BC, Hill PC, Aiken A, et al. Progression to active tuberculosis, but not transmission, varies by Mycobacterium tuberculosis lineage in The Gambia. J Infect Dis 2008;198(7):1037–1043. [PubMed: 18702608]
8. Winglee K, Manson McGuire A, Maiga M, et al. Whole Genome Sequencing of Mycobacterium africanum Strains from Mali Provides Insights into the Mechanisms of Geographic Restriction. PLoS neglected tropical diseases 2016;10(1):e0004332. [PubMed: 26751217]
9. Li WX LG, Yao L, Shen G, Yang R, Qiu FW, Ma Y. Identification of a novel HLA-A allele, HLA-A*02:505, by sequence-based typing in a patient with tuberculosis. HLA 2017;90(2):106–107. [PubMed: 28378528]
10. Toyo-Oka L, Mahasirimongkol S, Yanai H, et al. Strain-based HLA association analysis identified HLA-DRB1*09:01 associated with modern strain tuberculosis. HLA 2017;90(3):149–156. [PubMed: 28612994]
11. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988;334(6181):395–402. [PubMed: 3043226]
12. Thorsby E, Berle E, Nousiainen H. HLA-D region molecules restrict proliferative T cell responses to antigen. Immunol Rev 1982;66:39–56. [PubMed: 6182089]
13. International HapMap C. A haplotype map of the human genome. Nature 2005;437(7063):1299–1320. [PubMed: 16255080]
14. Wamala D, Buteme HK, Kirimunda S, Kallenius G, Joloba M. Association between human leukocyte antigen class II and pulmonary tuberculosis due to mycobacterium tuberculosis in Uganda. BMC Infect Dis 2016;16:23. [PubMed: 26803588]
15. Amirzargar AA, Yalda A, Hajabolbaghi M, et al. The association of HLA-DRB, DQA1, DQB1 alleles and haplotype frequency in Iranian patients with pulmonary tuberculosis. Int J Tuberc Lung Dis 2004;8(8):1017–1021. [PubMed: 15305487]
16. Dubaniewicz A, Lewko B, Moszkowska G, Zamorska B, Stepinski J. Molecular subtypes of the HLA-DR antigens in pulmonary tuberculosis. Int J Infect Dis 2000;4(3):129–133. [PubMed: 11179915]

17. Goldfeld AE, Delgado JC, Thim S, et al. Association of an HLA-DQ allele with clinical tuberculosis. JAMA 1998;279(3):226–228. [PubMed: 9438744]
18. Singh SP, Mehra NK, Dingley HB, Pande JN, Vaidya MC. Human leukocyte antigen (HLA)-linked control of susceptibility to pulmonary tuberculosis and association with HLA-DR types. J Infect Dis 1983;148(4):676–681. [PubMed: 6415179]
19. Vejbaesya S, Chierakul N, Luangtrakool K, Srinak D, Stephens HA. Associations of HLA class II alleles with pulmonary tuberculosis in Thais. Eur J Immunogenet 2002;29(5):431–434. [PubMed: 12358854]
20. Shankarkumar A, U S. Role of HLA-A, HLA-B, HLA-DRB1 and HLADQB1 alleles in HIV-1 patients with pulmonary tuberculosis co-infection from India. Int J Hum Genet 2012;12(1):11–13.
21. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol 1997;35(4):907–914. [PubMed: 9157152]
22. Cereb N, Maye P, Lee S, Kong Y, Yang SY. Locus-specific amplification of HLA class I genes from genomic DNA: locus-specific sequences in the first and third introns of HLA-A, -B, and -C alleles. Tissue Antigens 1995;45(1):1–11. [PubMed: 7725305]
23. Koehler RN, Walsh AM, Moqueet N, et al. High-throughput genotyping of KIR2DL2/L3, KIR3DL1/S1, and their HLA class I ligands using real-time PCR. Tissue Antigens 2009;74(1):73–80. [PubMed: 19522772]
24. Koehler RN, Walsh AM, Sanders-Buell EE, et al. High-throughput high-resolution class I HLA genotyping in East Africa. PLoS One 2010;5(5):e10751. [PubMed: 20505773]
25. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992;39(5):225–235. [PubMed: 1357775]
26. Togo ACG, Kodio O, Diarra B, et al. The most frequent Mycobacterium tuberculosis complex families in mali (2006–2016) based on spoligotyping. Int J Mycobacteriol 2017;6(4):379–386. [PubMed: 29171452]
27. Sveinbjornsson G, Gudbjartsson DF, Halldorsson BV, et al. HLA class II sequence variants influence tuberculosis risk in populations of European ancestry. Nature genetics 2016;48(3):318–322. [PubMed: 26829749]
28. Chen BF, Wang R, Chen YJ, Zhu Y, Ding L, Wen YF. Association between HLA-DRB1 alleles and tuberculosis: a meta-analysis. Genet Mol Res 2015;14(4):15859–15868. [PubMed: 26634553]
29. Gagneux S, DeRiemer K, Van T, et al. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2006;103(8):2869–2873. [PubMed: 16477032]
30. Desmond E, Ahmed AT, Probert WS, et al. Mycobacterium africanum cases, California. Emerg Infect Dis 2004;10(5):921–923. [PubMed: 15200832]
31. Fenner L, Egger M, Bodmer T, et al. HIV infection disrupts the sympatric host-pathogen relationship in human tuberculosis. PLoS Genet 2013;9(3):e1003318. [PubMed: 23505379]
32. Hill AV, Allsopp CE, Kwiatkowski D, et al. Common west African HLA antigens are associated with protection from severe malaria. Nature 1991;352(6336):595–600. [PubMed: 1865923]
33. Lyke KE, Fernandez-Vina MA, Cao K, et al. Association of HLA alleles with Plasmodium falciparum severity in Malian children. Tissue Antigens 2011;77(6):562–571. [PubMed: 21447146]
34. Valluri V, Mustafa M, Santhosh A, et al. Frequencies of HLA-A, HLA-B, HLA-DR, and HLA-DQ phenotypes in the United Arab Emirates population. Tissue Antigens 2005;66(2):107–113. [PubMed: 16029430]
35. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. Nat Genet 2010;42(6):498–503. [PubMed: 20495566]

