'The struggle against TB … has not always developed in harmony with the rules of science, but it has originated in the people itself, which have finally correctly recognized its mortal enemy. It surges forward with elemental power, sometimes in a rather wild and disorganized fashion, but gradually more and more finding the right path.'

Robert Koch, Nobel Lecture, 12 December 1905
The Nobel Prize in Physiology or Medicine 1905

Determination – Agustín Bejarano
Mixed/canvas
(21 x 21 cm)

'...To the incoming moon
the world will be open again....'

To the Incoming Moon **(Poem)**
– Miguel Hernández

Agustín Bejarano
Camagüey, Cuba

Agustín Bejarano graduated from the Higher Institute of Art in Havana, Cuba. Since the 1990s he has taken part in various art exhibitions held in Canada, Cuba, France, Mexico, Puerto Rico, Spain, Switzerland, and the United States. He received an award from the National Salon of Engraving (San Juan, Puerto Rico) during its IX Bienal de Grabado Latinoamericano y Caribeño, and the Grand Prize of the National Salon of Engraving, the Provincial Center of Fine Arts and Design, Havana.

His works are found in the Museo Nacional de Bellas Artes, (Havana); Coleccion Blasco & Lausín (Zaragoza); Colección Meter Ludwig, (Cologne); Collection Christie (Mexico); and at the Center for the Art (Florida), among others. He is a member of the Union of Writers and Artists of Cuba (UNEAC) and received a Distinction award from the Ministry of National Culture.

CHAPTER 11

Strategies for New Generation Vaccine Development

Angel Cataldi, Fabiana Bigi, Norazmi Mohd-Nor, María Elena Sarmiento, and Armando Acosta

Various chapters in this book alluded to the inability of BCG to confer protection against adult pulmonary TB and its failure to block the transmission of the disease. In addition, there are problems associated with latent TB and the appearance of MDR strains. The HIV-TB coinfection has further complicated the problem, and hence there is an urgent need to develop new generation vaccines against TB.

Currently, with the advent of new technologies, including those in genomics, proteomics, bioinformatics, nanotechnology, new adjuvants/formulations, and whole genome sequence of MTB and other mycobacteria, many different strategies are being used to develop new candidate vaccines against TB. These new technological approaches have enhanced the exploitation of new knowledge related to the MTB mechanisms of infection and virulence as well as host immune mechanisms of defense against the bacteria.

In various chapters of the book, new TB vaccines are described from a functional point of view. This chapter provides a brief overview of the different candidate antigens and the delivery strategies employed (Table 11.1), experimental strategies that are being used (Table 11.2), mutant live vaccines under investigation (Table 11.3), adjuvants used (Table 11.4), routes of administration employed (Table 11.5), and mycobacterial glycolipides (Table 11.6) for new generation TB vaccine development. The reader is recommended to refer to the listed literature to obtain more detailed information on each of these topics.

Table 11.1 Antigens Used in Vaccines in Different Delivery Strategies

Antigen (synonym, Rv number)	Delivery Strategy
Ag85A (FbpA: Rv3804)	DNA (1, 2), r85A boosting DNA (3) rListeria (4), rBCG (5), Vaccinia (6) MVA boosting DNA (7), Adenovirus (8–10), r85A (11), VSV (12), alphavirus (13)
Ag85B (FbpB: Rv1886)	DNA (1, 2), rListeria (4), fused to ESAT-6 (14), rBCG (5, 15–17), Vaccinia (6), Adenovirus (9, 10)
Ag85C (FbpC: Rv0129)	rBCG (3) Vaccinia (6)
ESAT-6 (EsxA: Rv3875)	DNA (1, 19–21), fused to 85B (14), rBCG (5), Vaccinia (6), fused to CFP10 (22), Influenza virus (23, 24)
CFP-10 (EsxB: Rv3874)	DNA (25, 25) r CFP10 boosting BCG (26) fused to ESAT-6 (22)
EsxR (Rv3019)	Boosting BCG (28)
TB10.4 (EsxH)	Adenovirus (8–10), fused to 85B (29)
65-kDa (GroEl2, Rv0440)	DNA (11, 30–34), DNA boosted by BCG (35, 36), DNA boosted by r65kDa (37)
HSP70 (DnaK, Rv0350)	DNA boosted by BCG (35, 36), DNA (32)
16-kDa (HspX. Rv2031c)	DNA (19)
PPE44 (Rv2770c)	rPPE44 (38)
PPE57 (Rv3425)	rBCG (15)
PE_PGRS62 (Rv3812)	DNA (39)
PE 20 (Rv1806)	DNA (39), rRv1806 (40)
PE_PGRS 16 (Rv0977)	DNA (41)
PE_PGRS 26 (Rv1441c)	DNA (41)
PE_PGRS 33 (Rv1818c)	DNA (41, 42)
PE_PGRS (Wag22, Rv1759)	Subunit (43)
Mtb39A (PPE18, Rv1196)	Vaccinia (44)
19-kDa (LpqH, Rv2290)	DNA (19, 45)
38-kDa (Psts1, Rv0934)	rBCG (46)
Apa (45/47-kDa, Rv1860)	DNA and boosted by BCG (35, 36)
CFP-21 (Rv1984)	Boosting BCG (27)
HBHA (Rv0475)	rHBHA (47, 48), DNA (21)
HtpX (Rv0563)	DNA (2)
KatG (Rv1908)	DNA (21)
LppX (22-kDa, Rv2945)	DNA (49)

