Future peptide vaccine for TB endemic regions: Challenges and solutions

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Epidemiology of Tuberculosis

Best Prophylactic measure

Vaccine

BCG

Total no. of new TB cases
9 million

Total no. of deaths
1.5 million

BCG Failure

Previously treated
85%

New cases
15%

Emergence of drug resistant

WHO Global tuberculosis report , 2014
Reasons for BCG failure

Lack of enduring immunologic memory

Interference by environmental mycobacteria (Masking and Blocking hypotheses)

Antigen processing and presentation- Blocking phagosome maturation and export of major histocompatibility complex (MHC) molecules

Helminth infections

Regulatory T cells
Limitations of DOTs

Time consuming (3-12 months), so chances of intermittent therapy increases, resulting in increased risk of drug resistance.

Side Effects.

No cure for latent infection.

Urgent need to develop: A potent construct with two edge sword (Prophylactic and Therapeutic potential)
Future TB vaccine: Epitope based vaccine

✓ Minimal antigen processing

✓ Minimal presence of mycobacterial components (except antigenic portions)

✓ Robust Th1 response (even in a Th2 bias environment)

✓ Enduring T cell memory

✓ No preformed antibodies in the population

✓ Vaccine candidate with both prophylactic and therapeutic properties

✗ Poor immunogenicity

✗ Binds to very few HLA-alleles
### Which peptide to choose?
Is promiscuous peptide right choice?

<table>
<thead>
<tr>
<th>HLA DRB1</th>
<th>Binding Affinity F91-110</th>
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<tbody>
<tr>
<td>0101</td>
<td>++++</td>
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<tr>
<td>0103</td>
<td>++++</td>
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<td>0301</td>
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<td>0401</td>
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<td>0701</td>
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<td>1101</td>
<td>++++</td>
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<tr>
<td>1301</td>
<td>+++</td>
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<td>1501</td>
<td>++++</td>
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<tr>
<td>1601</td>
<td>++++</td>
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</tbody>
</table>

++++ indicates high-affinity binding (IC\(_{50}\)<10µM); +++ intermediate affinity (10 µM<IC\(_{50}\)<100µM); and ++ indicates low affinity (100-1000µM).

Can coupling promiscuous peptides to Pam2Cys render them immunogenic?

Pam2Cys S-[2,3-is(palmitoyloxy)propyl]cysteine induces effective Th1 immune responses, by evoking DCs to secrete IL-12 (Thoma et al 2000, Jackson et al 2004, Zho et al 2004, Ghosh et al 2006)

Dendritic cells copiously express TLR2 so Pam2Cys (TLR2 agonist) can be used as vaccine delivery module (Jackson et al 2004, Zho et al 2004)

Pam2Cys has ability to mature and activate DCs (Hertz et al 2001, Jackson et al 2004, Zho et al 2004)

Safe for Human use (Zeng et al 2002)

(91-110)
SEFAYGSFVRTVSLPVGADE

-K

L91

Pam2Cys
Induction of MHC expression
Induction of microbicidal activity
NOS2 generation

Mechanism of L91

IFNγ, IL-17, TNFα
L91 confers better protection than BCG in Guinea pig model of tuberculosis.
Whether L91 has therapeutic potential?
L91 efficiently decreases bacterial burden in the lungs and spleen in conjunction with anti-TB drugs.
L91 immunization reduces *Mtb* induced lung pathology
L91 has enough therapeutic potential to confine bacterial growth in lungs.

Further, L91 restricts dissemination of bacteria in spleen.
Mechanism of protection?
L91 stimulation elicits innate immunity

- iNOS
- ACTIN

- NF-κB

Materials used:
- FB
- US
- F91
- Pam
- L91
- LPS
L91 immunization elicits secretion of protective cytokines against *Mtb*.

- Single cell suspension from Lungs
- In vitro stimulation with L91
- T cell response

**Graphs:**
- IL-1β (S.I) levels for PBS, Drug, L91, Drug+L91
- IFNγ (S.I) levels for PBS, Drug, L91, Drug+L91
- IL-17A (S.I) levels for PBS, Drug, Drug+L91, L91

Significance levels: ns, **, ***
L91 immunization induces generation of multifunctional CD4 T cells

IFN-γ+ TNF-α+ CD4 T cells (%)

IL-17+IFN-γ+ CD4 T cells (%)

Placebo  Drug  L91  D-L91

Medium  Pam2Cys  L91
L91 immunization generates specific subtype of Th17 cells
L91 immunization rescues CD4 T cells from exhaustion
L91 immunization engender long lasting memory CD4 T cells
L91 immunization eradicate bacteria by eliciting strong Th1 and Th17 response

L91 immunization generates unique subset of IFN-γ⁺IL-17⁺ polyfunctional Th17 cells

L91 engenders memory generation
Whether immunotherapy with L91 elicits immune response in TB patients?
Lipidated peptide exhibits T cell proliferation in PBMCs isolated from TB patients and their close contacts.

PBMCs (TB patients) + L91 + IL-2 → 96h → Immune response

T cell proliferation (Stimulation index)

n= 53 (TB patients)
L91 stimulation elicits IFN-γ secretion by the CD4 T cells of TB patients.

<table>
<thead>
<tr>
<th>Condition</th>
<th>IFN-γ (CD4+) T cells (%)</th>
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</thead>
<tbody>
<tr>
<td>Medium</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>Pam2Cys</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>L91</td>
<td>9.7 ± 1.5</td>
</tr>
</tbody>
</table>

Significance:
- ***: p < 0.001
- **: p < 0.01
- ns: no significance
L91 stimulation induces IL-17 secretion by the CD4 T cells of TB patients.
L91 stimulation generates polyfunctional Th17 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>IFN-γ+IL-17A+ CD4 T cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>2.8±0.4 (n=14)</td>
</tr>
<tr>
<td>Pam2Cys</td>
<td>3.7±0.6 (n=12)</td>
</tr>
<tr>
<td>L91</td>
<td>8.3±1.2 (n=14)</td>
</tr>
</tbody>
</table>

Significance:
- ***: p < 0.001
- **: p < 0.01
- ns: not significant
L91 expands the pool of memory T cell

CD45RA+ CD45RO+ CD4 T cells (%)

Medium: 3.8±0.4 (n=28)
Pam2Cys: 4±0.4 (n=15)
L91: 7.7±0.8 (n=21)

***

**

ns
F91 specific antibodies are absent in TB endemic population
Conclusions

✓ Promiscuous lipidated peptide can elicit robust Th1 and Th17 response in human PBMCs isolated from TB patients and their closed contacts

✓ L91 immunization induces unique subset of co-expressing CCR6 and CXCR3 Th17 cells, which secretes both IFN-γ and IL-17

✓ No anti-peptide antibodies in population so L91 has great potential to be a future vaccine candidate for TB endemic area

✓ Immunotherapy with L91 may reduces risk of generation of drug resistant Mtb
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