HOSTED BY

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Mycobacterium tuberculosis polyclonal infections and microevolution identified by MIRU-VNTRs in an epidemiological study

Elisabeth Streit, Julie Millet, Nalin Rastogi *

WHO Supranational TB Reference Laboratory, TB & Mycobacteria Unit, Institut Pasteur de la Guadeloupe, F97183 Abymes, Guadeloupe

ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form
5 May 2015
Accepted 6 May 2015
Available online 3 June 2015

Keywords:
Mycobacterium tuberculosis
Epidemiology
Spoligotyping
MIRU-VNTRs
Polyclonal infections
Microevolution

ABSTRACT

Introduction: The advent of molecular typing using MIRU-VNTR mini-satellites has largely facilitated tuberculosis (TB) molecular epidemiological studies. Apart from detecting the chains of transmission and risk factors, these markers have also allowed to study the phenomena of mixed strain infections versus microevolutionary events.

Methods: An initial set of *Mycobacterium tuberculosis* strains (n = 161) genotyped using spoligotyping and MIRU-VNTRs in Guyana and Suriname was evaluated for indications mixed strain infections (characterized by the detection of double alleles in 2 or more MIRU loci) versus "in-patient" microevolutionary events (characterized by the detection of double alleles in a single locus).

Results: The present study hereby reports evidence of microevolution in 3.7% (*n* = 6/161) of the studied population, vs. 0.6% (n = 1/161) for mixed infection. The strains belonged to three different spoligotyping-based lineages, namely the T (SITs 44, 53, and 1081), Haarlem (SIT47), and EAI (SITs 72 and 349) lineages, while 1 isolate (SIT237) could not be assigned to any lineage.

Discussion: By comparing these results on microevolutionary cases (*n* = 6) to 112,000 strains present in the SITVIT2 database, evidence is presented that in 2/6 cases (each case corresponding to 2 patterns due to MIRU double bands), one of the patterns corresponded to a shared type found exclusively in Suriname or Guyana. Phylogenetic analysis showed that no spoligotyping lineage in particular was more prone to microevolutionary events in this study's sample. Overall, the observations fortify the awareness regarding the existence of microevolution and polyclonal TB infections which has important implications for patient care.

Introduction

Tuberculosis (TB) remains a major global health problem, having caused 9 million new cases and 1.5 million deaths in 2013 [1]. It has long been assumed that a patient is infected by a single strain of *Mycobacterium tuberculosis* (MTB) at a time. Nonetheless, the advent of molecular epidemiological techniques has led to increasing reports of mixed strain infections

* Corresponding author at: WHO Supranational TB Reference Laboratory, TB & Mycobacteria Unit, Institut Pasteur de la Guadeloupe, Morne Jolivière, BP 484, F97183 Abymes, Guadeloupe. Tel.: +590 590 897661.
E-mail address: nrastogi@pasteur-guadeloupe.fr (N. Rastogi).

Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2015.05.005

(patients harboring more than one MTB clone at a time) over the past 15 years [2–6; reviewed in 7]. MIRU-VNTR typing [8,9], widely applied in molecular epidemiologic studies, facilitates the detection of polyclonal infections as the presence of more than one strain in a sample is likely to lead to the detection of multiple alleles in a number of loci. Depending on the number of implicated loci, two distinct mechanisms are considered responsible for the presence of such multiple alleles; "in-patient" microevolution of the infecting clone in case of the detection of double alleles in a single locus as opposed to simultaneous infection with two distinct MTB strains when two or more loci are concerned [2–4,10]. Microevolution can of course affect any genotypic marker, for example, a strain may evolve by losing a spacer of the initial spoligotype profile. As the molecular clock of spoligotyping profiles is lower than that of MIRU-VNTRs [11], evolutionary changes are much less frequent in the former. Moreover, "in-patient" microevolution within the DR-locus (i.e., the locus targeted by spoligotyping technique) is not detectable unless single colonies from serial isolates (containing the initial isolate vs. recently evolved isolates) are obtained and analyzed separately. Since the present report presents essentially MIRU-VNTR data, hereafter the focus will be on microevolution concerning this particular marker. Most often, studies on polyclonal and multiple strain infections were conducted in settings with high TB burden [2–5]. Nevertheless, they have also been observed in areas with moderate TB incidence [6]. The present study reports evidence for clonal heterogeneity and mixed-infection observed in a recent epidemiologic study on isolates from Guyana and Suriname [12] based on MIRU-VNTRs.

