

Excretory- Secretory Proteins of Mycobacterium Tuberculosis Strain H37Rv And Local Clinical Isolates- A Comparison



Medical Science

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ABSTRACT

Proteins that are secreted actively by Mycobacterium tuberculosis in invitro cultures (ES proteins) are said to be equivalent to those released in vivo. The ES proteins have been reported to be immunodominant. In this study, a comparison of the ES proteins of the prototype strain H37Rv with that of local clinical isolates has been done by One dimensional Polyacrylamide gel electrophoresis. A thick sharp band was observed in the region between 66kDa and 44kDa in the PAGE of all clinical isolates, which was not visible for H37Rv. The salient antigens from clinical isolates of Mycobacterium tuberculosis associated with active disease may substantially improve the presently available diagnostic tools and may be explored for improving the efficacy of vaccines.

Introduction

Tuberculosis is a communicable disease with a heavy toll on the prime period of life and on health resources. Hence, rapid and specific diagnostic methods should be available to initiate prompt treatment. Development of improved vaccines is also considered a high priority in the effort to control tuberculosis. For advances in both these areas, the appropriate antigenic fractions of the bacterium are required.

The main antigenic fractions of Mycobacterium tuberculosis (M.tb) are the sub - cellular fractions (cell wall, cell membrane and cytoplasm) and the excretory- secretory (ES) proteins which are released into the culture medium during growth of M.tb. Comparatively the ES proteins are better defined in terms of function, immunogenicity and composition. Hence, these proteins are good candidates as early diagnostic markers and for vaccines.

The current studies on Mycobacterium tuberculosis are based on the ES proteins obtained from M. tuberculosis H37Rv. H37Rv strain was first isolated in 1905. ¹Since then, it has been continually passaged and hence there is likely to be changes in its antigens. Hence, a comparison of the ES proteins of H37Rv with that of local clinical isolates may determine its relevance for use in studies aimed at development of TB diagnostic tests and vaccines.

In this study we have compared the protein profiles of the ES proteins of M. tuberculosis H37Rv with 9 local clinical isolates of Mycobacterium tuberculosis.

Materials and methods

Preparation of excretory- secretory proteins

Excretory- secretory proteins called detergent soluble sonicate (DSS) was prepared from Mycobacterium tuberculosis. Briefly, bacilli were 5% phenol inactivated in 0.5M phosphate buffer (PBS, pH7.2) and incubated with sodium dodecyl sulphate (SDS) extraction buffer. The supernatant was dialysed against 0.01M PBS, pH 7.2 and used as an antigen source.²

DSS protein was thus prepared from H37Rv strain and 9 isolates of Mycobacterium tuberculosis as confirmed by biochemical tests and MPT 64 antigen test (SD Bioline). The protein concentration in each of the preparation was determined by the Lowry method of protein concentration³ as seen in Table 1. Polyacrylamide gel electrophoresis (PAGE) was performed using 12% gel under standard conditions.

Results

Table 1: DSS Protein concentration in each isolate as estimated by Lowry method

Sl No	ID No of isolate	DSS Protein concentration
1	H37Rv	2.227 mg/ml
2	T60	2.045 mg/ml
3	T21	3.545 mg/ml
4	T16	4.272 mg/ml
5	T05	3.454mg/ml
6	T13	1.113mg/ml
7	T11	1.590mg/ml
8	T34	2.363mg/ml
9	T30	2.954mg/ml
10	T06	5.545mg/ml

Results of PAGE performed on H37Rv and clinical isolates

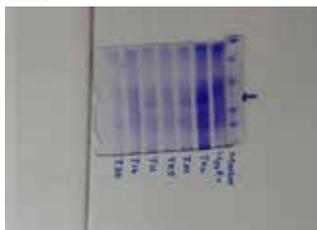
Many bands were seen on PAGE done for H37RV and the clinical isolates. These bands were more concentrated in the region between 29 and 66kDa.

The band pattern on PAGE of all the clinical isolates, were similar visually. There were some minor differences in the intensity of bands which is relative to the amount of protein.

The clinical isolate T06 showed sharper and thicker bands compared to all other clinical isolates.

A thick sharp band was observed in the region between 66kDa and 44kDa in the PAGE of all clinical isolates, which was not visible for H37Rv as seen in Figure 1.

Fig 1: One dimensional PAGE on H37Rv and Clinical Isolates



Discussion

Mycobacterium tuberculosis contains a large number of different components, which elicit an immune response and also play an important role in the pathogenesis of tuberculosis. The main groups of Mycobacterial components are lipids and carbohydrates of the cell wall, and proteins of different molecular weights which are cytoplasmic (enzymes and stress proteins) or secreted (cell membrane or cell wall associated and exported to the exterior).

In this study, we have compared the antigenic profile of the ES proteins obtained from the prototype strain (H37Rv) and 9 clinical isolates from our clinical lab. In agreement with previous studies on antigenic differences between *Mycobacterium tuberculosis* strain H37Rv and clinical isolates determined by PAGE, this study also revealed that most antigens expressed by H37Rv and the clinical isolates are similar. However, the clinical isolates revealed a band between 66kDa and 44kDa, which was not seen in H37Rv. There was no significant difference in the band patterns among clinical isolates. The clinical isolate T06 showed more intensely stained bands among clinical isolates. This may be because the protein concentration was higher in T06 DSS protein (5.545mg/ml), compared to the other clinical isolates. This ability of some strains to express higher concentration of proteins may have a bearing on the pathogenesis and prognosis of the disease.

There have been very few studies on comparison of the protein profile of H37Rv and clinical isolates. In a study conducted by Carmen Pheiffer et al,⁴ a comparison of the proteome of *M. tuberculosis* H37Rv and two local clinical strains, one belonging to the Beijing strain family and another clinical strain (F23) was done. Polyacrylamide gel electrophoresis of the culture filtrate proteins of the above strains revealed a 38kDa antigen that was present in Beijing strain and clinical strain (F23), but not in H37Rv and a 60kDa protein that was present only in H37Rv and not in the clinical isolates.

Rum Shin et al⁵ also compared the proteins present in culture filtrates from *Mycobacterium tuberculosis*. They used strain K, which is the dominant clinical isolate in Korea and *M. tuberculosis* H37Rv. Several differences in expression were detected between the two strains for those proteins with a molecular mass <20 kDa.

Hence, these studies indicate that significant differences in protein profile exists in H37Rv strain from local clinical isolates. There is a possibility that the salient antigens from clinical isolates of *Mycobacterium tuberculosis* (Mtb) associated with active disease may substantially improve the presently available diagnostic tools and may be explored for improving the efficacy of vaccines.

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