Antigen (synonym, Rv number)	Delivery Strategy
MPT51 (FbpD: Rv3803)	rListeria (4)
MPT64 (Rv1980)	DNA (50, 21), boosting BCG (27)
MPB(T)83 (Rv2873)	DNA (50–52)
MPB(T)70 (Rv2875)	DNA (51), DNA boosted by rMPT70 (51)
Mtb9.8 (Rv0287)	rMtb9.8 (53)
RpfA (Rv0867)	Subunit (54)
SOD (Rv3846)	DNA (19)
Rv0111	DNA (39)
Rv0198c	DNA (39)
Rv1411 (LprG, P27)	Fused to ESAT-6 (55)
Rv3407	DNA (56)
18-kDa protein of *M. leprae*	Vaccinia (57), rBCG (57).
36-kDa proline-rich antigen of *M. leprae*	DNA (34, 20)
72f	Fusion of Rv0125 and Rv1196 (58)
T&B multiepitopic constructions (more than 2 proteins)	ESAT-6+Ag85A+Ag85B+HSP65+Mtb39A (in MVA, 44); Ag85A+Ag85B+TB10.4 (rBCG, 59), Ag85B+Mpt64+Mtb8.4 (Rv1174) (rBCG, 60), Rv1813+Rv3620+Rv2608 (rProtein, 61), Ag85B epitopes + Mtb8.4 + multiple B epitopes from different MTB proteins (rBCG, Norazmi MN, Sarmiento ME, Acosta A, unpublished results)
Mix of native antigens	RUTI in liposomes (62, 63), culture filtrate (64, 65), Triton X-100-soluble cell wall proteins (66), low MW culture filtrate (68, 68),
LAM	Conjugated to tetanus toxoid (69)

Note: Only candidate vaccines tested for protective efficacy, irrespective of the final outcome, are listed.

Table 11.2 Antigen Delivery Strategies and Experimental Vaccination Strategies

Delivery Strategy	Antigen	Experimental Strategy
Adenovirus (Ad)	Ag85A	Immunization of mice with a naked DNA or BCG and boosted by recombinant replication-deficient adenovirus (70*, 71*). Immunization of mice with recombinant adenovirus and boosted by recombinant VSV (72).
	Ag85A-Ag85B-TB10.4, Ag85-TB10.4	Immunization of mice with recombinant replication-deficient adenovirus expressing fusion proteins (10**, 8*).
BCG	Ag85A	Immunization of cynomolgus monkeys (73*), and rhesus monkeys (74*) with rBCG.
	Ag85B	Immunization of mice with rBCG secreting Ag85B -murine IL-15 fusion protein (16*). Immunization of guinea pigs with rBCG30 (75*).
	Ag85C	Immunization of guinea pigs with rBCG (18*, 76*).
	ESAT-6	Immunization of mice with rBCG expressing ESAT-6 gene linked to BCG hsp60 or ESAT -6 with secretory sequence (77**).
	72f	Vaccination of cynomolgus monkey with rBCG (78*).
	Ag85B-Mpt64(190–198)-Mtb8.4	Immunization of mice with rBCG (60*).
	Ag85B-ESAT-6 or ESAT-6-Ag85C	Immunization of mice with rBCG alone (79**, 80*) or boosted by LTK-63-adjuvanted recombinant fusion protein (14**).
BCG modified	-	Immunization of mice with a urease C-deficient hly+ rBCG (DeltaureC hly+ rBCG) strain (81*).
	Ag85	Immunization of guinea pigs with a replication-limited rBCG strain (82*).
	Ag85A+Ag85B +EsxH (TB10.4)	Immunization of mice with AFRO-1 strain, an ureC-BCG strain expressing a mutant form of perfringolysin O (pfoA) and overexpressing the MTB antigens (83*).
DNA	Ag85A	Immunization of mice with naked DNA alone (1, 3, 84, 85) or absorbed to poly (DL-lactide-co-glycolide) (PLGA) (86). Immunization of guinea pigs with naked DNA (87, 88) alone or boosted by peptides (89).
	Ag85B	Immunization of mice with naked DNA (1, 90, 91**, 92) alone or boosted by BCG (93*).
	AhpC (Rv2428)	Immunization of mice with naked DNA (94).
	Apa or Pro	Immunization of mice with naked DNA (95). DNA prime-BCG boost vaccination of cattle (96*).

