EDITORIAL COMMENTARY

Mycobacterium tuberculosis Lineage—What's in Your Lungs?

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(See the article by Click et al, on pages 211–9.)

Although the most common form of tuberculosis affects the lung, tuberculosis can affect almost any part of the body. Extrapulmonary tuberculosis (EPTB) accounts for 15%–53% of reported cases in countries with comprehensive diagnostic and reporting systems. In industrialized countries in which the site of disease is systematically collected as part of national surveillance systems, the proportion of cases of EPTB has been reported to be increasing over time [1, 2]. In the Netherlands and the United States, the increase in the proportion of EPTB cases has been attributed in part to the steady decline in the number of pulmonary tuberculosis (PTB) cases, in conjunction with a stable [2] or slower annual decline [1] of EPTB cases. In these countries, EPTB has been observed primarily among foreign-born patients; however, the determinants for EPTB remain largely unknown. Other than host factors, such as the immunosuppressed state, causes of which include human immunodeficiency (HIV) coinfection, other factors, including those related to *Mycobacterium tuberculosis* (MTB), have not been fully evaluated [3, 4].

In this issue, Click et al [5] describe a cross-sectional study that concludes that some lineages of MTB are more likely to cause EPTB than others. The authors used systematically collected demographic, clinical, and radiographic data on all culture-confirmed tuberculosis cases reported to the Centers for Disease Control and Prevention's National Tuberculosis Surveillance System from 2004 through 2008 for which isolate-specific spoligotyping data were also available. They used these spoligotyping data to categorize MTB strains into 1 of the 6 lineages originally described, using large sequence polymorphism (LSP) analysis [6], and then evaluated the association between MTB lineage and site of disease. The authors found that the percentages of cases that were exclusively EPTB differed by lineage and that the East Asian lineage had the smallest proportion of EPTB cases, after adjustment for age, sex, HIV infection status, race/ethnicity, and region of birth. The East African–Indian and Indo-Oceanic LSP-based lineages were prevalent among patients born in Southeast Asia, the Eastern Mediterranean, or the Western Pacific. The authors also evaluated the relationship between EPTB and lineage, limiting the analysis to US-born patients to control for the possibility that the associations were due to some factor related to region of birth, and the results remained unchanged: compared with the East Asian lineage, the odds of exclusively EPTB were greater for Euro-American, Indo-Oceanic, and East African–Indian lineages.

These findings add to a series of studies that have reported that MTB from different lineages has different clinical phenotypic properties. Recent studies have demonstrated lineage-specific effects on the outcome of MTB infection and disease in various settings. In the Gambia, transmission of MTB to household contacts (measured using skin test conversions) was similar among MTB strains from different lineages. However, the proportion of contacts developing active tuberculosis within the 2-year follow-up period varied, with active tuberculosis developing in 1% of people exposed to isolates belonging to the West African 1 and 2 lineages, in 5.6% exposed to isolates belonging to the East Asian lineage, and in 1.2%–3.9% exposed to isolates belonging to the Euro-American lineage [7]. In California, we previously reported that isolates from the East Asian lineage were associated with an elevated proportion of genotypic clustering (a molecular epidemiologic proxy for transmission with rapid progression to disease in secondary cases), while Indo-Oceanic strains produced no secondary cases [8]. In Vietnam, patients with tuberculosis meningitis caused by strains of the East Asian lineage had a shorter duration of the disease and fewer lymphocytes in their cerebrospinal fluid at presentation,

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suggesting a differential intracerebral inflammatory response [9].

Table 1 is a summary of select studies published on the association between specific lineages of MTB and site of disease [11–12, 14, 16–21]. There is a lack of consistency among these studies in terms of the findings reported, complicating our understanding of the purported relationships between lineage and phenotype. The reasons for the inconsistencies noted are not completely understood but may in part be due to small sample sizes with inadequate distribution of lineages [14, 16, 20]; use of classifications of MTB strains that are not phylogenetically robust [11, 21]; lack of details on, or lack of control for, possible confounders [19] or known risk factors for EPTB, such as HIV infection [14, 19]; and use of different operational definitions for EPTB [11, 17]. With these limitations in mind, 2 of the studies reported an association between pulmonary site of disease and strains belonging to the Euro-American lineage [16, 20], and 1 study found this association with strains from the East Asian lineage (ie, the Beijing strain) [21]. Two studies associated EPTB with the East Asian lineage [11, 18], and 1 study associated it with the East African–Indian LSP-based lineage [20]. There are also studies that found no difference [17, 19]. In contrast to these smaller studies, Click et al [5] used a large national database with data collected systematically by use of a standard case report form, used the most accepted definitions for pulmonary and EPTB, evaluated patients in 3 disease-site categories (PTB, EPTB, and both), used a phylogenetically robust method to classify MTB lineage, and controlled for known factors associated with EPTB, as well as for confounders for lineage and site of disease.

