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Vaccine development for Tuberculosis: Past, Present and Future Challenges

Dileep Tiwari[1,2,3]*, Shafiul Haque[4,5] and Ramesh Chandra [2,3]

1. Gennova Biopharmaceuticals Limited, I. T.- B. T. Park, Phase-II, Hinjwadi Pune-411057, India
2. Ambedkar Center for Biomedical Education and Research 154, SFS, Ashok Vihar Phase-III, Delhi-110052, India.
3. Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, Delhi-110007, India.
4. Centre for Drug Research, Faculty of Pharmacy, Viikki Biocentre-2, P.O. Box No. 56, Viikinkaari 5E, FIN-00014, University of Helsinki, Helsinki, Finland.
5. Gene Expression Laboratory, Department of Biotechnology, Jamia Millia Islamia (A Central University), New Delhi-110025, India

Abstract

About one third of the world's population is infected with *Mycobacterium tuberculosis* (*M. tb*), and new infections occur at a rate of about one per second. Additionally, more people in the developed world contact tuberculosis (TB) because their immune systems are more likely to be compromised due to higher exposure to immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe, still the treatment is difficult and requires long courses of multiple antibiotics. However, antibiotic resistance is a growing problem in multidrug-resistant (MDR) tuberculosis. But mostly the prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guérin (BCG) vaccine. BCG is the most commonly used vaccine worldwide, but not as a powerful vaccine. BCG also provides some protection against severe forms of pediatric TB, but has been shown to be unreliable against adult pulmonary TB which accounts for most of the disease burden worldwide. Currently, there is an urgent need for novel, more effective vaccine that can prevent all forms of TB including drug resistant strains for all age groups and among people with HIV. The first recombinant tuberculosis vaccine rBCG30, entered clinical trials in year 2004, but, still no effective vaccine is available in a market. Study showed that DNA TB vaccine given with conventional chemotherapy can accelerate the disappearance of bacteria as well as protect against re-infection in mice and it is quite effective against TB. A very promising TB vaccine, MVA85A, is currently in phase II trials and is based on a genetically modified vaccinia virus. Many other strategies are also being used to develop novel vaccines, including both subunit vaccines such as Hybrid-1, HyVac4 or M72, and recombinant adenoviruses such as Ad35. Some of these vaccines can be effectively administered without needles making them preferable for areas where HIV is very common and few of these vaccines have been successfully tested in humans and are now in extended testing in TB-endemic regions. To encourage further discovery, researchers and policymakers across the globe are promoting new economic models of vaccine development including prices, tax incentives and advance market commitments. This review gives the basic idea of various vaccine development approaches and its effective application in TB control.

*Corresponding author, Mailing address:
Dr. Dileep Tiwari, Gennova Biopharmaceuticals Ltd., P-1, I.T.-B.T. Park Phase -II, MIDC
Hinjwadi, Pune-411057, India
E-mail: dileep5000@yahoo.co.in, dileep.tiwari@gennova.co.in

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Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis*, remains a major global health problem. TB, AIDS and malaria are the 'big three' killer infectious diseases worldwide. TB causes ~2 million

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deaths annually and latently infects one-third of the world population (estimated ~2 billion). Successful global TB control faces many obstacles including the difficulty of timely diagnosis, lack of effective vaccines, and the fact that TB treatment requires many months of chemotherapy. The situation has been further compounded with the advent of *M. tb*/HIV co-infection and the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. In 2006, the global burden of MDR-TB, defined as resistance to isoniazid and rifampin, was estimated at 500,000 cases. Additionally, the incident of XDR-TB, caused by MDR strains that are also resistant to fluoroquinolone and at least one second-line injectable agent (amikacin, kanamycin, or capreomycin), is increasing in many countries. A deadly association between HIV and TB has been known since the start of the HIV epidemic. Of the 1.7 million people who died from TB in the year 2010, approximately 200,000 were co-infected with HIV. In light of these developments, a new and effective vaccine is urgently needed, which is essential for reducing the estimated 8-10 million new TB infections that occur annually. According to the Global plan to Stop TB (2006-2015), the introduction of effective TB vaccines will be an essential component of any strategy to control TB by 2050

History of BCG Vaccine

In 1908, Camille Guerin and Albert Calmette initiated their attempts to produce an anti-TB vaccine from a virulent bovine strain. In 1921, vaccination with BCG, an attenuated vaccine, was introduced (Sakula, 1983). The efficiency of the BCG vaccine has been questioned since its early use and therefore, a large number of trials have been carried out to determine its efficacy. In these studies it was found that, the BCG vaccine protected efficiently against leprosy (Fine and Rodrigues, 1990) as well as childhood manifestations of TB (disseminated TB) (Rodrigues *et al.,* 1993). However, the protective efficacy against pulmonary TB was limited (Tuberculosis Research Centre [ICMR], Chennai, India, 1999). Fig.:1