Materials and methods

Clinical isolates

The MTB bacterial isolates ($n = 7$) described in this study were genotyped in the course of a recent epidemiological study on clinical isolates ($n = 161$) from Guyana and Suriname [12]. They were part of a convenience sample of clinical isolates sent to the Caribbean Epidemiology Centre (CAREC), Trinidad, for identification and drug susceptibility testing (DST) [13].

Genotyping and database comparison

The initial set of strains ($n = 161$) was subjected to standard spoligotyping [14] and 15-loci MIRU-VNTR typing [9] in the following order: MIRU-4, MIRU-10, MIRU-16, MIRU-26, MIRU-31, MIRU-40, ETR-A, ETR-C, QUB-11b, QUB-26, QUB-4156, Mtub04, Mtub21, Mtub30, and Mtub39. The results from each of the 15 loci were combined to create a 15-digit allelic profile, and a cluster was defined as two or more strains sharing identical spoligotypes and 15-loci MIRU patterns. The obtained profiles were compared with SITVIT2, a proprietary database of the Pasteur Institute of Guadeloupe which is an updated version of SpolDB4 [15] and SITVITWEB [16], and assigned to a SIT (spoligo-international-type) and/or 15-MIT (15-loci MIRU-international-type) respectively, if they matched at least one other profile in the database or classified as an orphan (no match found).

From the initially published study [12], strains for which multiple bands were repeatedly obtained for $\geqslant 1$ locus were excluded from epidemiological analysis ($n = 7$), and were further investigated in the present study to elucidate the phenomena of polyclonal infections versus microevolutionary events identified by MIRU-VNTRs. For this purpose, all polymerase chain reactions (PCRs) were repeated twice to confirm the presence of multiple alleles in a given MIRU locus.

Phylogenetical analysis

Major phylogenetic clades were assigned according to the signatures provided in the database defining 62 genetic lineages/sublineages. These include various MTB complex members, as well as rules defining major lineages/sublineages for MTB *sensu-stricto*, namely: Beijing clade, the Central Asian (CAS) clade and 2 sublineages, the East African-Indian (EAI) clade and 9 sublineages, the Haarlem (H) clade and 3 sublineages (including H3/Ural-1 and H4/Ural-2 sublineages), the Latin American-Mediterranean (LAM) clade, its 12 sublineages (note that sublineages LAM7-TUR and LAM10-CAM are now referred to as Turkey and Cameroon lineages), the ancestral "Manu" family and 3 sublineages, the S clade, the IS*6110*-low-banding X clade and 3 sublineages, and an ill-defined T clade with 5 sublineages.

BioNumerics v6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium) was used to construct a minimum spanning tree (MST) based on spoligotypes and 15-loci MIRU VNTR profiles and nodes differing in a maximum of two genotypic markers (i.e., VNTR locus or spoligo-spacer) were grouped in order to highlight single locus variants (SLVs) and double locus variants (DLVs).

Results

The results obtained along with schematic representation of microevolution and polyclonal infection, the mechanisms involved, and genotyping results are summarized in Table 1 and Figs. 1 and 2. As listed in Table 1, 7 patient isolates ($n = 5$ from Suriname and $n = 2$ from Guyana) repeatedly displayed multiple alleles in one or more MIRU locus. As schematically illustrated in Fig. 1, they were considered suggestive of microevolution if a single locus ($n = 6$ strains) was implicated, and mixed infection if several loci were concerned ($n = 1$ strain). Given that the original epidemiological study [12] covered $n = 154$ isolates with unambiguous profiles, the rate of patients harboring clonal subpopulations was 3.7% ($n = 6/161$) in the studied population, and 0.6% ($n = 1/161$) showing evidence of mixed strain infection. Their genotypic profiles and basic demographic data are shown in Table 1; the strains belonged to three different spoligotyping-based lineages, namely the T (SITs 44, 53, and 1081), Haarlem (SIT47), and EAI (SITs 72 and 349) lineages. One isolate (SIT237) could not be assigned to any lineage. The patients' mean age was 48 years, ranging from 14 to 83 years. With the exception of the youngest patient (a female), all other patients were male. All strains were pan-susceptible.

In $n = 6/7$ samples double alleles were detected in a single locus. In accordance with current conventions [2–5,10], these