Table 1.

Demographic characteristics of study participants with *M. tuberculosis*, *M. africanum* and healthy controls

Parameter		***M. tuberculosis* LAM10 n (%) N=20**	***M. africanum* n (%) N=20**	**Controls n (%) N=20**	**Chi-square P value***
Male sex		18 (90%)	15 (75%)	11 (55%)	0.114
Age	[18–30]	11 (55%)	7 (35%)	16 (80%)	0.047
	[30–45]	9 (45%)	10 (50%)	3 (15%)	0.291
	[45–60]	0 (0%)	2 (10%)	1 (5%)	-
	[60–75]	0 (0%)	1 (5%)	0 (0%)	-
Smoking (current and past)	Yes	11 (55%)	4 (20%)	n/a	0.667
House hold contact	Yes	3 (15%)	6 (30%)	n/a	-
BCG Scar noted		18 (90%)	17 (85%)	n/a	0.944
Chest radiograph pattern	Bilateral infiltrate	3 (15%)	6 (30%)	n/a	-
	Cavitary lesions	4 (20%)	3 (15%)	n/a	-
	Miliary pattern	2 (10%)	0 (0%)	n/a	-
	Unilateral infiltrate	3 (15%)	3 (15%)	n/a	-

* Chi-square P-values were calculated when n > 4 for all three groups (at least 5)

Table 2.

Differential HLA allele frequencies of individuals infected with *M. africanum* and *M. tuberculosis* compared to healthy controls