Various hypotheses have been suggested to explain the low protective efficacy of BCG against pulmonary TB. These hypotheses include inappropriate treatment and storage of the vaccine, the use of different strains of BCG (Fine, 1995), and lack of an effective stimulation of the optimal blend of T-cell populations and in particular that of the $CD8^+$ T-cells (Hess and Kaufmann, 1999). In addition to these hypotheses, the currently used intradermal route of immunization has been suggested as another factor influencing the capacity of BCG to induce optimal immunity in the lungs. In this regard, intranasal (i. n.) route of immunization has recently been evaluated as a possible route for BCG delivery, in mouse experimental models. Results from this study showed a high degree of protection against challenge with *M. tb* in BALB/c mice, following BCG vaccination (Falero-Diaz *et al.,* 2000). In a similar model, vaccination with BCG conferred as good, if not better protection than subcutaneous (s. c.) route, against challenge with virulent *M. bovis* (Lyadova *et al.,* 2001).

Prospects for new vaccines

Given the limitations of BCG in protection against adult pulmonary TB, there is a considerable scope for improved vaccination strategies. Immunological research has a key position in understanding the pathogenesis of TB, and thereby in developing novel designs for effective prophylactic vaccination, immunodiagnostic tools and immunotherapeutic agents. Two approaches have been considered for vaccine development. One involves the replacement of BCG by a more potent vaccination inducing immune responses capable of either complete elimination of the bacilli, or of reliable containment of persistent infection. The second approach involves the post-exposure vaccination to boost immunity in

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individuals whose natural immunity has already been primed by infection or BCG vaccination (Young and Stewart, 2002). Indeed, over the past decade research efforts have been directed to evaluate potential vaccine candidates as well as alternative routes of vaccine delivery, such as the intranasal (i. n.) route, in order to improve protection.

New vaccine candidates

A wide range of potential vaccine candidates have been generated and subjected to tests for protective efficacy in experimental model of infection. New vaccine candidates include live attenuated vaccines, subunit vaccines and DNA vaccines.

Live attenuated vaccines

Advances in the techniques required to genetically modify Mycobacteria, as well as the increase in the knowledge of the pathogenesis of the microorganism have made possible to delete genes encoding for potential virulence factors in *M. tb*, thereby enabling the generation of attenuated mutants. In addition to attenuated strains of *M. tb*, the natural attenuated Mycobacteria, such as *M. vaccae* and *M. microti* are being studied as possible vaccine candidates (Nor and Musa, 2004). Another approach has been the improvement of the BCG immunogenicity by the addition of genes encoding cytokines, such as IFN-γ (Murray *et al*., 1996) or Mycobacterial proteins, such as the antigen 85 complex (Ag85) (Horwitz *et al.,* 2000). Although encouraging results have been obtained in challenge experiments (Horwitz *et al.,* 2000; Smith *et al.,* 2001), but, still a major consideration for the clinical use of live vaccines is safety, specificity when considering TB vaccination strategies for AIDS patients.

Subunit vaccines

Subunit vaccines are currently the most widely studied. This type of vaccine has been focused in particular on proteins present in filtrates prepared from *in vitro* cultures of *M. tb*, although non-secreted antigens have also been shown to induce protective responses in experimental studies (Coler *et al.,* 2001; Skeiky *et al.,* 2000). The most extensively studied antigens are members of the Ag85 complex, a family of mycolyl transferases enzymes involved in cell wall biosynthesis and present in culture filtrates (Belisle *et al.,* 1997). The Ag85 has been reported to induce strong activation of T-cells in several studies (Andersen *et al.,* 1995; Mustafa *et al.,* 1998). Other antigens being studied are:

(i) Early secreted antigenic target (ESAT-6), which has been reported to be absent from all BCG vaccine strains and to induce very strong T-cell and antibody responses (Brodin *et al.,* 2004).

(ii) Heat-shock proteins (HSP) such as HSP-65 and HSP-70, found to induce a prominent immune response at both, the antibody and the T cell levels (Silva, 1999).

(iii) PstS-1 (38 kDa protein), a glycoprotein exposed on the surface of the *Bacillus* and reported to be a powerful B and T-cell antigen (Bothamley *et al.,* 1992; Lefevre *et al.,* 1997).

(iv) 19 kDa protein, a lipoprotein found to induce the expression of IL-12 and iNOS inmonocytes and dendritic cells through its binding to TLR2 (Brightbill *et al.,* 1999; Thoma-Uszynski *et al.,* 2000) and to promote neutrophil activation (Neufert *et al.,* 2001).

