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Proteomic Approach to Identify Surrogate Biomarkers for Evaluation of Efficacy After Prime Boost Vaccination

KEYWORDS: BCG, Ag85B, Ag85 peptide, Proteomic approach, biomarkers

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ABSTRACT *The main objective of the study was to identify proteomic markers, expressed in serum of mice after prime boost strategies using BCG as priming agent followed by booster doses with BCG, Ag85B and also Ag-85complex peptide. Mice serum samples were subjected for two dimensional electrophoresis based on which, we identified number of biomarkers. Some were found to down regulate after primary BCG vaccination but were again expressed after booster doses with Ag85 B and Ag85 peptide but not with BCG. We also identified some proteomic markers that were specifically expressed only after booster doses with BCG, Ag85B and Ag85 peptide. To conclude, serum proteomic profiling provides us useful information in identification of biomarkers. Characterization of such protein bands further by LC-MS in near future will help us in identification of such surrogate biomarkers which can be correlated with improved efficacy of prime boost vaccination strategies.*

Introduction
Advances in Tuberculosis (TB) research over the last few years have been driven by genomics, proteomics, and immunomonics leading to the development of improved vaccination strategies for increasing the efficacy of Bacillus Calmette Guerin (BCG) vaccine (1).Heterologous boosting is among one of the widely used approaches used in recent times to boost waning BCG induced immune response. Numerous studies have shown that use of heterologous prime/boost combinations can result in the induction of vastly improved vaccine-specific T-cell responses and improved protection in animals (2).

Although prime boost strategies have been widely explored with promising results in animal studies, identification of appropriate protective biomarkers that's can be co-related with protective efficacy of such regimes are urgently needed. Even to test the new candidate vaccines, we do not have surrogate markers of protection, which can help pronounce the merit of a new vaccine (3). Existing biomarkers such as T cell specific cytokines: IFN-γ, IL-12 although, have been widely explored, but their exact role as protective biomarker is however debated by various investigators. Our earlier studies in PBMC and animal models also suggested similar observation where use of prime boost approach provided increased immune response (4-5), however identification of protective biomarkers leading to such response was were still needed to be identified, which we tried to evaluate in present study.

Proteomic, large scale analysis of cellular protein, is a powerful tool for protein identification.

Proteomics strategy when combined with immunomics (looking for immunogenic proteins) and vaccinomics (characterization of host response to immunization), delivers valuable information on pathogen–host cell interaction. . New antigens discovered by proteomic approaches are useful for new multivalent vaccine construction or alternatively may be included into already existing preparations to improve their efficacy (6-8). Studies on serum protein profiling after prime boost strategies with antigen and their peptides may help us in identification of number of immunogenic proteins expressed which may be further correlated other known serological markers and ultimately with improved efficacy of vaccine.

Objective of the present paper, was to study proteomic profile in serum of mice collected after single dose of BCG vaccination and after administration of booster dose with BCG (in homologous approach) and with i) Ag85B ii) Ag85 peptide (in heterologous approach). From this study we intended to identify course of potential proteins markers expressed after prime boost strategies in week wise manner, which can be ultimately used in future vaccination regimes for predicting vaccine efficacy

Materials and Methods
Mice- Female inbred Mice strain (Swiss Albino) 6-8 weeks old, 25-30g were purchased from Shree Farms, Bhandara (M.S).All the mice were housed in Animal house facility developed by Central India Institute of Medical Sciences (CIIMS) at Nagpur Veterinary College, Nagpur.

Ethical Consideration- The protocols for Animal experiments were approved by Institutional Animal Ethics Committee of CIIMS, Nagpur and conform to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004).

Vaccine and Reagents- BCG vaccine (Moscow strain) was

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obtained from Serum Institute of India, Pune and stored at 4-8°C before use. Peptides of Ag85 complex were designed by method described earlier by Kolaskar et al and developed and supplied by Caslo laboratories, Denmark. Purified Ag85B was supplied by Colorado State University, USA.

Experimental Design and Sample Collection-
Mice were divided into three different experimental groups (n=10 each group). A control group (n=4) of mice was separately maintained. All groups of mice were vaccinated subcutaneously with BCG (10^5 CFU) on 0th day followed by booster doses with BCG (Group I), Ag85B (Goup II) and Ag85 complex peptide (group III) at 7th week post vaccination. Control group was vaccinated with sterile saline. Blood samples were collected at 0th, 1st, 4th, 7th week post vaccination and 2nd week post booster dose with BCG, Ag85B, & Ag85 complex peptide. Serum was separated and stored at 4°C until use. A detailed experimental sketch is given in figure 1.

