International Journal of Antimicrobial Agents 48 (2016) 353–360

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Review

Drug-resistant tuberculosis viewed from bacterial and host genomes

Imen Ben-Kahla, Sahal AL-Hajoj *

Mycobacteriology Section, Department of Infection and Immunity, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 13 March 2016
Accepted 15 July 2016

Keywords:
Mycobacterium tuberculosis
Drug resistance
Molecular biology

ABSTRACT

The outcome of infection with *Mycobacterium tuberculosis* (MTB) is largely influenced by the host–pathogen interaction in which both the human host and the MTB genetic backgrounds play an important role. Whether this interaction also influences the selection and expansion of drug-resistant MTB strains is the primary focus of this review. We first outline the main and recent findings regarding MTB determinants implicated in the development of drug resistance. Second, we examine data regarding human genetic factors that may play a role in TB drug resistance. We highlight interesting openings for TB research and therapy.

1. Introduction

The emergence of *Mycobacterium tuberculosis* (MTB) strains resistant to the majority or all effective antibiotics is a real threat for the control of tuberculosis (TB). We actually see appearing different severe forms of resistant TB that are difficult and sometimes impossible to treat successfully even in developed healthcare settings [1,2]. The risk for ongoing and nosocomial transmission of resistant bacilli is further alarming [3–6]. The World Health Organization (WHO) considers drug resistance as a priority issue in the post-2015 global strategy for TB control, which aims to reduce the global incidence of TB by 90% before 2035 [7]. To reach this goal, it is important to understand how MTB successfully eludes almost every drug administered in current TB treatment and what factors favour the acquisition of this resistance.

It has been reported that the genetic diversity between MTB strains has a major impact on bacterial behaviour and disease outcome, including geographic distribution [8], virulence [9], host immune regulation [10,11], disease severity [12,13] and transmission [14]. With regard to drug resistance, inconclusive results have been reported. For example, Beijing/W (lineage 2) strains have been associated with drug resistance in particular clinical studies [15–18], whilst in others no specific correlation was detected [19,20]. These contradictory results have been obtained even within the same country and have been attributed to differences in study sample size or design. MTB intrafamily heterogeneity could explain the difference in the association with drug resistance. Nevertheless, one could hypothesise that the host genomic background may interfere in the selection of drug-resistant bacteria. The human role in the development of drug-resistant TB is generally linked to behaviour attitudes, such as improper drug use. However, development of drug-resistant TB has been observed despite good patient adherence and strict application of the WHO directly observed treatment, short-course (DOTS) strategy [21]. Host individual immune status, smoking and early-life antibiotic treatment may influence the ability of a pathogen to develop drug resistance in the human host [22,23]. In addition, the possible role of human genetic variation in the selection of drug-resistant micro-organisms is becoming more suggested in infectious diseases such as malaria [24]. For TB, some characteristics may argue for this notion. For example, based on statistical analysis, human ethnicity has been reported to affect the frequency of multidrug-resistant (MDR)-TB and the nature of the mutation conferring drug resistance [25,26]. In addition, it was shown that the ability of each individual to generate a pro- or anti-inflammatory response is a potential determinant in MDR-TB [27,28]. In correlation with these data, specific polymorphisms within particular immune genes have been identified as possible susceptible factors for the development of drug-resistant TB [29,30].

The aim of this review was to outline the factors that may favour the development of drug-resistant TB both from the bacterial and host point of view. We first review the recent determinants of MTB drug resistance and specific factors that may contribute to the development of drug resistance in particular MTB lineages/strains. Second, we review available data that may support the notion of the influence of human genetic background in the development of bacterial drug resistance. We conclude with future considerations for TB treatment strategies.

* Corresponding author. Mycobacteriology Section, Department of Infection and Immunity, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. Fax: +966 1 442 7854.
E-mail address: hajoj@kfshrc.edu.sa (S. AL-Hajoj).

http://dx.doi.org/10.1016/j.ijantimicag.2016.07.010
0924-8579/

2. *Mycobacterium tuberculosis* determinants involved in the development of drug resistance

2.1. Mycobacterium tuberculosis genetic diversity and influence on drug resistance level and cost

The latest phylogenetic analysis classified MTB strains into five major lineages: lineage 1, Indo-Oceanic; lineage 2, East Asian; lineage 3, Central Asian; lineage 4, Euro American; and lineage 7, Ethiopia (lineages 5 and 6 correspond to *Mycobacterium africanum* West African 1 and West African 2, respectively) [31]. Based on the TbD1 deletion, lineages 1, 5 and 6 are considered evolutionary 'ancient', lineages 2, 3 and 4 as 'modern' and lineage 7 as 'intermediate' [12]. Most TB cases, severe cases and outbreaks in the world were caused by MTB strains belonging to the 'modern' lineages. MTB lineages 2 and 4 have also been correlated with poor response to anti-TB treatment [32]. Specifically, multiple studies revealed a strong association between the Beijing (lineage 2), Haarlem (lineage 4) and LAM (lineage 4) families with treatment failure and drug-resistant outbreaks [33–35]. Recently, Ford et al. demonstrated that MTB lineage 2 strains are able to rapidly acquire drug resistance in vitro compared with other strains [36]. Although the number and type of strains in the study by Ford et al. could not be effectively representative of all MTB lineages, and the fact that this association has not been confirmed by other studies, did not rule out the higher success of particular MTB strains [37,38]. Indeed, the majority of MDR-TB and extensively-drug resistant (XDR)-TB outbreaks reported from different geographic locations were caused by particular MTB strains belonging to lineage 2 or 4.