However, despite the methodologic advantages of the study by Click and colleagues over those of prior publications, there are still a number of other limitations that need to be considered. First, determination of exclusively PTB or exclusively EPTB was based in part on symptoms, physical signs, and results of diagnostic tests, but also on the experience of the healthcare personnel. There is evidence that patients with PTB often have undiagnosed extrapulmonary or disseminated disease [23] that may have become clinically evident with time. This may be one of the explanations of why Click et al [5] found that the associations seen with exclusively PTB were similar to those seen with combined PTB and EPTB. Second, MTB strains belonging to a LSP-based lineage can be further categorized into sublineage strata, using LSP or single-nucleotide polymorphism analysis. In fact, Kong et al [12] reported that specific sublineages of the East Asian lineage (ie, RD142 and RD150 [2 of 5 or 7, depending on the genetic markers used]) were associated with extrathoracic tuberculosis (defined as tuberculosis disease outside of the lung, pleura, and intrathoracic lymph nodes with or without concurrent disease within the thoracic cavity). This suggests that within a broader lineage categorization, there may be specific sublineages that are driving the associations reported in the study by Click and colleagues and in studies published elsewhere. Interestingly, the frequency of the different sublineages of the East Asian lineage varies depending on the study site, which may explain in part why some studies associate Beijing strains with EPTB and others associate Beijing strains with PTB or none.

The finding by Click and colleagues that the East Asian lineage is less likely to be associated with EPTB, and conversely, compared with the other lineages, that the East Asian lineage is relatively more likely to be associated with exclusively PTB, adds to the growing body of data supporting the notion that the Beijing family of strains (which belongs to the East Asian lineage) may be more pathogenic (defined as the ability to be transmitted to other hosts and to cause disease) and, perhaps, more virulent (defined as the ability to cause severe disease), compared with strains belonging to other lineages. A strain that causes PTB has a higher likelihood of "perpetuating itself" by transmitting and causing disease in other hosts [24]. Indeed, strains from the Beijing family have been identified throughout the world, with the exception of South America, and have been linked with reports of outbreaks. Some of the hypotheses proposed for the extensive global dissemination of the Beijing family of strains include the following: (1) dissemination may be related to the large global migrations of persons from Asia in the 20th century, (2) BCG vaccine may have positively selected for the Beijing genotype by having less protective efficacy against this lineage, and (3) Beijing strains may be more pathogenic and/or more virulent than other strains of MTB.

MTB strains causing EPTB, it is proposed, have a better ability to invade macrophages, have a higher replication rate in macrophages and animal models, can disseminate, and are associated with decreased survival among animals, compared with strains that cause non-EPTB [3, 4]. However, the exact interpretation of these results with regard to the pathogenesis of EPTB is not known. Correlation of the human response to MTB infection with disease types caused in animals is very difficult, and data from animal models need to be interpreted carefully. A study using a rabbit model of tuberculosis disease showed that Beijing strains caused persistent bacillary loads, caused prolonged inflammation, and had a propensity to disseminate, compared with the non-Beijing strains [25]. Does this mean that strains that have these characteristics in the rabbit model will have the ability to cause EPTB in humans? Not necessarily. Unfortunately, there is no experimental model that can definitively address the ability of a given strain of MTB to cause EPTB. This is due to the gaps in knowledge about the pathogenesis of tuberculosis, the basics

Table 1. Select Studies in Which the Association Between Lineage of *Mycobacterium tuberculosis* and Extrapulmonary Tuberculosis Was Evaluated

Site	Period	Participants, No.	Classification Method	Comparison Groups	Results	Strengths	Comments	Reference
Arkansas	Jan 1996–Dec 2000	679	Based on the 3 principal genetic groups [10]	Group 1 versus group 2 and group 3; within group 1, Beijing versus others in group 1	Beijing (which is part of the East Asian lineage) was associated with extrathoracic involvement (AOR, 3.06 [95% CI, 1.39–6.73])	Population-based study	Extrathoracic tuberculosis was defined as disease outside of the lung, pleura, and intrathoracic lymph nodes with or without concurrent disease within the thoracic cavity	[11]
Arkansas	Jan 1996–Dec 2000	679	Based on the 3 principal genetic groups [10]	Sublineages of the East Asian lineage versus other lineages	Sublineage 142 (AOR, 3.05 [95% CI, 1.58–5.90]) and sublineage 150 (AOR, 11.09 [95% CI, 4.27–28.80]) were associated with extrathoracic tuberculosis	Population-based study	Extrathoracic tuberculosis was defined as disease outside of the lung, pleura, and intrathoracic lymph nodes with or without concurrent disease within the thoracic cavity	[12]
Taipei, Taiwan	Jan 2001–Jan 2004	249	Spoligotyping [13]	Beijing and non-Beijing; main objective was to evaluate treatment outcome	No difference	…	Small sample; hospital based; no HIV data	[14]
Ho Chi Minh City, Vietnam	Mar 2000–Apr 2003 for tuberculosis meningitis. Sept 2003–Dec 2004 for PTB	Tuberculosis meningitis, 187; PTB, 234	LSPs [15]	Tuberculosis meningitis and PTB	Euro-American lineage was associated with PTB (AOR for meningitis, 0.40 [95% CI, .20–.83]; $P = .013$)	HIV-negative adults; case-control study	Proportion of Euro-American lineage was relatively small; hospital-based population	[16]
Cape Town, South Africa	Dec 2000–Dec 2003	285 children (<14 years old)	Spoligotyping [13]	PTB (including pleural effusion) versus EPTB (including cases with PTB and EPTB)	47% of cases with EPTB; no differences between W-Beijing and LAM3/F11 (which is part of the Euro-American lineage [6])	High number of EPTB cases	Hospital-based population; EPTB definition different from that in the study by Click et al [5]	[17]
Western Cape Province, South Africa	Mar 2003–Aug 2005	392 children (<13 years old)	Spoligotyping [13]	MTB genotypes: Beijing, LAM, S, and other; site of disease: intrathoracic in 75% vs extrathoracic in 25%	Beijing (AOR, 2.36 [95% CI, 1.21–4.60]) and S (which is part of the Euro-American lineage [6]; AOR, 3.47 [95% CI, 1.26–9.56]) were more likely to be in subjects with extrathoracic disease, compared with LAM (which is part of the Euro-American lineage [6])	…	Disease was classified as extrathoracic or thoracic on the basis of the site from which the mycobacterial isolate was cultured, irrespective of clinical disease	[18]

