

A self-assembling and self-adjvanting multiepitope peptide nanoparticle vaccine improves the efficacy and immunogenicity of Bacille Calmette-Guérin

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Background

- TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS)¹
- The global burden of LTBI was 23.0%
- Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat.
- After one century, BCG still remains the only licenced TB vaccine
- Previously we discovered that a 15 leucine polypeptide moiety as a self-assembling and self-adjvanting inducer of peptide-based vaccine²

Vaccine design

Linker, increase the hydrophilicity

N- LLLLLLLLLLLLLLLLLL - KKK - TB epitope - KKK - HLA-E binding moiety - C

15 Leucine polypeptide moiety, self-assembled from CFP10, tein, high-affinity binding moiety, self-adjvanting inducer

15 amino acids epitopes Derived from Mtb protein Ag85b, HspX proteins of from mice, nonhuman primates and humans³

Immune cells, Antibodies, Cytokines, Sonicate/Dissolve

PNx6 physicochemical property characterization

Morphology and size of PNx6 analyzed by TEM

Secondary structure of PNx6 analyzed by CD

Peptide	Size (nm)	Zeta potential (mV)	α-helix %
Peptide 1	22 ± 4; 134 ± 70	29 ± 10	83.00
Peptide 2	34 ± 7; 162 ± 34	37 ± 6	83.10
Peptide 3	20 ± 7; 201 ± 46	14 ± 5	81.20
Peptide 4	22 ± 2; 141 ± 13	34 ± 8	81.80
Peptide 5	98 ± 37; 418 ± 192	48 ± 11	83.50
Peptide 6	10 ± 2; 61 ± 25	19 ± 8	81.90
PNx6	27 ± 6; 256 ± 36	48 ± 6	

- All peptide building blocks for PNx6 have α-helix structure
- PNx6 are able to self-assemble into nanoparticles
- PNx6 nanoparticles are positively charged

Vaccine synthesis

- Fmoc solid-phase peptide synthesis
- 2-Chlorotrityl chloride resin
- All peptide building blocks were successfully synthesized
- Purity ≥ 95%

Mass spectrum results of building block 5

HPLC results of building block 5

Immunology evaluation of PNx6

Day 0, Day 14, Day 28, Day 42, Day 60

- s.c. BCG + s.c. PNx6 induced highest amount of IFN γ -secreting cells
- s.c. BCG + s.c. PNx6 induced significantly higher titer of antigen-specific antibodies than s.c. BCG
- s.c. BCG + s.c. PNx6 induced higher frequency of immune cells
- s.c. BCG + s.c. PNx6 induced significantly higher frequency of polyfunctional cytokine secreting T cells

Group	Treatment	Mice No.	Dose (per mouse, per administration)	Legend
A	unvaccinated	n=10		
B	s.c. BCG	n=10	s.c. 1x10 ⁸ BCG (in 200 μ L PBS) on Day 0	
C	s.c. PNx6	n=10	s.c. 50 μ L (3mg/mL) PNx6 on Day 0, 14, 28 and 42	
D	s.c. BCG + s.c. PNx6	n=10	s.c. 200 μ L BCG on Day 0 + s.c. 50 μ L (3mg/mL) PNx6 on Day 14, 28 and 42	
E	i.n. BCG	n=10	i.n. 1x10 ⁸ BCG (in 40 μ L PBS) on Day 0	
F	i.n. PNx6	n=10	i.n. 50 μ L (3mg/mL) PNx6 on Day 0, 14, 28 and 42	
G	i.n. BCG + i.n. PNx6	n=10	i.n. 40 μ L BCG on Day 0 + i.n. 50 μ L (3mg/mL) PNx6 on Day 14, 28 and 42	
H	s.c. BCG + i.n. PNx6	n=10	s.c. 200 μ L BCG on Day 0 + i.n. 50 μ L (3mg/mL) PNx6 on Day 14, 28 and 42	
I	i.n. BCG + s.c. PNx6	n=10	s.c. 200 μ L BCG on Day 0 + i.n. 50 μ L (3mg/mL) PNx6 on Day 14, 28 and 42	

Protection efficacy evaluation of PNx6

Day 0, Day 14, Day 28, Day 42, Day 60, Day 105

Lung CFU (Log10)

Spleen CFU (Log10)