What makes specific strains from lineages 2 and 4 more successful compared with other strains or lineages remains largely unknown, but several genetic factors could have a potential impact. For example, the stepping stone mutations observed in different MDR- and XDR-TB strains may constitute a successful strategy for the acquisition of higher resistance [34,39,40]. Moreover, it has been shown that the drug resistance level conferred by each mutation is influenced by the strain genetic background. For example, *katG*(S315T) has been reported to be associated with high-level isoniazid resistance for different MTB strains except for lineage 1 strains [41]. Recently, Sun et al. reported that Beijing strains (lineage 2) were significantly associated with the *rpsL* K43R gene mutation ($P < 0.01$) conferring high-level streptomycin resistance [42]. The strain genetic background has also been reported to influence the 'fitness cost' of resistance-conferring mutations. For example, the 'fitness cost' of the *rpoB* H526D mutation conferring rifampicin resistance differs between MTB strains of different lineages [43]. Such a 'fitness cost' could be restored or even increased by second-site mutations, making the pathogen able to spread and cause drug-resistant outbreaks [34,44,45]. This compensation could be a key mechanism for MTB strains frequently associated with drug resistance (for instance particular strains belonging to Beijing and LAM families).

The greater ability of particular MTB strains belonging to lineages 2 and 4 to acquire drug resistance has also been suggested to be due to their higher ability to mutate [36]. Indeed, in different studies of transmission chains, the substitution rate was found to be within 0.5 single nucleotide polymorphisms (SNPs) per genome per year, and most strains taken from the same patient or from a household contact did not diverge from each other by more than 5 SNPs [39,46,47]. Analysis of serial XDR clinical isolates belonging to lineage 4 that had evolved from susceptible ancestors in single patients or in epidemiologically related patients revealed higher mutation rates reaching 4.3 SNPs per genome per year [34,48]. Likewise, De Steenwinkel et al. showed that MTB Beijing (lineage 2) strains exhibit significantly higher mutation rates (1.6×10^{-5} to 5.4×10^{-3}) compared with strains from lineage 3 (6.3×10^{-8} to 3.8×10^{-4}) when tested in vitro for rifampicin resistance [49]. Specific polymorphisms in *mutT* and stress-inducible SOS response genes have been suggested to generate the mutator phenotype of particular Beijing strains, but this notion remains controversial [40,50–52]. Increased mutagenesis has also been linked to upregulation of the inducible putative error-prone DNA polymerase *dnaE* gene. Boshoff et al. demonstrated that rifampicin resistance occurs rarely or not at all in the absence of the *dnaE* gene [52], a result that was strengthened by Bergval et al. who found that S522L, H526D and S531W *rpoB* mutations significantly correlate with higher expression of the *dnaE* gene [53]. It is, however, unclear to what extent SOS-induced mutagenesis influences the evolution of drug-resistant MTB strains.

2.2. Mycobacterium tuberculosis virulence factors and the development of drug resistance

The tremendous success of particular MTB strains within lineages 2 and 4 compared with other lineages has been linked to their higher virulence [12,54]. Higher virulence strains, in contrast to less virulent strains, may have more chance to be exposed to antibiotics and acquire resistance because they will not be eradicated by the immune system. Furthermore, from a functional point of view, some determinants identified as virulence factors for MTB strains are known to play a role in the development of drug resistance [55,56].

2.2.1. Mycobacterium tuberculosis biofilm formation and drug resistance

It has been demonstrated that micro-organisms growing within biofilm are more tolerant to antibiotics than those growing under planktonic conditions owing to reduced permeability. The biofilm mode of growth is increasingly recognised as a key factor for bacterial infection chronicity, transmission and antibiotic resistance [57–59]. Several mycobacteria species, including MTB, have been described to grow in vitro as a pellicle at the air–media interface, a biofilm-like structure that harbours drug-tolerant bacilli able to survive exposure to higher concentrations of isoniazid and rifampicin [60–62]. When it comes to human disease, fewer data are available, particularly for TB. Recently, Qvist et al. demonstrated the presence of biofilm aggregates in chronic pulmonary *Mycobacterium abscessus* complex infection [63]. For TB, it has been shown that in the cavitary form MTB grow almost exclusively near the interface of air–fluid cavities and this mode of growth has been expected to be a biofilm-like [64]. Further studies using a guinea pig infection model have revealed viable extracellular MTB bacilli after drug treatment within a defined acellular rim within the macrophage [65,66]. Interestingly, the morphology of this rim in the guinea pig shows a strong similarity with human TB lesions in which extracellular bacilli growing in multicellular structures have been also described [65]. All of these in vitro and in vivo data, together with the clinical features of TB (chronicity and difficulty to threat), endorse the possible contribution of biofilm formation in MTB pathogenesis. Furthermore, it has been reported that the growth of several bacterial species under biofilm conditions induces a higher mutation rate compared with planktonic condition, which consequently may favour the emergence of drug-resistant mutants [59,67,68]. Thus, the persistence of MTB in biofilms may be at least one possible reason for the higher prevalence of drug-resistant TB cases among previously treated patients [7].

A global survey of MTB clinical isolates from different lineages revealed that in vitro biofilm formation is not specific to a particular lineage, but is specific to each strain [69]. The biofilm phenotype is highly variable among MTB strains, ranging from non-producer to thick biofilm-producer [60,69]. It was shown that methoxy-mycolic acid (one of the major structural mycolic acids in the MTB cell wall) is a major component of the extracellular matrix in biofilm growth and participates in biofilm maturation [60]. A comparative