Alleles	*M. tuberculosis* n (%) N=20	*M. africanum* n (%) N=20	Controls n (%) N=20	P-value (*Mtb vs Controls*)	P-value (*Maf vs Controls*)	P-value (*Mtb vs Maf*)
HLA-A						
A*02:05	6 (30%)	10 (50%)	0 (0%)	**0.020**	**0.001**	0.333
A*29:02	2 (10%)	5 (25%)	0 (0%)	0.487	**0.047**	0.407
A*30:02	1 (5%)	2 (10%)	0 (0%)	**0.001**	0.487	1.000
A*34:02	13 (65%)	14 (70%)	1 (5%)	**0.001**	**0.001**	0.736
A*66:01	13 (65%)	13 (65%)	1 (5%)	**0.001**	**0.001**	1.000
A*68:02	13 (65%)	14 (70%)	1 (5%)	**0.001**	**0.001**	0.736
HLA-B						
B*07:02	15 (75%)	9 (45%)	1 (5%)	**0.001**	**0.008**	0.053
B*08:01	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*14:02	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*15:03	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*15:10	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*18:01	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*42:01	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*42:02	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
B*51:01	15 (75%)	9 (45%)	1 (5%)	**0.001**	**0.008**	**0.053**
B*81:01	15 (75%)	8 (40%)	1 (5%)	**0.001**	**0.019**	**0.025**
HLA-Cw						
Cw*02:10	4 (20%)	2(10%)	0 (0%)	0.106	0.487	0.661
Cw*04:01	6 (30%)	2 (10%)	1(5%)	0.091	1.000	0.235
Cw*06:02	5 (25%)	2 (10%)	0 (0%)	**0.047**	0.487	0.407
Cw*08:10	5 (25%)	2 (10%)	1 (5%)	0.181	1.000	0.407
Cw*18:01	5 (25%)	2 (10%)	1 (5%)	0.181	1.000	0.407
HLA-DR						
DRB1*01:01	2 (10%)	0 (0%)	0 (0%)	0.487	NA	0.487
DRB1*01:03	2 (10%)	0 (0%)	0 (0%)	0.487	NA	0.487
DRB1*03:01	11 (55%)	9 (45%)	0 (0%)	**0.001**	**0.001**	0.487
DRB1*03:02	4 (20%)	3 (15%)	0 (0%)	0.106	0.231	1.000
DRB1*04:01	1 (5%)	1 (5%)	0 (0%)	1.000	1.000	1.000
DRB1*04:11	1 (5%)	1 (5%)	0 (0%)	1.000	1.000	1.000
DRB1*07:01	2 (10%)	1 (5%)	0 (0%)	0.487	1.000	1.000
DRB1*07:02	2 (10%)	1 (5%)	0 (0%)	0.487	1.000	1.000
DRB1*08:01	2 (10%)	0 (0%)	0 (0%)	0.487	NA	0.487
DRB1*08:04	2 (10%)	0 (0%)	0 (0%)	0.487	NA	0.487
DRB1*09:01	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA

Alleles	*M. tuberculosis* n (%) N=20	*M. africanum* n (%) N=20	Controls n (%) N=20	P-value (*Mtb vs Controls*)	P-value (*Maf vs Controls*)	P-value (*Mtb vs Maf*)
DRB1*10:01	1 (5%)	0 (0%)	1 (5%)	1.000	1.000	1.000
DRB1*11:01	7 (35%)	3 (15%)	0 (0%)	**0.008**	0.231	0.144
DRB1*11:04	6 (30%)	3 (15%)	0 (0%)	**0.020**	0.231	0.451
DRB1*12:01	3 (15%)	0 (0%)	0 (0%)	0.231	NA	0.231
DRB1*12:02	3 (15%)	0 (0%)	0 (0%)	0.231	NA	0.231
DRB1*13:01	1 (5%)	3 (15%)	0 (0%)	1.000	0.231	0.605
DRB1*13:02	4 (20%)	6 (30%)	0 (0%)	0.106	**0.020**	0.465
DRB1*13:03	3 (15%)	4 (20%)	0 (0%)	0.231	0.106	1.000
DRB1*13:04	3 (15%)	4 (20%)	0 (0%)	0.231	0.106	1.000
DRB1*13:05	4 (20%)	3 (15%)	0 (0%)	0.106	0.231	1.000
DRB1*14:01	0 (0%)	0 (0%)	0 (0%)	1.000	NA	NA
DRB1*14:02	4 (20%)	3 (15%)	0 (0%)	0.106	0.231	1.000
DRB1*14:03	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA
DRB1*14:04	0 (0%)	0 (0%)	0 (0%)	NA	NA	NA
DRB1*15:01	0 (0%)	0 (0%)	1 (5%)	1.000	1.000	NA
DRB1*15:02	0 (0%)	0 (0%)	1 (5%)	1.000	1.000	NA
DRB1*16:01	14 (70%)	7 (35%)	0 (0%)	**0.001**	**0.008**	**0.027**
DRB1*16:02	9 (45%)	7 (35%)	0 (0%)	**0.001**	**0.008**	0.058
DRB3*01:01	10 (50%)	10 (50%)	0 (0%)	**0.001**	**0.001**	1.000
DRB4*01:01	4 (20%)	4 (20%)	1 (5%)	0.342	0.342	1.000

HLA, human leukocyte antigen; NA, Not Applicable; Mtb, *Mycobacterium tuberculosis*; Maf, *Mycobacterium africanum*; Bold text indicates level of significance $P < 0.05$ (Chi-square).

Table 3.