A major limiting factor of the subunit vaccines is the need of adjuvant for vaccine delivery. Currently research studies are focused on the choice of which adjuvant to use and whether immuno-modulatory, such as cytokines, could be used. Despite this drawback subunit vaccines based on recombinant protein antigens are attractive because the techniques for its production are fully established and this type of vaccines are expected to satisfy the regulatory requirements for use in humans more easily than the live vaccines.

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DNA vaccines

Administration of naked DNA has the potential of eliciting both cellular and humoral immunity against encoded antigens. Several Mycobacterial antigens including PstS-1, HSP-65 and Ag85 have been studied and found that they are inducing protection in animal models (Bonato *et al.,* 1998; Fonseca *et al.,* 2001; Huygen *et al.,* 1996). Although the results are promising, various concerns about the safety of DNA vaccination have been raised mainly regarding the possibility of DNA integration into the host genome affecting oncogenes or tumor suppressor genes and thereby inducing the development of cancer. However, the risk of integration has been reported to be low under a variety of experimental conditions (*Manam et al.,* 2000; Martin *et al.,* 1999).

Experimental animal models in TB

Discussions about the value of experimental animal models in TB research have a longstanding history. Experimental animal models are critical for delineating the general mechanisms underlying natural resistance and acquisition of a protective immune response against TB. However, assessment of this information using experimental animals should be conducted carefully since there are differences in the host defense mechanisms between experimental animals and humans.

Many experimental animal species such as mouse, guinea pig and non-human primates have been used for deciphering the mechanisms involved in TB. The mouse, without any doubt it is a very sophisticated and cost-efficient animal model. The immune response of the mouse is very well understood and biological reagents such as monoclonal antibodies against surface antigens and cytokines are available. Moreover, the genetic manipulation of mouse species is highly advanced. Trans-gene expression, gene knockout, gene knock-in have all become standard technologies, and a large variety of mouse mutants with defined immune-deficiencies are available to researchers studying the role of distinct cells and effector molecules in the *in-vivo* setting of TB. Furthermore, the recent elucidation of the murine genome promises to open a new area of research with enormous impact on our understanding of genetic disorders and also of host mechanisms in TB (Kaufmann, 2003).

Currently, two main vaccination strategies are being pursued. The first strategy uses subunit vaccines in the form of protein-adjuvant formulation, naked DNA, or recombinant bacterial or viral carriers that express defined antigens. Till now, some very promising results have been obtained but so far no vaccine candidate tested in animal models has proven to be better than already available BCG vaccine. The second strategy, comprising viable Mycobacterial vaccines, either attenuated viable *M. tb* or BCG, or recombinant BCG over expressing certain antigens or immuno-modulator is also being pursued and shows promise.

Recently, a lot of attention has been focused on secretory protein antigens of *Mycobacterium tuberculosis*, which are synthesized by actively growing *M. tb* culture or by induction of desired immune response (Anderson and Heron, 1993; Anderson, 1994). These proteins have also been termed as culture filtrate proteins and known to elicit strong immune reaction in humans and animals infected with *M. tb*/ *M. bovis* (Anderson *et al,* 1991 b; Orme *et al,*1992; Romain *et al,*1993; Anderson, 1994). As a result of the combined efforts of several laboratories, more than 30 secretory proteins of *M. tuberculosis* have been characterized (Andersen *et al.,* 1991; Anderson, 1994, Kamath *et al.,* 1999; Sonnenberg and Belisle, 1997; Ingrid *et al.,* 2000; Karin *et al.,* 1998; Gennaro, 2000; Kanaujia *et al.,* 2004; Orme *et al.* 1992; Romain *et al1993;* Spencer *et al., 2004;* Sable *et al., 2005;* Young *et al., 2004).*

The secretory proteins have been demonstrated to be strongly recognized by T-cells isolated from human (Tuberculosis) TB patients (Orme, 1997; Spencer *et*

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al., 2004) as well as mice and cattle experimentally infected with TB (Anderson and Heron, 1993; Pollock and Anderson, 1997; Lanbo *et al.,* 2004). Experimental work in animal models suggests that both CD_4^+ and CD_4^+ T-cells are required for optimal protection against tubercle bacillus (Orme *et al.,* 1992; Bonato *et al.,* 1998; Flynn *et al.,* 1992; Pais *et al., 1998;* Spencer *et al.,* 2004). These proteins have been the focus of much of the research directed at identifying antigens that induce protective immunity or those that elicit immune responses of diagnostic value (Aub *et al.,* 2002; Lein *et al.,* 1999; Young *et al.,* 2004; Paolo *et al.,* 2004).