Two Dimensional SDS PAGE-
For the first dimension, 125 µl (150 ug protein) of week wise pooled sample from the mice groups were applied to a Bio-Rad IPG strip (pH 3-10, 7 cm) and then it was subjected to isoelectric focusing (IEF). Briefly, the IPG strips were rehydrated overnight and IEF was then carried out at 20 °C in a Protean IEF unit (Bio-Rad, USA). Prior to second dimension electrophoresis, the IPG gel strips were immersed in equilibration buffer I & II solution each for 15 min, followed by incubation in SDS buffer for 15 min. The second dimension separations were carried out at 10 °C using SDS slab gels (10%) without stacking gels and a mini-protean tetra cell electrophoresis system (Bio-Rad). The IPG strips were embedded on the top of the gels with 0.5% agarose, and electrophoresis was performed at 30 mA/gel for 1 h. The gels were fixed with a methanol: acetic acid: water (5:1:5) solution, stained with Coomassie brilliant blue Stain and destained in a solution of 10% methanol and 7% acetic acid. Gel images were taken using the gel documentation system (Bio-Rad) and were imported into the PD Quest (Bio-Rad) 2D gel analysis software package. For detection of spots a master gel image was created by combining all of the spots that were present in BCG, Ag85B and Ag85 peptide, vaccinated and control groups followed by analysis.

Results
In the present study, protein profiling was carried out in serum of mice with aim to identify certain protective markers expressed in prime boost vaccination strategies with BCG/ BCG, BCG/ Ag85B and BCG/Ag85 complex peptide.

Based on proteomic approaches, we found expression of 70kDa protein in samples with boosted BCG (homologous appraoch). However expression of this protein was shortly down regulated at 7th week post BCG vaccination and remained the same even after booster dose with BCG (fig 2). In contrast, we reported up regulation of this protein after hetrologous boosting with BCG/Ag85B and BCG/Ag85 complex peptide (fig 3 and 4). Similar expression profile of 97kDa protein was also identified in samples of mice boosted with BCG/Ag85B (fig 3). Apart from this, we also identified proteins which were expressed and specific to respective booster regimes. Expression of a 43 kDa protein was noted specifically in samples boosted with BCG (homologous boosting) (fig 1) while expression of 75kDa and 29kDa protein were observed in samples serum samples of mice boosted with BCG/Ag85B (fig 2) and BCG/Ag85 peptide (fig 3).

Discussion
Prime boost immunization strategies have been widely explored by number of investigators towards improvement in efficacy of currently available BCG vaccine. Importantly, this vaccination regimen for generating highly effective protective immunity can also be used to identify in vitro correlates of antimycobacterial protection (9-10). Proteomics based approaches can be routinely used in such regimes for expression studies and identification of protective proteomic markers. In present study, proteomic profiling was done in weekwise pooled serum samples collected from prime boost approaches in mice to study the expression of protective protein markers expressed after vaccination and after administration of booster dose with BCG (homologous), Ag 85 B and Ag 85 peptide (heterologous). Based on protein profiling studies, we identified course of two proteins, whose expression were found to down regulate shortly after primary vaccination with BCG but was up regulated after booster doses with Ag85B and Ag85 peptide. Protein such as 70 kDa particularly showed increased expression after booster doses with Ag85B and Ag85 peptide which was not observed in cased of samples boosted with BCG only. Thus based on proteomic profiling we observed that combination of vaccines may be better than a single vaccine in boosting BCG primed immune response.

Although success of genomics based approaches has helped towards characterization of genes and antigens of MTB, in recent times proteomic studies have absolutely accelerated the research at the vaccine level leading to the confirmation on usefulness of novel candidates on subunit vaccine construction (10). The studies by various investigators explain the importance of proteomics in improvement of the protective efficacy of BCG vaccine against tuberculosis. Elzbieta et al studied the impact of proteomic on anti-mycobacterium tuberculosis vaccine development; based on comparative proteomic analysis in virulence vs. attenuated strains of MTB and identified 1,750 proteins spots which have potential to boost BCG induced immune response (11). Another study carried out by Jungblut P. R et al and Mattow J et al, aimed at the identification of proteins differentially expressed in BCG and MTB which resulted in detection of more than 60 proteins (12-13). Malen et al used two complimentary proteomic technologies, which revealed 257 M. Tuberculosis H37Rv secreted proteins (14). It is generally accepted that antigens expressed or up regulated after vaccination and after booster doses are more effective antigens. Thus identification of course of such antigens by serum profiling in prime boost regimes is of utmost important. Moreover it may be also beneficial in monitoring protective efficacy of candidate antigens molecule that are used for boosting BCG induced immune response. Form our proteomic studies we have tried to study expression profile of such antigens.