Delivery Strategy	Antigen	Experimental Strategy
	ESAT-6	Immunization of mice (1, 20, 21) and guinea pigs (19) with naked DNA encoding the wild type protein (1, 19, 20) or linked to tissue plasminogen activator (TPA) signal sequences (21). DNA prime-BCG boost vaccination of mice (97*).
	ESAT-6-Ag85B	Immunization of mice with BCG and boosted by DNA vaccination of mice (98*).
	ESAT-6-CFP10	Vaccination of cattle with BCG and boosted by ESAT-6:CFP10 DNA in combination with GM-CSF and CD80/CD86 (99*).
	KatG, HBHA	Immunization of mice with naked DNA encoding the protein fussed to TPA (21).
	HSP65	Immunization of mice with naked DNA (20**, 31**, 100) or co-encapsulated with trehalose dimicolate (TDM) into biodegradable poly(DL-lactide-co-glycolide) (PLGA) microspheres (101). Vaccination of cynomolgus monkey with DNA expressing HSP65 and IL-12 encapsulated into hemagglutinating virus of Japan (HVJ)-liposome (78*). Immunization of mice with a BCG prime DNA boost vaccination regime (102*).
	HSP70	Immunization of mice with naked DNA (20).
	LppX	Immunization of mice with naked DNA (49).
	MPT64	Immunization of mice with naked DNA encoding the protein linked to ubiquitin protein (UbA or UbGR) (103) or TPA (21).
	MPT/MPB83–70	Immunization of mice (104*) or guinea pigs (88) with naked DNA. DNA prime-BCG or DNA prime-protein boost vaccination of cattle (51).
	PE_PGRS 16, PE_PGRS 26 or PE_PGRS 33	Immunization of mice with naked DNA (41).
	PPE44	Immunization of mice with naked DNA (38**).
	PstS-1, PstS-2, or PstS-3	Immunization of mice with naked DNA (105, 106**, 107**).
	P27	Immunization of mice with naked DNA (108).
	Rv0111, Rv0198c, Rv3812 or Rv1806–1807	Immunization of guinea pigs with a DNA recombinant vector carrying TPA signal (39, 40).
	Rv3407	BCG prime-DNA boost vaccination of mice (56*).

(*continued*)

Table 11.2 (*continued*)

Delivery Strategy	Antigen	Experimental Strategy
	Sod or Rv2031c	Immunization of guinea pigs with a DNA recombinant vector carrying CpG motifs and TPA signal (19).
	19-kDa	Immunization of mice with naked DNA (95).
	36-kDa proline-rich antigen from *M. leprae*	Immunization of mice with naked DNA (20**, 31**,).
	72f	Immunization of guinea pigs with naked DNA (109**).
	1818PE_PGRS, 1818PE	Immunization of mice with naked DNA (42).
	Ag85A + ESAT-6	DNA prime- WAg520 attenuated *M. bovis* strain or BCG boost vaccination of mice (110).
	Ag85A + ESAT-6 -Ag85B	Vaccination of mice with naked Ag85B-ESAT-6 DNA vaccines (111**).
	Ag85A + PstS-3	Immunization of mice with naked DNA (112).
	Ag85B + MPT-64 + MPT-83	Immunization of calves with naked DNAs (113*) and boosted by BCG.
	Ag85B + MPT-83 + ESAT-6	Immunization of mice with naked DNAs (114).
	CFP10 + CFP21 + Ag85B	Immunization of mice with naked DNAs (115**).
	Multiepitope	Vaccination of mice with combinations of naked DNA encoding ESAT-6, MPT64, MPT63, MPT8e, 85B, KatG, MTB12, MTB8.4, MTB39, Rv1818c and IL12, fused (116**, 117) or not (118, 119, 120) to TPA and ubiquitin (Ub). Immunization of cattle with combined DNA vaccines expressing Ag85B, MPT64, ESAT-6 and MPT83 in the presence of dimethyldioctyldecyl ammonium bromide (DDA) (121*, 122). Immunization of mice (35*) and cattle (36*) with a DNA cocktail encoding Hsp65, Hsp70 and Apa, and boosted by BCG. Immunization of mice with a DNA cocktail encoding A85B, MPT64, MPT83 and IL2 (123).
Recombinant proteins produced in *Escherichia coli*	Ag85B -TB10.4	Immunization of mice with recombinant fusion protein emulsified with MPL-TDM (29**).