The recent identification of novel secreted proteins of *M. tb* open the way to study their immunological characterization of these protein to define their potential for immonological diagnosis of TB or vaccine design. A few numbers of secretory antigenic protein and peptides from *M. tb* have already been evaluated as antigens for the immunodiagnosis and vaccines research of TB.

purification by immunological methods, and by screening of expression libraries of *M. tb* DNA with anti-culture filtrate sera (Wolinsky and Schaeferm, 1973; Ginsberg, 1998; Grange and Laszlo, 1990; Bothamley *et al,* 1991; Altamirano *et al,* 1992; Sorensen *et al,* 1995; Mileler *et at,* 1994; Bellete *et al,* 2002). The early secretory antigenic target (ESAT)-6, purified protein and peptides from *M. tb* have been already under in-depth evaluation as antigens for the immunodiagnosis of TB. Some important antigen molecules (like, 38 KDa, 30/31 KDa, 40 KDa, 42 KDa, SOD, 30 KDa MSP, 85B, ESAT-6, and CFP10 etc,), have been found to be secreted by *M. tb*. These Mycobacterial antigens are highly effective for vaccine development for tuberculosis.

Biomarkers for TB

Biomarkers have been described as distinct molecular features that indicate a defined status of the host in relation to any process or involvement. For development of TB biomarkers, different conditions are desired. These conditions include protection by vaccination, discrimination of latent and active disease to facilitate quick treatment outcome or assess relapse risk. A number of TB biomarkers have been reported based on liposomes and including a synthetic *Mycobacterium* glycolipids as immunomodulator, it induces strong and protective T-helper-1 and T-helper-17 adult murine responses to Ag85B-ESAT-6, another DNA-based, extracellular proteins, C-reactive protein (Kamath *et al.,* 2009; Hanekom *et al.,* 2008; Zarate-Blades *et al.,* 2011). Now a days several adjuvants are available in market including AS01, AS02, ISCOMS, Aluminum salts (alum), MF59, IFA (McKee1 *et al.,* 2010), these biomarkers would be/ may be of usefull value to evaluate new candidate subunit vaccines in drug development strategy.

Currently our group is working on novel adjuvant PEGylated liposome (Data not show) for *M. tb*, which is significant effort in the direction of development of novel, more efficacious vaccine against TB these may/ will be become reliable biomarkers of protective immunity against *Mycobacterium tuberculosis*.

Challenges from the disease

Though, the TB vaccine research has gained momentum in recent years, there are still major obstacles. A new generation of TB vaccines must offer greater protection than currently used BCG and be safe enough to be used in HIV-endemic countries. Currently, none of the subunit vaccines that are in clinical trials have exhibited greater efficacy than BCG in animal model studies, which is the reason that they are considered as a booster rather than a replacement for BCG. Although recombinant BCG strains (rBCG30, rBCG::Δ*ureC-llo*+) and the attenuated *M. tb phoP* mutant consistently reduced the *M. tb* burden by ~1.0 log compared to BCG alone, but, it is not clear that whether this level of improvement is sufficient. In addition to above, the safety of these recombinant BCG and attenuated *M.*

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tb strains remains a question. In 2007, WHO revised its policy to recommend that BCG not be given to children known to be HIV-positive, even if asymptomatic, because of substantial high risk of BCG-induced disseminated disease in HIV-infected individuals. All clinical trials of new TB vaccines have so far excluded HIV positive individuals. While this cautious approach is logical that there is a urgent need to evaluate new TB vaccines in HIV-infected populations because with an annual TB incidence rate of 5-10% they are among the most in need of a new and effective vaccine.

Viewpoint with potential instructions

While TB vaccine development has come a long way in the last decade there are still some major impediments ahead of us. For the leading candidates there is no guarantee that they will progress through phase-III clinical trials and registration. Experiences from HIV and malaria vaccine trials have taught us that it is important to continue pre-clinical research and keep developing new and even better vaccines for the pipeline. Another important point for the development of new vaccines in the future is that the human-adapted members of the *M. tb* complex are more genetically diverse than generally recognized and has recently been linked to changes in human demography and to both ancient and recent human migrations.

Such diversity is most likely to have functional consequences for the mycobacterial strains and could affect the efficacy of new vaccines. In addition to that, vaccines may show different protective efficacies against different mycobacterial strains. Consequently, the efficacy of new vaccines should ideally be tested against several clinical *M. tb* isolates and preferably against strains from all 6 major human MTBC lineages. Although, it is clear than ever that designing a vaccine, which can cope with many strategies that *M. tb* has evolved to escape the host's immune response will be very complex, there remain reasons to be optimistic. The first new vaccine against *M. tb* in half a century is progressing through clinical trials at a rapid pace. Phase-II trials are already underway with two vaccines and at least two more expected to reach that stage over the next 1 or 2 year. At the same time vaccines which have shown detectable activity against the latent form of the disease in animal models are already in late pre-clinical stages and several new adjuvants effective at stimulating cell-mediated responses are apparently safe in humans are also in trials. As we scrutinize the immune response against *M. tb* and the pathogen's response, we are becoming capable of designing novel vaccine strategies which could let us tip the balance in the host's favour.