Mtb bacterial burden in lung and spleen were analyzed

Lung damage was analyzed by the percentage of infiltration area

Profile of cytokines and chemokines in serum

Cytokine	A	B	C	D	E	F	G	H	I
GRO alpha (CXCL1)	0.210	0.420	0.201	0.262	0.207	0.252	0.248	0.154	0.222
IFN gamma	0.392	0.207	0.208	0.108	0.064	0.198	0.084	0.071	0.075
IL-1 beta	0.142	0.108	0.114	0.232	0.116	0.106	0.166	0.209	0.125
IL-2	0.263	0.144	0.113	0.121	0.113	0.111	0.183	0.068	0.270
IL-5	0.073	0.052	0.278	0.079	0.041	0.067	0.123	0.097	0.125
IL-6	0.086	0.323	0.091	0.100	0.072	0.068	0.064	0.102	0.154
IL-9	0.075	0.000	0.100	0.000	0.000	0.000	0.058	0.000	0.000
IL-10	0.091	0.005	0.005	0.000	0.052	0.013	0.111	0.000	0.050
IL-12p70	0.122	0.132	0.092	0.065	0.162	0.115	0.053	0.044	0.135
IL-13	0.279	0.224	0.229	0.224	0.224	0.224	0.206	0.224	0.224
IL-17A (CTLA-8)	0.134	0.125	0.174	0.245	0.163	0.144	0.184	0.239	0.186
IL-18	0.189	0.074	0.147	0.000	0.111	0.095	0.000	0.000	0.046
IL-22	0.061	0.029	0.112	0.058	0.055	0.148	0.036	0.010	0.023
IL-23	0.054	0.019	0.088	0.035	0.042	0.140	0.039	0.014	0.024
IL-27	0.081	0.031	0.181	0.050	0.038	0.029	0.035	0.050	0.041
IP-10 (CXCL10)	0.701	0.658	0.697	0.563	0.580	0.708	0.515	0.656	0.646
MCP-1 (CCL2)	0.192	0.202	0.105	0.175	0.136	0.232	0.155	0.170	0.227
MCP-3 (CCL7)	0.452	0.483	0.438	0.416	0.418	0.475	0.458	0.421	0.501
MIP-1 alpha (CCL3)	0.252	0.190	0.276	0.154	0.139	0.228	0.116	0.130	0.172
MIP-1 beta (CCL4)	0.373	0.507	0.268	0.271	0.371	0.250	0.247	0.287	0.325
MIP-2 alpha (CXCL2)	0.414	0.556	0.420	0.320	0.408	0.378	0.364	0.258	0.384
RANTES (CCL5)	0.442	0.488	0.423	0.392	0.344	0.444	0.339	0.429	0.402
TNF alpha	0.347	0.328	0.180	0.153	0.140	0.138	0.150	0.187	0.174

Effect of antigen stimulation on IFN γ secretion of PNx6 was analyzed by ELISpot

Titers of antigen-specific antibodies in serum and BALF were analyzed by ELISA

Frequencies of T cells were analyzed by FACS

Lung CD4⁺ cells

Lung CD8⁺ cells

Spleen CD4⁺ cells

Spleen CD8⁺ cells

Frequencies of polyfunctional cytokine secreting T cells were analyzed by FACS

Summary

- s.c. BCG + s.c. PNx6 vaccination induced much stronger immune response than s.c. BCG only
- s.c. BCG + s.c. PNx6 immunity demonstrated better protection efficacy than s.c. BCG vaccination only
- As previous study proved that the 15 leucine polypeptide moiety is non-cytotoxic, the safety of s.c. BCG + s.c. PNx6 vaccination should be similar to s.c. BCG
- More Mtb peptide epitopes and binding peptides could be incorporated into the PNx6 platform to produce a stronger booster vaccine for BCG

Advantages of PNx6

- Cold-chain and adjuvant independent
- Use of cross-species validated epitopes and binding peptides could facilitate potential clinical translation
- The modular building block approach allows for rapid, economical, and customized vaccine production
- The peptides can be stored and transported as freeze-dried solids at room temperature
- PNx6 can be prepared on-site by convenient two steps (dissolve, sonicate) within 2 minutes
- Infinite potential to optimize by including more peptide epitopes
- Produce customizable vaccine by incorporating specific peptide epitopes

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