Table 1 continued.

Site	Period	Participants, No.	Classification Method	Comparison Groups	Results	Strengths	Comments	Reference
Sweden (mainly Gothenburg and surrounding areas)	2001–2005	349	Spoligotyping [13]	Impact of immigration on tuberculosis epidemiology in Sweden; 59% with PTB vs 41% with EPTB	No differences	…	Small number per group; 74% were foreign born; no HIV data; no definition of EPTB	[19]
Tuscany, Italy	Jan 2002–Dec 2005	1009	Spoligotyping [13]	EPTB versus PTB	EPTB was associated with *Mycobacterium bovis* (AOR, 3.2 [95% CI, 1.2–8.1]) and Central Asian family (part of the East African Indian lineage by LSP; AOR, 2.3 [95% CI, 1.0–5.1]); PTB was associated with Harlem family (part of the Euro-American lineage; AOR, 1.6 [95% CI, 1.1–2.5])	…	Not clear whether study was population based	[20]
London, United Kingdom	Jul 1995–Dec 1997	2490	IS*6110*-based superfamilies	Comparison of different superfamilies	The following superfamilies were associated with PTB: sfam6, sfam7, L3, and sfam5 (which includes the Beijing strains) (AOR, 1.90 [95% CI, 1.17–3.09])	Population-based study	MTB classification was based on IS*6110;* no HIV data	[21]
United States	2004–2008	33 554	Spoligotyping [13] was used to determine the lineage (originally described using LSP [6, 22])	EPTB versus PTB	Compared with East Asian lineage, EPTB was associated with Euro-American (AOR, 1.3 [95% CI, 1.1–1.4]), Indo-Oceanic (AOR, 1.7 [95% CI, 1.5–1.9]), and East-African Indian (AOR, 1.6 [95% CI, 1.4–1.9]) lineages	Population-based study	…	[5]

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; EPTB, extrapulmonary tuberculosis; HIV, human immunodeficiency virus; IS, insertion sequence; LAM, Latin-American-Mediterranean; LSP, large sequence polymorphism; MTB, *Mycobacterium tuberculosis*; PTB, pulmonary tuberculosis; S, spoligotype family originally described as prevalent in Sicily and Sardinia.

of the cellular processes involved in the pathogenesis of the disease, and the host-pathogen interaction.

Nonetheless, as noted by Click and colleagues, a better understanding of the role of different lineages (and sublineages) of MTB in causing EPTB would provide insights into pathogenicity, infectiousness, progression from infection to active disease, and, perhaps, response to drug therapy. If there are lineages that are associated with EPTB, it would be useful to determine the degree to which the global epidemiology of EPTB is driven by the spread of "EPTB-type" lineages. It would be informative to evaluate rates of EPTB in countries where specific lineages predominate [22], such as China, for the East Asian lineage; the Philippines, for the Indo-Oceanic lineage; northern India, for the East African–Indian LSP-based lineage; and Mexico, for the Euro-American lineage. Ideally, however, this type of comparative analysis would need to be done in a setting in which there was an adequate number of cases of tuberculosis (pulmonary and extrapulmonary), as well as a broad diversity of lineages of MTB, as was done in the study by Click and colleagues. From here, we need to conduct additional research to understand the degree to which host factors, pathogen factors, and environmental factors (such as exposure intensity and type) contribute to the determination of the site of disease and, thereby, gain a window through which we can start to see what role lineage plays in our global tuberculosis epidemiology.

Notes

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