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Recombinant DNA techniques, synthetic peptides and bioinformatics identify proteins and peptides of Mycobacterium tuberculosis-specific genomic regions important for development of new vaccines against tuberculosis

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Introduction: Comparative genomic studies have identified 11 regions of differences (RDs) between the pathogenic Mycobacterium tuberculosis and vaccine strains of M. bovis BCG. The bioinformatics analyses of these RDs for open reading frames (ORFs) suggested that they may encode 89 M. tuberculosis-specific proteins. By using the techniques of recombinant DNA cloning and expression, synthetic chemistry and bioinformatics in this study, the proteins and peptides of RDs have been identified with potentials in development of new vaccines against tuberculosis (TB).

Methods and Results: Attempts were made to clone RD genes in expression plasmids, express the cloned genes as recombinant proteins in Escherichia coli, purify the recombinant proteins and test them for cellular and humoral immune responses. However, this approach could not be applied to all the genes/proteins of RDs because of the problemsinherently associated with mycobacterial gene cloning,their expression and purification of mycobacterial proteins expressed in E. coli. Therefore, overlapping synthetic peptides (n= 1,648), covering all of the open reading frames identified in the above RDs were synthesized using fmoc chemistry and tested in cellular immune responses, i.e. protective T helper (Th)1 and pathologic Th2, using cells from HIV negative pulmonary TB patients and PPD+ healthy humans and M. bovis-infected cattle. The results showed that the highest Th1-responses were induced by RD1 peptides and the highest Th2 responses were induced by RD12 and RD13 peptides. Further tests showed that four RD proteins, i.e. PE35, PPE68, ESXA and ESXB were the best stimulators of Th1 cells. These proteins also induced delayed type hypersensitivity responses in guinea-pigs infected with M. tuberculosis, but not in animals infected with other mycobacteria. The bioinformatics analysis for binding to human leukocyte antigen (HLA)-DR molecules revealed that all of these proteins were promiscuous HLA-DR binders. Further analysis of the individual peptides of these proteins showed that several peptides of ESXA and ESXB were HLA-DR binders and inducers of Th1-cell reactivity, whereas a single peptide of PPE68 was HLA-DR promiscuous and immunodominant. To evaluate the in vivo potentials, the genes of immunodominant antigens were cloned and expressed in DNA vaccine vectors (pUMVC6 and pUMVC7) and several mycobacterial hosts, i.e. BCG, M. vaccae and M. smegmatis. Immunizations of mice and guinea-pigs with the recombinant constructs induced antigen-specific cellular and humoral immune responses in these animals. Each of these proteins had several T and B cell epitopes scattered throughout their sequences, which confirmed their strong immunogenicity.

Conclusion: The study has identified proteins of M. tuberculosis-specific RDs with potentials as new vaccine candidates against TB.

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Biography

Abu Salim Mustafa, male, molecular biologist and immunologist; BSC (1972) and MSc (1974) from Aligarh Muslim University, Aligarh;and PhD (1979) from the All India Institute of Medical Sciences, New Delhi, India.
I have been a Scientist (1980-1987) at the Norwegian Cancer Research Institute, Oslo, Norway (1980 to 1987), Research Associate (1987-1989) at the Whitehead Institute for Biomedical Research, MIT, Cambridge, USA, and Assistant Professor (189-192), Associate Professor (1992-1996) and Full Professor (1996-till date) at the Faculty of Medicine, Kuwait University, Kuwait.I became a Fellow of the Royal College of Pathologists, UK in 1998 and was Visiting Professor (Resource Person Program) of American Society for Microbiology/UNESCO in 2001 and 2004.
At Kuwait University, I became Research Coordinator in 1992, Graduate Program Director in 1998, Member of Medical Research Council in 2001 and Director of Research Core Facility in 2012.
Till date, I have published 255 full-length papers, 375 conference abstracts and edited 3 books and conference proceedings. I am a recipient of 25 national and international awards in recognition of excellence in research. I am a recipient of more than 50 funded research grants from national and international funding bodies, and have refereed more than 60 research projects submitted for funding to national and international institutions.
I am a member of the editorial board of two international journals, member of 11 Scientific Societies, supervised more than 25 graduate research thesis, invited speaker in 50 scientific conferences and chaired scientific sessions in 15 conferences.
In the recent years, the focus of my research has been molecular biology and immunology of infectious diseases with specific reference to diagnosis and identification of new vaccine candidates against tuberculosis and other mycobacterial diseases.

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