Belisle et al. (1997) reported a carboxylesterase domain within the amino acid sequences of Ag85A, B, and C, and stated that each protein acted as a mycolyltransferase involved in the final stages of mycobacterial cell wall assembly. This was revealed by direct enzyme assay and site-directed mutagenesis. In the same year Montgomery et al. (1997) explored the regulatory requirements for live TB vaccines to enter Phase I trials, in particular those based on attenuated *Mycobacterium tuberculosis*. The vaccine was extensively characterized and was analyzed for side effects and emergence of disease, including issues of attenuation, the presence of antibiotic resistance markers in live vaccines and the nature of any attenuated vaccine phenotype. Lozes et al. (1997) investigated the utility of nucleic acid vaccination for induction of immune responses against mycobacterial antigens. They found that Ag85A and Ag85B encoding plasmids induced a robust Th1-like response towards native Ag85, characterized by elevated levels of interleukin (IL)-2, interferon-gamma, and TNF-alpha.

Yildir (1998) show induction parameters including inducer concentration, period of induction and the cell concentration. These parameters were optimized in order to increase the yield of the *Eco*RI restriction endonuclease isolated from recombinant *E. coli*. He observed maximum specific activity of the enzyme to be attained in six hours after the addition of IPTG. The addition of IPTG in the early or the late exponential phase did not have any meaningful effect on the time course of induction of enzyme synthesis. Baldwin et al. (1999) contributed in studies analyzing immunogenicity and protective efficacy of a DNA vaccine encoding Ag85A from *Mycobacterium tuberculosis*. The optimal adjuvant and various delivery systems shown to be promising candidates in enhancing immunogenicity when associated with subunit and peptide based vaccines.

Tanghe et al. (2000) compared immunogenicity and protective efficacy of a DNA vaccine encoding Ag85A from *Mycobacterium tuberculosis* in BALB/c and C57BL (B6 and B10) mice immunized by intramuscular (i.m.) needle injection or epidermal gene gun (gg) bombardment. The results indicate that i.m. DNA vaccination is the method of choice for the induction of protective Th1 type immune responses with the Ag85A tuberculosis DNA vaccine. Armitige et al. (2000) carried out a study whose purpose was to generate strains of *Mycobacterium tuberculosis* deficient in expression of the principal members of this complex in
order to determine their role in the pathogenesis of the bacteria. Constructs of Fibronectin binding protein A (fbpA) Fibronectin binding protein B (fbpB) disrupted with the kanamycin resistance marker OmegaKm and containing varying amounts of flanking gene and plasmid vector sequences were then introduced as linear fragments into H37Rv by electroporation. The results show that the fbpA mutant LAa1 grew similarly to the parent H37Rv in enriched laboratory media but exhibited little or no growth in nutrient-poor media and macrophage-like cell lines.

Mustafa (2001, 2002) tested plasmid DNA expression vectors encoding *Mycobacterium tuberculosis* antigen 85 (Ag85) as vaccines in preclinical animal models. The technique used was recombinant DNA, synthetic peptides, antigen-specific antibodies and T-cells to overcome the problem of short-lived immunity associated with protein and peptide based vaccines. Kremer et al., (2002) investigated three abundantly secreted proteins (FbpA, FbpB and FbpC2) which play a key role in the pathogenesis of tuberculosis and also exhibit cell wall mycolyltransferase activity. An experimental set-up was carried out to determine whether FbpC1 may also possess mycolyltransferase activity, a characteristic feature of the Ag85 complex.

Pym et al., (2003) stated that Ag85A per say does not have effect on cellular and humoral immunity and therefore, possibly can serve as candidate for possible vaccine against tuberculosis and other infective diseases. Taylor et al., (2003) reported that a DNA vaccine encoding the hsp60 molecule of *Mycobacterium leprae* which has previously shown to protect against intravenous infection of mice with *Mycobacterium tuberculosis* in both prophylactic and immunotherapeutic modes however; was not effective in latent infection of tuberculosis in the lungs. Besides, when given in an immunotherapeutic model the immunized mice developed classical Koch reactions characterized cellular necrosis throughout the lung granulomas. Their result states unanticipated safety problem indicating that DNA vaccines should be used with caution in individuals who may have already been exposed to tuberculosis.

describe two crystal structures: the structure of antigen 85C co-crystallized with octylthioglucoside as substrate, resolved to 2.0 Å, and the crystal structure of antigen 85A, which was solved at a resolution of 2.7 Å. The structure of 85C with the substrate analog identifies residues directly involved in substrate binding. Elucidation of the antigen 85A structure, the last of the three antigen 85 homologs, shows that the active sites of the three antigen 85 proteins are virtually identical, indicating that they share the same substrate.

Haile (2005) used the techniques of recombinant DNA (Stanley and Boyer, 1974) to express major antigens of *Mycobacterium tuberculosis* like heat shock protein (hsp) 60, hsp70, Ag85, ESAT-6 and CFP10 etc. as new candidate vaccines and diagnostic reagents against TB. Horwitz et al., (2006) compared the genome sequence of *Mycobacterium tuberculosis* with BCG and other mycobacterium, thus unraveling *Mycobacterium tuberculosis* specific regions and genes. He inferred that expression and immunological mapping of these regions and genes can potentially identify antigens of *Mycobacterium tuberculosis* important for developing new vaccines like live attenuated *Mycobacterium tuberculosis* vaccines, recombinant BCG, DNA vaccines, subunit vaccines, fusion proteins with novel adjuvants, delivery systems and specific diagnostic reagents against TB. Hunter et al., (2006) studied the effect of concentration based IPTG induction and duration of induction time on expression of Fcc-Bik.