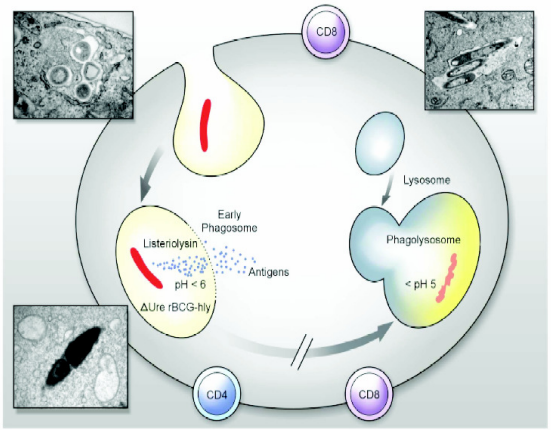

Figure-1: Source: Max-Planck-Institute for Infections biology

References

1) Andersen P., Andersen A. B., Sorensen A. L. and Nageli S., 1995, Recall of long-lived immunity to *Mycobacterium tuberculosis* infection in mice. *J. Immunol.,* 154: 3359-3372.

2) Andersen P. and Heron I., 1993, Specificity of a protective memory immune response against *Mycobacterium tuberculosis*. *Infect. Immun.,* 61: 844-851.

3) Andersen, P., Askgaard D., Ljungqvist L., Bennedsen J., and Heron I., 1991. Proteins released from *Mycobacterium tuberculosis* during growth. Infect. Immun. 59:1905-1910.

Review & ResearchPaper
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4) Altamirano M., Kelly M. T., Wong A., Besuille E. T., Black W. A., Smith J. A., 1992. Characterization of a DNA probe for detection of *Mycobacterium tuberculosis* complex in clinical samples by polymerase chain reaction. *J Clin Microbiol.* 30:2173-2176.

5) Anderson P., 1994.Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted Mycobacterial proteins. *Infect. Immun.* 62: 2536-2544.

6) Anderson P., Askgaard D., Ljungqvist L., Benzton M. W. and Heron ,1991b . T cell proliferative response to antigens secreted by *Mycobacterium tuberculosis*. *Infect. Immune.,* 59: 1558-1563.

7) Azuma I, Thomas DW, Adam A, Ghuysen JM, Bonaly R, Petit JF, Lederer E., 1970 Jun. Occurrence of N-glycolylmuramic acid in bacterial cell walls. A preliminary survey. *Biochim Biophys Acta*.; 208(3):444–451.

8) Belisle J. T., Vissa, V. D., Sievert T., Takayama K., Brennan P. J., Besra G. S., 1997. Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science* 276: 1420-1422.

9) Bonato V. L., Lima V. M., Tascon R. E., Lowrie D. B., Silva C. L., 1998.

10) Identification and characterization of protective T cells in hsp65 DNA-vaccinated and *Mycobacterium tuberculosis*-infected mice. *Infect. Immun.* 66: 169-175.

11) Bothamley G. H., Rudd R., Festenstein F., Ivanyi J., 1992. Clinical value of the measurement of *Mycobacterium tuberculosis* specific antibody in pulmonary tuberculosis. *Thorax* 47: 270-275.

12) Bothamley G., Swansonberk J., Britoon W., Ivanyi J., 1991. Antibodies to *Mycobacterium tuberculosis* specific antigen in lepromatous leprosy. *Clin Exp Immunol.* 86:426-432.

13) Brightbill, H. D., Libraty D. H., Krutzik S. R., Yang R. B., Belisle J. T., Bleharski J. R., Maitland M., Norgard M. V., Plevy S. E., Smale S. T., Brennan P. J., Bloom B. R., Godowski P. J., Modlin R. L., 1999. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 285: 732-736.

14) Brodin P., Rosenkrands I., Andersen P., Cole S. T., Brosch R., 2004. ESAT-6 proteins: protective antigens and virulence factors? *Trends Microbiol.* 12: 500-508.

15) Bellete B, Cobercle J, Barnes GL, *et al.* 2002. Evaluation of a whole-blood interferon-gamma release assay for the detection of *Mycobacterium tuberculosis* infection in 2 study populations. *Cli Infect Dis*. 34: 1445-1456.

16) Calmette A., 1927. La vaccination preventive control tuberculosis. 250 p. Masson et. Cie. Paris.

17) Coler R. N., Campos-Neto, A., Ovendale P., Day F. H., Fling S. P., Zhu L., Serbina N., Flynn J. L., Reed S. G., Alderson M. R., 2001. Vaccination with the T cell antigen Mtb 8.4 protects against challenge with *Mycobacterium tuberculosis*. *J. Immunol.* 166: 6227-6235.