Apart from 97kDa and 70 kDa proteins which were found to be expressed both after primary vaccination and booster doses, there were certain proteins which were specifically expressed after booster doses with BCG (43kDa,), Ag85B (75kDa) and Ag85 complex peptide (29kDa,). Ag85 B molecule has been largely used in number of vaccination protocols and has also advanced into clinical trials. However there is need for search of new biomarkers that can be used in assessment of vaccination regime with such molecule. Our studies thus may be useful in future prospects with respect to quest for of improved biomarkers in TB vaccination. The availability of true TB biomarkers of protection more accurately termed "surrogate endpoints of protection against active TB thus would greatly facilitate and accelerate TB vaccine development and importantly increase the likelihood of success. Surrogate endpoints could also be used to determine which of the vaccines currently in clinical trials – which were often developed relatively early in the new TB vaccine era – will prove to be the most efficacious. The availability of TB biomarkers of protection will allow more rapid and rationalized down selection of the best candidates and strategies at an earlier stage in the development pipeline, which may include selection of the most relevant antigens, delivery systems and routing, live vaccines, vaccine dosing and timing. Finally, such biomarkers could help in the decision process for combination vaccination, combining the best prime candidate(s) with the most appropriate boost candidate (15).

In our studies we have also used Ag 85 peptide for boost-

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ing. Peptide vaccination is an entirely appraoch with limited data available, and therefore proteomic studies in samples boosted with Ag85 peptide can open new avenues in vaccine research. . From our earlier studies (unpublished data) in mice we have shown capability of both Ag85B and Ag85 peptide to boost BCG induced immune response. We found that course of immune response induced by single doses reaches maximum at 4th week and declined steadily after that, but was again increased after booster doses with Ag85B and Ag85 peptide. Similar kind of results was also observed in our homologous studies in mice [6]. In this study we specifically selected week wise samples from our earlier studies, where course of immune response was found to increase and then decline. Our proteomic studies identified certain proteins whose weekwise expression correlated with immune response observed in earlier prime boost studies in animals.

Thus from this study, identification of proteins like a 97kd and 70kd protein in week wise prime boost samples along with other markers identified may be useful in future studies with such immunization strategies. Future characteriation of such proteins will exactly tell us about kind of protein are expressed which will be helpful in monitoring the protective efficacy of prime boost regimes.

Conclusion: Serum protein profiling in prime boost vaccination regimes may be helpful in identification of new potential surrogate markers of protection, which can be related to protective efficacy of a vaccine and solve our ultimate goal for quest of improved biomarkers in TB vaccine research.

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Conflict of Interest
None

Figure Legends

Figure 1: Schematic Representation of experimental sketch

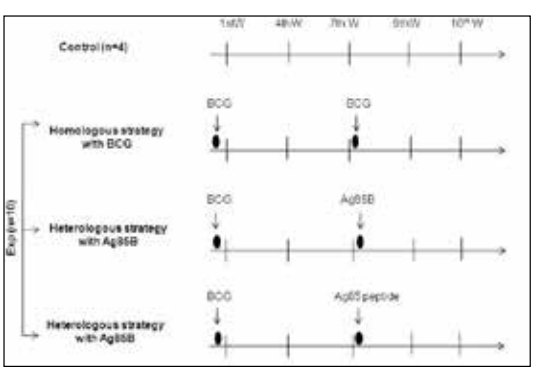


Figure 2

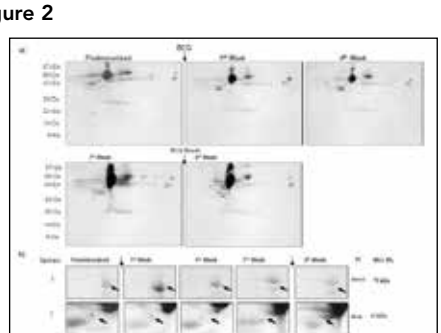


Figure 2: a) Two dimensional SDS PAGE profile in selected week wise pooled serum samples of mice (n=8) before vaccination, after vaccination and after booster dose with BCG indicated by an arrow ( ). b) Montex images of expression profile of proteins identified by 2- Dimensional SDS PAGE (indicated by an arrow) in week wise samples of mice

Figure 3:

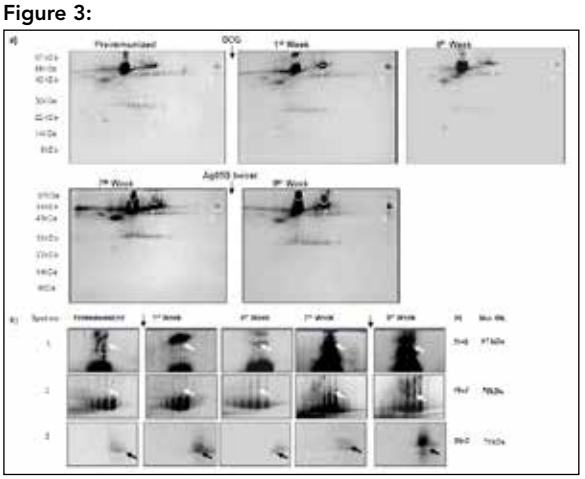
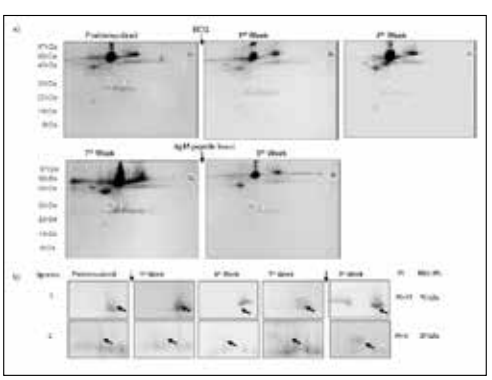


Figure 3: a) Two dimensional SDS PAGE profile in selected week wise pooled serum samples of mice (n=8) before vaccination, after vaccination and after booster dose with Ag85B, indicated by an arrow ( ). b) Montex images of expression profile of proteins identified by 2- Dimensional SDS PAGE (indicated by an arrow) in week wise samples of mice.
Figure 4: a) Two dimensional SDS PAGE profile in selected week wise pooled serum samples of mice (n=8) before vaccination, after vaccination and after booster dose with Ag85 complex peptide, indicated by an arrow ( ). b) Montex images of expression profile of proteins identified by 2- Dimensional SDS PAGE (indicated by an arrow) in week wise samples of mice.

Figure 4.



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REFERENCE 1. Parthasarathy A & Sharma H, BCG Vaccination: Is there light at end of the Tunnel? Paediatrics 2010 2:48. | 2. Hoft DF, Tuberculosis vaccine development: goals, immunological design, and evaluation. Lancet 2008 372: 164. | 3. Monika AP, Sergiusz M, Elzbieta K & Jagusztyn K, Proteomics for development of vaccine. Proteomics 2011 74:2596. | 4. Kashyap RS, Husain AA, Morey SH, et al, Assessment of immune response to repeat stimulation with BCG vaccine using in vitro PBMC model. J Immune Based Ther Vaccines 2010 8:12. | 5. Husain AA, Kashyap RS, Kalorey DR, et al, Effect of repeat dose of BCG vaccination on humoral response in mice model. Indian J Exp Biol 201049:7 | 6. Tyagi AK, Nangpal P & Satchidanandam V, Development of vaccines against tuberculosis. Tuberculosis 2011 1:469. | 7. Sheng G, Jin C, Dobos KM, et al, Comprehensive Proteomic Profiling of the Membrane Constituents of a Mycobacterium tuberculosis Strain. BMC Microbiology 2011 11:80. | 8. Agranoff D, Fernandez-RD, Papadopoulos MC, et al, Identification of diagnostics marker of tuberculosis by proteomic finger printing of serum. Lancet, 2006 368:1012. | 9. Ferraz JC, Stavropoulos E, Yang M, et al, A heterologous DNA priming-Mycobacterium bovis BCG boosting immunization strategy using mycobacterial Hsp70, Hsp65, and Apa antigens improves protection against tuberculosis in mice. Infect Immun 2004 72: 6945. | 10. Mollenkopf HJ, Grode L, Mattow J, et al, Application of mycobacterial proteomics to vaccine design: improved protection by Mycobacterium bovis BCG prime-Rv3407 DNA boost vaccination against tuberculosis. Infect Immun 2004 72:6471. | 11. Elzbieta KJ, Roszczenko P & Grabowska A, Impact of proteomic on anti-mycobacterium tuberculosis (MTB) vaccine development, Polish journal of microbiology 2009 58:281. | 12. Jungblut PR, Schaible UE, Mollenkopf HJ, Zimny – Arndt U, et al, Comparative proteome analysis of mycobacterium tuberculosis and mycobacterium tuberculosis bovis BCG strains: towards functional genomics of microbial pathogens. Mol. Microbiol 1999 33:1103. | 13. Mattow J, Jungblut PR. Schaible UE, et al, Identification of proteins from mycobacterium tuberculosis missing in attenuated mycobacterium tuberculosis bovis BCG strains, Electrophoresis 2001 22:2936. |