Delivery Strategy	Antigen	Experimental Strategy
	Ag85B-ESAT-6	Vaccination of mice (124**, 125, 126**), guinea pigs (127) or cynomolgus monkeys (128) with the fusion protein alone or emulsified with different adjuvants (DDA/MPL Detoxified monophosphoryl lipid A (MPL), AS02A, LTK63).
	ESAT-6-MPT64	Immunization of mice with recombinant fusion protein (129).
	HSP65 or HSP65 - hIL-2	Immunization of mice with recombinant HSP65 (31). Immunization of mice with recombinant fusion protein emulsified in the adjuvant combination DDA and monophosphoryl lipid A (130**).
	HTPX	Immunization of mice with recombinant *E. coli* expressing recombinant protein (2).
	MPT51	Immunization of mice with recombinant protein with adjuvants (FIA and CpG DNA) (131).
	P27	Immunization of mice with recombinant protein emulsified with DDA, monophosphoryl lipid or Ribi (108).
	PE_PGRS Rv1759	Immunization of mice with recombinant PGRS domain by using a murine model of chronic TB (43).
	Rpf	Immunization of mice with recombinant proteins formulated in FIA (54).
	Rv0287	Immunization of mice with recombinant protein formulated in either AS02A or AS01B Adjuvant Systems (53**).
	Rv1174c	Immunization of mice with recombinant protein formulated in FIA (132**).
	Rv1806	Immunization of guinea pigs with recombinant protein formulated with DDA/TDB (40).
	Rv2770c	Immunization of mice with recombinant protein formulated in DDA (38).
	Rv3019c	Immunization of mice with a BCG prime- recombinant protein boost vaccination regime (133). Immunization of mice with recombinant proteins emulsified with MPL-TDM/DDA (28**).
	72f	Immunization of mice and guinea pigs with recombinant protein emulsified with AS01B adjuvant (109**).
	ESAT-6-+CFP21+MPT64	BCG prime- recombinant protein cocktail boost vaccination of mice emulsified with DDA co-adjuvanted with monophosphoryl lipid A (27*).
Influenza viruses	ESAT-6	Immunization of mice (23**, 24) and guinea pigs (23**) with recombinant viruses.

(*continued*)

Table 11.2 (*continued*)

Delivery Strategy	Antigen	Experimental Strategy
Listeria monocytogenes	Ag85A, Ag85B or MPB/MPT51	Mice immunized with self-destructing attenuated *L. monocytogenes* Delta 2 strains carrying eukaryotic expression plasmids (4).
Mycobacterium smegmatis	HBHA	Immunization of mice with recombinant protein emulsified in DDA and monophosphoryl lipid A (47).
	19 kDa	Immunization of mice with recombinant *M. smegmatis* (45).
	P27-ESAT-6	Immunization of mice with recombinant fusion protein formulated in DDA adjuvant with or without monophosphoryl lipid A (55*).
Mycobacterium tuberculosis	CFP-25+ CFP-20.5+ Ag85B+ Ag85A +CPF32	Immunization of mice with protein emulsified with DDA-MPL adjuvants (67**).
	TB10.4+ ESAT-6+ CFP8+ CFP10 +CFP15	Immunization of mice with a native protein cocktail (134).
	CFP11+ CFP21+ CFP22.5+ MPT64 +CFP31	Immunization of mice with native protein cocktail (134**).
Salmonella typhimurium	Ag85A	Oral immunization of mice with a *S. typhimurium* aroA strain that secretes Ag85A (135**).
	ESAT-6	Oral immunization of mice with a *S. typhimurium* aroA strain that secretes ESAT-6 via the HlyB/HlyD/TolC export machinery (136**). Immunization of mice with attenuated *Salmonella typhimurium* strain that secretes ESAT-6 via the hemolysin secretion system of *E. coli* (137).
Sindbis virus (pSINCP)	Ag85A	Immunization of mice with pSINCP expressing recombinant antigen (13).
Vaccinia	Ag85A	Immunization of mice with BCG (138*) or BCG(delta) ureC hly+ (139*) prime- modified Vaccinia virus Ankara (MVA) boost vaccination regimen. Immunization of guinea pigs with BCG boosted by MVA and fowlpox recombinant vectors (140*). BCG/ MVA prime-boost vaccination regimen in rhesus macaques (141*).
	ESAT-6-MPT63	Immunization of mice with DNA/Vaccinia virus Ankara prime-boost vaccination regimen (142**).
	ESAT-6- Ag85A-Ag85B, HSP65- MTB 39A	Immunization of mice with a recombinant MVA expressing fusion protein together with IL-15 (84).