Frequency of HLA types in individuals with active TB due to *M. tuberculosis* sensu stricto LAM10 **or *M. africanum* vs. healthy controls in Mali**

Allele	Pulmonary TB – All strains n (%) N=40	Healthy controls n (%) N=20	Chi square	P-value
HLA-A				
A*02:05	16 (40%)	0 (0%)	10.90	**0.001**
A*29:02	7 (17.5%)	0 (0%)	3.96	0.084
A*30:02	3 (7.5%)	0 (0%)	1.59	0.544
A*34:02	27 (67.5%)	1 (5%)	20.92	< **0.001**
A*66:01	26 (65%)	1 (5%)	19.39	< **0.001**
A*68:02	27 (67.5%)	1 (5%)	21.03	< **0.001**
HLA-B				
B*07:02	24 (69%)	1 (5%)	16.59	< **0.001**
B*08:01	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*14:02	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*15:03	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*15:10	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*18:01	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*42:01	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*42:02	23 (57.5%)	1 (5%)	15.31	< **0.001**
B*51:01	24 (69%)	1 (5%)	16.59	< **0.001**
B*81:01	23 (57.5%)	1 (5%)	15.31	< **0.001**
HLA-Cw				
Cw*02:10	6 (12.5%)	0 (0%)	3.33	0.165
Cw*04:01	8 (20%)	1(5%)	2.35	0.249
Cw*06:02	7 (17.5%)	0 (0%)	3.96	0.084
Cw*08:10	7 (17.5%)	1 (5%)	1.80	0.249
Cw*18:01	7 (17.5%)	1 (5%)	1.80	0.249
HLA-2				
DRB1*01:01	2 (5%)	0 (0%)	1.03	0.548
DRB1*01:03	2 (5%)	0 (0%)	1.03	0.548
DRB1*03:01	20 (50%)	0 (0%)	15.00	< **0.001**
DRB1*03:02	7 (17.5%)	0 (0%)	3.96	0.084
DRB1*04:01	2 (5%)	0 (0%)	1.03	0.548
DRB1*04:11	2 (5%)	0 (0%)	1.03	0.548
DRB1*07:01	3 (7.5%)	0 (0%)	1.57	0.544
DRB1*07:02	3 (7.5%)	0 (0%)	1.57	0.544
DRB1*08:01	2 (5%)	0 (0%)	1.03	0.548
DRB1*08:04	2 (5%)	0 (0%)	1.03	0.548

Allele	Pulmonary TB – All strains n (%) N=40	Healthy controls n (%) N=20	Chi square	P-value
DRB1*09:01	0 (0%)	0 (0%)	NA	NA
DRB1*10:01	1 (2.5%)	1 (5%)	0.25	1.000
DRB1*11:01	10 (25%)	0 (0%)	6.00	**0.023**
DRB1*11:04	9 (22.5%)	0 (0%)	5.29	**0.023**
DRB1*12:01	3 (7.5%)	0 (0%)	1.57	0.544
DRB1*12:02	3 (7.5%)	0 (0%)	1.57	0.544
DRB1*13:01	4 (10%)	0 (0%)	2.14	0.291
DRB1*13:02	10 (25%)	0 (0%)	6.00	**0.025**
DRB1*13:03	7 (17.5%)	0 (0%)	3.96	0.084
DRB1*13:04	7 (17.5%)	0 (0%)	3.96	**0.048**
DRB1*13:05	7 (17.5%)	0 (0%)	3.96	0.084
DRB1*14:01	0 (0%)	0 (0%)	NA	NA
DRB1*14:02	7 (17.5%)	0 (0%)	3.96	0.084
DRB1*14:03	0 (0%)	0 (0%)	NA	NA
DRB1*14:04	0 (0%)	0 (0%)	NA	NA
DRB1*15:01	0 (0%)	1 (5%)	2.03	0.333
DRB1*15:02	0 (0%)	1 (5%)	2.03	0.333
DRB1*16:01	21 (52.5%)	0 (0%)	16.15	< **0.001**
DRB1*16:02	20 (50%)	0 (0%)	15.00	< **0.001**
DRB3*01:01	20 (50%)	0 (0%)	15.00	< **0.001**
DRB4*01:01	8 (20%)	1 (5%)	2.35	0.249

HLA, human leukocyte antigen; NA, Not Applicable; Mtb, *Mycobacterium tuberculosis*; Maf, *Mycobacterium africanum*; Bold text indicates level of significance P < 0.05 (Chi-square).