18) Fine P. E., 1995. Variation in protection by BCG. Implication of and for heterologous immunity. *Lancet,* 346: 1339-1345.

19) Falero-Diaz G., Challacombe S., Banerjee D., Douce G., Boyd A., Ivanyi J., 2000. Intranasal vaccination of mice against infection with *Mycobacterium tuberculosis*. *Vaccine* 18: 3223-3229.

20) Flynn, J. L., Goldstein, M. M., Triebold, K. J., Koller, B., Bloom, B. R., 1992. Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. *Proc. Natl. Acad. Sci. U.S.A* 89: 12013-12017.

21) Fine P. E. and Rodrigues L. C., 1990. Modern vaccines. Mycobacterial diseases. *Lancet* 335: 1016-1020.

22) Freund J., 1956. The mode of action of immunological adjuvants. *Adv. Tuberc. Res*., 1: 130-1487.

23) Fonseca D. P., Benaissa-Trouw B., van Engelen M., Kraaijeveld C. A., Snippe H., Verheul A. F., 2001. Induction of cell-mediated immunity against *Mycobacterium tuberculosis* using DNA vaccines encoding cytotoxic and helper T-cell epitopes of the 38-kilodalton protein. *Infect. Immun.* 69: 4839-4845.

Review & Research Paper
Covered in Official Product of Elsevier, The Netherlands

24) Gennaro M. L. 2000. Immunologic Diagnosis of Tuberculosis, *J. Clin. Infec. Dis*. 30(Suppl 3): S243–6.

25) Ginsberg AM. 1998. The tuberculosis epidemic. Scientific challenges and opportunities. Pubbluc Health Rep. 113:128-136.

26) Grange JM, Laszlo A., 1990. Serodiagnostic test for tuberculosis: a need for assessment of their operational predictive accuracy and acceptability. WHO Bull OMS, 68:571-576.

27) Hess J. and Kaufmann S. H., 1999.Live antigen carriers as tools for improved antituberculosis vaccines. *FEMS Immunol. Med. Microbiol.* 23: 165-173.

28) Hirayama, Shin, Shiraishi, Takeshi , Shirakusa, Takayuki , Inuzuka, Koji , Iwasaki, Akinori ,Kawahara, Katsunobu ,2005. Pulmonary Paragonimiasis: Report of Two Cases and a Review of the Japanese Literature. Journal of Bronchology. 12(2):116-118.

29) Horwitz M. A., Harth G., Dillon B. J., Maslesa-Galic S., 2000. Recombinant bacillus calmette-guerin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc. Natl. Acad. Sci. U. S. A* 97: 13853-13858.

30) Huygen, K., Content J., Denis O., Montgomery D. L., Yawman A. M., Deck R. R., DeWitt C. M., Orme I. M., Baldwin S., D'Souza C., Drowart A., Lozes E., Vandenbussche P., Van Vooren J. P., Liu M. A., Ulmer J. B., 1996. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nat. Med.* 2: 893-898.

31) Ingrid Olsen,Liv J. Reitan,and Harald G. Wiker. 2000. Distinct Differences in Repertoires of Low-Molecular-Mass Secreted Antigens of *Mycobacterium avium* Complex and *Mycobacterium tuberculosis* ,J. Clini. Microbiol. 4453-4458, Vol. 38, No. 12.

32) Kanaujia G. V., Motzel S., Garcia M. A., Andersen P., and Gennaro M. L., 2004. Recognition of ESAT-6 Sequences by Antibodies in Sera of Tuberculous Nonhuman Primates. Clin Diagn Lab Immunol; 11:1:222-226.

33) Kamath, A. T., Feng C. G., Macdonald M., Briscoe H., Britton W. J., 1999. Differential protective efficacy of DNA vaccines expressing secreted proteins of *Mycobacterium tuberculosis*. Infect. Immun. 67:1702–1707.

34) Kaufmann, S. H. 2003.Immune response to tuberculosis: experimental animal models. *Tuberculosis. (Edinb.)* 83: 107-111.

35) Karin weldingh, Ida Rosenkrands, Susane Jacobsen, Peter Birk Rasmussen, Martin J. Elhay, and Peter Andersen,1998. Two-Dimensional Electrophoresis for Analysis of *Mycobacterium tuberculosis* Culture Filtrate and Purification and Characterization of Six Novel Proteins. J. Infec. and Immun., 66:3492–3500.

36) Lanbo Shi, Robert North, and Maria Laura Gennaro. 2004. Effect of Growth State on Transcription Levels of Genes Encoding Major Secreted Antigens of *Mycobacterium tuberculosis* in the Mouse Lung. Infec. Immun. 72; 4:2420–2424.