Delivery Strategy	Antigen	Experimental Strategy
	19-kDa, 38-kDa	Mice inoculated with recombinant Vaccinia virus (143).
	18-kDa from *M. leprae*	Immunization of mice with recombinant Vaccinia virus (VV18) (57).
Vesicular stomatitis virus (VSV)	Ag85A	AdAg85A prime-VSVAg85A boost mice vaccination regime (12).

*Better protection than BCG
**Equivalent or similar protection to BCG
DDA dimethyl dioctadecylammonium bromide
FIA Freund's incomplete adjuvant

Table 11.3 Attenuated MTB Complex Mutant Vaccines Generated by Genomic Manipulation

Mutated Gene	Function	MTB Complex Species	Reference
purC	Purine biosynthesis	MTB, *M. bovis*	144
leuD	Leucine biosynthesis	MTB, BCG	145
trpD	Trytophan biosynthesis	MTB	146
panC, purD	Pantothenate and purine biosynthesis	MTB	147
fadD26	Involved in PDIM synthesys	MTB	148
mce2	unknown	MTB	149
phoP	Central regulator	MTB	150
cysH	Cysteine biosynthesis	MTB	151
lpqH	19-kDa Lpp, unknown	MTB	151
mmpL4, *mmpS4*, *ufaA1*, *sigK*	Complex lipid transfer and metabolism and sigma factor	*M. bovis*	153
Rv0192, Rv0201c	Unknown and Unknown	*M. bovis*	153
fbpA (85A)	Mycolyl transferase	MTB	154
r-BCGΔure:Hly	Urease interrupted by listeriolysin (Hly) of Listeria monocytogenes	BCG	155
lysA	Lysine biosynthesis	MTB	156
Delta RD1	EsxA export system	MTB	157
mbtB	synthesis mycobactin (siderophore)	rBCG (hyperattenuated)	82
secA2	Component of a protein secretion system	MTB	158

Table 11.4 Adjuvants

Adjuvant	Reference
DDA	27, 28, 30, 40, 47, 55, 67, 108, 121, 122, 128, 130
As02A /AS01B	159
IC31	160, 161
cationic liposomes	162, 163, 164, 165
liposomes	166
LTK3	167
CTA1-DD/ISCOMs	168
Lactoferrin	169, 170
Alum	171
Eurocine L3	172
CpG DNA	131

Table 11.5 Routes of Administration

Route	Reference
Intradermal	61
Subcutaneous	173, 174, 175
Intramuscular	25, 176
Mucosal	
• intranasal	177, 178
• oral	179
• rectal	180
• respiratory	181

Table 11.6 Glycolipides from Mycobacterial Origin and Glyconjugates Used in Vaccine Formulations

Antigen (synonym)	Delivery Strategy
Chloroform: methanol extracts enriched in glucose monomycolate (GMM) and mycolic acid	liposomes plus QS-21 adjuvant, subcutaneous (sc) route (182)
GMM	sc route (183) NP
Arabinomannan conjugated to Ag 85b	sc route and nasal booster (184)
Arabinomannan conjugated to tetanus toxoid	sc route and nasal booster (184)
mannophosphoinositides (PIMs)	In liposomes (185)
Monomycolated glycerol (MMG)	Adjuvant, not a protective antigen (186)
Trehalose mycolate (cord factor)	Combined with another antigen, intravenous (iv) route (184) or alone (190)
Trehalose-6,6-dimycolate (TDM) and Trehalose-6,6-dibehenate	Combined with MTB Ag85B and ESAT-6. (187)
Trehalose dimicolate (TDM) into biodegradable poly(DL-lactide-co-glycolide) (PLGA) microspheres	Combined with DNA-hsp65 (188, 189)
Ac2SGL (dyacilated sulphoglycolipid)	No published protection assay. Listed as candidate vaccine in http://www.stoptb.org/ (191)

Conclusion

The main objective of this overview chapter is to highlight the main trends in TB vaccine development. These registers of research activities are not exhaustive, but they reflect the highly complex technologies and strategies that are being attempted in the development of new vaccines against TB. The following chapters will provide more extensive analyses of some of these strategies in the hope that some of these pipeline candidate vaccines will be able to enter or complete clinical trials in the future.

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