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Micro-array analysis of tuberculosis and BCG

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Keywords

BCG, micro-arrays, tuberculosis, vaccine

Comments

This report demonstrates a number of interesting points. First that large scale (4.4 Mb) genomic comparison is possible and can be automated. The widespread use of microarray technology is clearly on the horizon. Specifically, the identification of deleted regions of tuberculosis will allow investigators to focus on parts of the genome which may contribute to pathogenicity or some other phenotypic difference. It is interesting to note that these deletions not only include genes with some putative pathogenic role but also genes involved in transcriptional regulation. Thus phenotypic expression may be determined by both quantitative as well as a qualitative factors. Comparative genomics has also allowed a possible evolutionary relationship between different strains of *M. bovis* BCG to be postulated. The genomic era is here. With the sequencing of the human genome, applications such as those demonstrated in this paper will have increasing relevance to treating and combating human disease.

Introduction

Tuberculosis is still the single most deadly pathogen to afflict man with total deaths for this decade predicted to exceed 80 million. Diagnosis and treatment of the disease is hampered by the organism's slow culture time and fastidious nature and the failure of skin tests to differentiate between active and latent disease and those previously vaccinated with BCG. The BCG vaccine has been widely used since it was first developed by Calmette and Guerin in 1921. It was developed by serial passage of a strain of *Mycobacterium bovis* (a member of the *M. tuberculosis* complex) over 13 years, until it became non-pathogenic to man. Since then several daughter strains have been propagated and used for vaccination. However there is considerable variation in BCG's efficacy and this may be related to the different BCG strains used for vaccination. Previous studies comparing the genomes of *M. bovis* BCG and *M. tuberculosis* have demonstrated deletions from the *M. bovis* BCG genome. These deleted areas may relate to those genes which confer pathogenicity to *M. tuberculosis*. The identification of further genomic deletions would help narrow down the list of candidate pathogenic genes. In turn this may allow the development of better vaccines and/or improved tests to detect tuberculosis. The completed sequencing of a strain of *M. tuberculosis* has facilitated further deletions to be identified.

Aims

To identify regions of *M. tuberculosis* that have been deleted from different strains of *M. bovis* BCG using DNA microarrays.

Methods

The sequencing of the *M. tuberculosis* strain H37Rv allowed the polymerase chain reaction (PCR) amplification of internal fragments representing 3902 of the 3924 predicted open reading frames which make up the H37Rv genome. Each of these PCR fragments was then spotted and bound to a glass slide. Genomic DNA from a test *M. bovis* strain and H37Rv were randomly labelled with a fluorescent nucleotide analog (red in the case of H37Rv and green for the test *M. bovis* strain). These labelled DNAs were then mixed and applied to the glass slide. If a particular DNA had a sequence corresponding to a fragment it would bind to the fragment. Binding of the genomic DNAs were then assessed using a scanning laser microscope to assess the fluorescence emitted from each 'spot'. Red flourescence would indicate H37Rv binding predominantly; green *M. bovis* predominance and yellow indicating that both had bound. Since the screening of the genomes was based on PCR fragments based on the *M. tuberculosis* sequence, this method only allowed a unidirectional comparison of the *M. tuberculosis* genome with different strains of *M. bovis*. Thus deletions present in *M. tuberculosis* were not looked for.

Results

Eight different strains of *M. bovis* and 13 different M. bovis BCGs were screened. From this a total of 16 deleted areas from *M. bovis* BCG/ *M. bovis* were identified, four of these have previously been documented. Nine deletion areas are absent from all BCGs and *M. bovis* strains, one from all BCG strains, four from certain BCG strains only and two are missing from BCG and some virulent *M. bovis* strains. A genealogic relationship between different strains of BCG based on their different deletion patterns is also created.

Discussion

By identifying differences in the regions of the *M. tuberculosis* and *M. bovis* , insights into the phenotypically differences between these two bacterial strains may be possible. Among the possible causes of reduced virulence of *M. bovis* compared to that of *M.tuberculosis* may be the absence of three phospholipase C genes from *M. bovis* . Phospholipase C activity is known to contribute to the virulence

of other lung pathogens. Clinically, the differences in open reading frames could be used to develop a diagnostic test, to distinguish between infections caused by *M. tuberculosis* and those who have been vaccinated by BCG, which at present are indistinguishable. This would be of great use for vaccination programs in countries that have a low prevalence of the wild-type infection. The loss of virulence of the BGC vaccine may be attributed to the deletion areas from the *M. bovis* strain. The authors investigated the predicted function of these deletion areas using previously published data on the *M. tuberculosis* gene. None of the deletion areas corresponded to virulence elements, but a variety of functions, such as transcriptional regulation, was suggested. Loss of efficacy of the BCG vaccine could be attributed to the ongoing deletion of genetic material. The authors propose that the deletions detected in the BCG vaccine, compared to the *M. bovis* gene, reflect a progressive adaptation to laboratory conditions. This may affect their capacity to survive within the host and therefore impair the ability to stimulate a durable immune response.

References

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