37) Lefevre P., Braibant M., de Wit L., Kalai M., Roeper, D., Grotzinger J., Delville J. P., Peirs P., Ooms J., Huygen K., Content J., 1997. Three different putative phosphate transport receptors are encoded by the *Mycobacterium tuberculosis* genome and are present at the surface of *Mycobacterium bovis* BCG. *J. Bacteriol.* 179: 2900-2906.

38) Lien, E., Sellati T. J., Yoshimura A., Flo T. H., Rawadi G., Finberg R. W., Carroll J. D., Espevik T., Ingalls R. R., Radolf J. D., Golentick D. T., 1999. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem. 274:33419.*

39) Lyadova I. V., Vordermeier H. M., Eruslanov E. B., Khaidukov S. V., Apt A. S., Hewinson R. G., 2001. Intranasal BCG vaccination protects BALB/c mice against virulent *Mycobacterium bovis* and accelerates production of IFN-gamma in their lungs. *Clin. Exp. Immunol.* 126: 274-279.

40) Mileler N, Hernandez SG, Cleary T., 1994. Evaluation of gene-probe amplification

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Covered in Official Product of Elsevier, The Netherlands

Mycobacterium tuberculosis direct test and PCR for direct direction of *Mycobacterium tuberculosis* in clinical specimens. J Clin Microbiol. 32:393-397.

41) Mustafa A. S., Amoudy H. A., Wiker H. G., Abal A. T., Ravn P., Oftung F., Andersen P., 1998.Comparison of antigen-specific T-cell responses of tuberculosis patients using complex or single antigens of *Mycobacterium tuberculosis*. *Scand. J. Immunol.* 48: 535-543.

42) Manam S., Ledwith B. J., Barnum A. B., Troilo P. J., Pauley C. J., Harper L. B., Griffiths T. G., Niu Z., Denisova L., Follmer T. T., Pacchione S. J., Wang Z., Beare C. M., Bagdon W. J., Nichols W. W., 2000. Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. *Intervirology* 43: 273-281.

43) Martin T., Parker S. E., Hedstrom R., Le T., Hoffman S. L., Norman J., Hobart P., Lew D., 1999. Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. *Hum. Gene Ther*. 10: 759-768.

44) Nor N. M. and Musa M., 2004. Approaches towards the development of a vaccine against tuberculosis: recombinant BCG and DNA vaccine. *Tuberculosis. (Edinb.)* 84: 102-109.

45) Neufert, C., Pai, R. K., Noss, E. H., Berger, M., Boom, W. H., Harding, C. V., 2001. *Mycobacterium tuberculosis* 19-kDa lipoprotein promotes neutrophil activation. *J. Immunol.* 167: 1542-1549.

46) Nunc D. 1999. Bacterial type II protein export and pilus biogenesis: More than just homologies? *Trends Cell Biol.* 9: 402-408.

47) Orme I. M., Andersen P. and Boom W. H., 1992. T-cell response to *Mycobacterium tuberculosis*. *J. Infect. Dis.,* 167: 1481-1497.

48) Pais T. F. R., Silva R. A., Smedegaared B., Appelberg R. and Andersen P., 1998. Analysis of T-cells recruited during delayed type hypersensitivity to purified protein derivative (PPD) verses challenge with tuberculosis infection. *Immunology*, 95: 69-75.

49) Pollock J. M. and Andersen P., 1997. Predominant recognition of the ESAT-6 protein in the first phase of infection with *Mycobacterium bovis* in cattle. *Infect. Immun.,* 65: 2587-2592.

50) Romain F. A., Laquyecie P., Militizer P., Pescher P., Chavarot M., Lagranderie G., Auregen M., Merchal B. G., 1993. Identification of a *Mycobacterium bovis* BCG.

51) Rodrigues L. C., Diwan V. K., Wheeler J. G., 1993.Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int. J. Epidemiol.* 22: 1154-1158.

52) Sable S. B., Kumar Rajnish, Kalra M., Verma Indu, Khuller G. K. , Dobos K., Belisle J. T., 2005. Peripheral Blood and Pleural Fluid Mononuclear Cell Responses to Low-Molecular-Mass Secretory Polypeptides of *Mycobacterium tuberculosis* in Human Models of Immunity to Tuberculosis, *Infect. Immuni.* 73(6): 3547–3558.

53) Sakula A., 1983.BCG: who were Calmette and Guerin? *Thorax* 38: 806-812.

54) Silva C. L., 1999.The potential use of heat-shock proteins to vaccinate against Mycobacterial infections. *Microbes. Infect*. 1: 429-435.

55) Skeiky Y. A., Ovendale P. J., Jen S., Alderson M. R., Dillon D. C., Smith S., Wilson C. B., Orme I. M., Reed S. G., Campos-Neto A., 2000. T cell expression cloning of a *Mycobacterium tuberculosis* gene encoding a protective antigen associated with the early control of infection. *J. Immunol.* 165: 7140-7149.