Dou et al., (2008) compared immune responses induced in mice by vaccination with DNA vaccine constructs expressing mycobacterial ag85A and interleukin-21 and Bacillus Galnette-Guérin (BCG) . He premediated that Ag85A DNA immunization against intravenous *Mycobacterium tuberculosis* challenge, resulted in reduced numbers of CFU in spleen and lungs, compared to animals vaccinated with control DNA.

Barker et al., (2009) identified the general criteria for further clinical development from Phase I through to Phase III to prevent TB. New tools for measuring and characterizing cell-mediated immune responses to *Mycobacterium tuberculosis* have furthered the assessment of these new vaccines in animal models and in human clinical studies including efficacy trials. Sugawara et al., (2009) reported that recombinant BCG Tokyo (Ag85A) is promising as a tuberculosis vaccine, demonstrating protective efficacy in cynomolgus monkeys. Rhesus monkeys were utilized because they are also susceptible to *Mycobacterium tuberculosis*
infection and mimics continuous course of infection resembling human tuberculosis. The recombinant BCG vaccine was administered once intradermally into the back skin to three groups of rhesus monkeys, and its protective efficacy was compared for 4 months with that of its parental BCG Tokyo strain. The result indicated that even in rhesus monkeys rBCG-Ag85A (Tokyo) induce higher protective efficacy than BCG Tokyo.

Borgdorff et al., (2010) investigated tuberculosis spread in the population of Netherland. He revealed that disease directly depends on secular trend, immigration and recent transmission. Dheda et al., (2010) emphasized the use of Bacille Calmette-Guérin (BCG) vaccine, focusing on its limitations and benefits in controlling tuberculosis (TB). Some new TB vaccines, which have entered or are expected to enter clinical trials, are highlighted in his paper. Deng et al., (2010) observed in his study that early secretory antigen target 6 (ESAT-6) is a dominant target for cell-mediated immunity in the early phase of tuberculosis (TB) in patients with TB, Mycobacterium tuberculosis ESAT-6 (MtBE SAT-6) shows cell-lysis activity. Its biological roles in pathogenesis have been implicated in rupture of the phagosomes for bacterial cytosolic translocation causing T-cell proliferation and gamma interferon (IFN-γ) production, which has been considered to be a protective antigen that can be used for future vaccine development. MtBE SAT-6 possesses a unique membrane-interacting activity which establishes the utility of rigorous biochemical approaches in dissecting the virulence of M. tuberculosis.

Mustafa et al., (2011) reported that cellular immune responses are responsible for both protection and pathogenesis in tuberculosis, and are mediated by a complex network of pro-inflammatory, T helper (Th) type 1 and type 2 cytokines. The secretion of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8 and IL-1β; Th1 cytokines interferon-gamma (IFN-γ), IL-2 and tumor necrosis factor-beta (TNF-β); and Th2 cytokines IL-4, IL-5 and IL-10 by the peripheral blood mononuclear cells (PBMCs) of pulmonary tuberculosis patients was studied. Aagaard et al., (2011) developed a multistage vaccination strategy in which antigens Ag85B and 6-kDa early secretory antigenic target (ESAT-6) are combined with the latency-associated protein Rv2660c (H56 vaccine). Boosting with H56 resulted in efficient containment of Mycobacterium tuberculosis infection and reduced rates of clinical disease, as measured by clinical parameters, inflammatory markers, and improved survival of the animals compared with BCG alone. Boosted animals showed reduced pulmonary pathology and
extrapulmonary dissemination, and protection correlated with a strong recall response against ESAT-6 and Rv2660c. Importantly, H56-vaccinated mice promotes a T cell responses against all protein components that is characterized by a high proportion of CD4 (+) T cells.

Greene (2012) evaluated the usefulness of the new commercially available GenoType *Mycobacterium tuberculosis* complex (MTBC) test for species differentiation within, the *Mycobacterium tuberculosis* complex as applied to routine mycobacterial cultures. The *Mycobacterium tuberculosis* complex is composed of the closely related species *Mycobacterium tuberculosi*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium canetti*. So far, rapid identification of the *Mycobacterium tuberculosis* complex has been achieved by using gene probes, which target the 16S Rrna. Recently, the GenoType MTBC (Hain Lifescience GmbH, Nehren, Germany)—a new commercially available DNA strip assay for the rapid identification of the members of the *Mycobacterium tuberculosis* complex—was evaluated by use of a well-characterized collection of *Mycobacterium tuberculosis* complex isolates and was proven to be useful for species differentiation. His work committed about the step towards the development of new vaccines to manage the disease to achieve tuberculosis-free world. Hong *et al.*, (2012) constructed a DNA vaccine based on the Ag85A/MPT64 gene of *Mycobacterium tuberculosis* (Mtbc) and analyze its immunogenicity by enzyme-linked immunospot (ELISPOT) assay. The fusion gene encoding Ag85A/MPT64 was amplified by PCR from the genome of the MTB H37Rv strain and cloned into a eukaryotic expression vector followed by confirmation using restriction endonuclease digestion and DNA sequencing.

Freches *et al.*, (2013) analyzed the role of IL-17A receptor signaling in immune defense. Their data suggested that early neutrophil recruitment is essential for IL-17A mediated long-term control of *Mycobacterium tuberculosis* infection and that a functional IFN-γ response is not sufficient to control *Mycobacterium tuberculosis* growth when the IL-17RA pathway is deficient.