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Mycobacterium tuberculosis lineage: A naming of the parts

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Summary There have been many reports of groups of related *Mycobacterium tuberculosis* strains described variously as lineages, families or clades. There is no objective definition of these groupings, making it impossible to define relationships between those groups with biological advantages. Here we describe two groups of related strains obtained from an epidemiological study in Tanzania, which we define as the Kilimanjaro and Meru lineages on the basis of IS*6110* restriction fragment length polymorphism (RFLP), polymorphic GC rich sequence (PGRS) RFLP and mycobacterial interspersed repeat unit (MIRU) typing. We investigated the concordance between each of the typing techniques and the dispersal of the typing profiles from a core pattern. The Meru lineage is more dispersed than the Kilimanjaro lineage and we speculate that the Meru lineage is older. We suggest that this approach provides an objective definition that proves robust in this epidemiological study. Such a framework will permit associations between a lineage and clinical or bacterial phenomenon to be tested objectively. This definition will also enable new putative lineages to be objectively tested.
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Introduction

Until relatively recently *Mycobacterium tuberculosis* was thought to be a highly homogeneous species with differences in disease presentation and complications being due to differences in host response.[1] The organism has proved itself very adaptable as demonstrated by the ability of mycobacterium to be transmitted and by its ability to adapt to new environments.[2] Study of the virulence of *M. tuberculosis* has been handicapped by the paucity of tools to differentiate the organism into different types.

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This situation has been transformed by the description of a number of methods of subdividing isolates of the genus including IS*6110* restriction fragment length polymorphism (RFLP), spoligotyping, polymorphic GC rich sequence (PGRS) typing, mycobacterial interspersed repeat unit (MIRU) and deletion analysis.[3–7] These techniques were first applied to epidemiological studies and outbreak investigations.[8,9] When applied to very large collections of strains, those strains with similarities have been identified. For example a group of strains has been identified by IS*6110* and spoligotyping and designated the Beijing family.[10] This is a group of strains of considerable importance as it includes the organisms implicated in the ''strain W'' outbreaks in the United States.[11] Also it has been suggested that Beijing strains may be associated with an enhanced febrile response in patients on treatment, and multiple drug resistance may be more common in strains of this family.[12]

It is generally accepted that 100% identity by IS*6110* type is found between strains that are related and may be defined as a 'cluster'.[13] Clustering is used as a surrogate marker for recent transmission, even when the direct relationships between the patients infected have not been established. For strains that are more distantly related this 100% rule is likely to be broken. Recent analysis of the evolutionary relationships between strains of *M. tuberculosis*, using deletion analysis, has been able to root studies of the molecular epidemiological associations of isolates in the evolutionary tree for this organism.[14] Analysis of sequential samples suggests that the mean time between IS*6110* transposition events is 0.5–5 years.[15] Thus, the speed of the molecular clock for deletions is likely to be at least an order of magnitude slower than that for the molecular markers used in epidemiological studies. Different research groups have variously applied different degrees of similarity as defined by the Dice coefficient of between 40% and 95% calling these 'families', 'groups' or 'clades'.[16–18] There are no agreed definitions of what constitutes a significant collection of isolates or indeed what it should be called. In this study we have adopted the term lineage.

It is clear that an objective definition of a lineage, or rules whereby a lineage can be identified and assessed, is required. To do this we studied two groups of related strains obtained in an epidemiological study in Tanzania which we typed by IS*6110* RFLP, PGRS RFLP and MIRU polymerase chain reaction (PCR) to determine the anatomy of a lineage, and to assist in the proposition of rules for lineage definition.

Methods

Bacterial isolates

Single *M. tuberculosis* isolates were prospectively collected from all culture-positive patients diagnosed by the National Tuberculosis and Leprosy Control Programme Reference Laboratory at Kibong'oto Hospital over the 6 month period April–September 1995. Speciation was confirmed by standard microbiological techniques. Isolates were maintained on Löwenstein–Jenson (LJ) slopes at 37 °C for a minimum of 4 weeks and subsequently transported to the Department of Medical Microbiology, Royal Free & University College Medical School.[19]

Clinical/epidemiological data

The following data were collated for each isolate; age, sex, district of domicile, tuberculosis smear status, HIV status. For analysis of these data, the Kruskal–Wallis test was used for non-parametric continuous data, i.e. age, and categorical data were compared using the χ^2 statistic.

Molecular analysis

We have previously reported the molecular analysis of these isolates[19] by IS*6110* and PGRS typing. In brief, isolates of *M. tuberculosis* were genetically fingerprinted using IS*6110* RFLP typing using the international standard protocol.[7] All patterns were entered by one researcher (SLB) onto a database using Bionumerics software (Applied Maths, Koutrai, Belgium). All available isolates were submitted to PGRS analysis. Genomic DNA was digested with *Alu* I restriction endonuclease and a Southern blot probed using an oligonucleotide consisting of two copies of the PGRS consensus repeat.[19]

MIRU typing was performed using the technique described by Supply et al.[6] PCR mixtures were prepared as follows, using the HotStartTaq DNA polymerase kit (Qiagen, Crawley, West Sussex, UK). A final volume of 50 μl containing 1 U of DNA polymerase, 10 μl of Q solution, 0.2 mM (each) dATP, dCTP, dGTP, and dTTP, 5 μl of × 10 PCR buffer, 0.4 μM (each) primer, 1 μl DNA, 25.8 μl of water and a final $MgCl_2$ concentration of 2.5 mM. The PCR reactions were carried out using a OmniGene thermocycler (Hybaid, Ashford, UK), starting with a denaturing step of 15 min at 95 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 59 °C, and 1 min 30 s at 72 °C, followed by a final incubation at 72 °C for 10 min. PCR products were sized using an 11 cm,

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