56) Smith DA, Parish T, Stoker NG & Bancroft GJ., 2001. Characterization of auxotrophic mutants of *Mycobacterium tuberculosis* and their potential as vaccine candidates. *Infection and Immunity* 69:1142–1150.

57) Spencer J. S., Jin Kim H., Marques A. M., Gonzalez-Juarerro M., Monica C. B. S. Lima, Vissa V. D., Truman R. W., Gennaro,Sang-Nae Cho M. L., Cole S. T., Brennan Patrick J. . 2004. Comparative Analysis of B- and T-Cell Epitopes of *Mycobacterium leprae* and *Mycobacterium tuberculosis* Culture Filtrate Protein 10. *Infec. Immun*. 72(6): 3161–3170.

58) Sonnenberg M. G. and Belisle J. T., 1997. Definition of *Mycobacterium tuberculosis* culture filtrate proteins by two-dimensional polyacrylamide gel

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electrophoresis, N-terminal amino acid sequencing, and electrospray mass spectrometry. Infect. Immun. 65:4515-4524.

59) Sorensen A. L., Nagai S., Houen G., Andersen P., Andersen A. B., 1995. Purification and characterization of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*. Infect. Immun. 63:1710-1717.

60) Singh AP and Khuller GK., 1993. Enhancement of immunogenicity of immunoprotective glycerophospholipid antigen of Mycobacteria using liposomes containing Lipid. *J. Lip. Res*., 3: 303-316.

61) Thoma-Uszynski S., Stenger S., Takeuchi O., Ochoa M. T., Engele M., Sieling P. A., Barnes P. F., Rollinghoff M., Bolcskei P. L., Wagner M., Akira S., Norgard M. V., Belisle J. T., Godowski P. J., Bloom B. R., Modlin R. L. , 2001. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 291:1544–1547.

62) Thoma-Uszynski S., Kiertscher S. M., Ochoa M. T., Bouis D. A., Norgard M. V., Miyake K., Godowski P. J., Roth M. D., Modlin R. L., 2000. Activation of tolllike receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10.*J. Immunol.* 165: 3804-3810.

63) Tuberculosis Research Centre (ICMR), Chennai. 1999. Fifteen year follow up of trial of BCG vaccines in south India for tuberculosis prevention. *Indian J. Med. Res*. 110:56-69.

64) World Health Organization, 2004. Monitors the tuberculosis epidemic, evaluating surveillance, planning, and financial data in support of national TB control programmes.

65) Wolinsky E. and Schaefer WB., 1973. Proposed scheme for Mycobacterial serotypes by agglutination. Int J Syst Bacteriol. 23:182-183.

66) Young Y. B., Suk A. K., Ji-Soo K, Hyung-Jin E., Bai G. H., Cho S. N., Yu Sam K. 2004. Antigens secreted from *Mycobacterium tuberculosis*: Identification by proteomics approach and test for diagnostic marker. *Proteomics;* 4: 3299–3307.

67) Young, D. B. and Stewart G. R., 2002. Tuberculosis vaccines. *Br. Med. Bull.* 62: 73-86.

68) Hanekom WA, HM Dockrell, THM Ottenhoff, TM Doherty, H Fletcher, H. McShane, F. F. W., D.F. Hoft, S. K. Parida, U. J. Fruth. 2008. Immunological outcomes of new tuberculosis vaccine trials: WHO panel recommendations, PLoS Med 5;7: e145.10.1371.

69) Kamath AT, A-F Rochat, D Christensen, EM Agger, P Andersen, P.H. Lambert, C.A. Siegrist. 2009. A Liposome-Based Mycobacterial Vaccine Induces Potent Adult and Neonatal Multifunctional T Cells through the Exquisite Targeting of Dendritic Cells. PLoS ONE 4;6: e5771.

70) McKee1 A. S, M. K L M.Leod1, J.W. Kappler1 and P. Marrack1. 2010. Immune mechanisms of protection: can adjuvants rise to the challenge?,BMC Biology , 1741-7007;8;37.

71) Wallis R. S., M. Pai, D. Menzies et al., 2010. "Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice," *The Lancet*, vol. 375, no. 9729;1920–1937.

72) Parida S. K. and S.H. E. Kaufmann. 2010. "The quest for biomarkers in tuberculosis," *Drug Discovery Today*, vol. 15, no. 3-4, pp. 148–157.

73) Zarate-Blades C. R., C.L.Silva,G.A. Passos .2011.The Impact of Transcriptomics on the Fight against Tuberculosis: Focus on Biomarkers, BCG Vaccination, and Immunotherapy, Cli. & Devel.Imm., 10; 1155; 6.

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