

A novel vaccine construct comprising of lipidated peptide protects against *Mycobacterium tuberculosis* by bolstering enduring memory T cell response

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Theme of the study

To develop a unique and novel construct that can function as a vaccine in TB-endemic regions, where BCG has failed

Failure of BCG

Despite nine decades of BCG vaccination, TB continues to be a major global health challenge.

Clinical trials worldwide have proved the inadequacy of the BCG vaccine in preventing the manifestation of pulmonary TB in adults.

Ironically, the efficacy of BCG is poorest in TB-endemic areas.

Factors such as non-tuberculous or environmental mycobacteria and helminth infestation have been suggested to limit the efficacy of BCG.

Hence, in high TB-burden regions, radically novel strategies of vaccination are urgently required.

Gowthaman & Agrewala. Trends Mol Medicine 2012

Antigen presentation

Recent series of studies have suggested that *M. tuberculosis* and environmental mycobacteria (EnM) actively inhibit bacterial antigen processing and presentation by MHC class I and class II pathways, thus slowing the emergence of protective adaptive immunity (Wolf et al. 2007; Soualhine et al. 2007; Gehring et al. 2003, Tobian et al, 2003).

BCG and EnM specifically blocks the surface export of mature class II molecules on APCs (Soualhine et al. 2007).

M. tuberculosis and EnM impairs *in vivo* antigen processing by DCs (Wolf et al. 2007).

Gowthaman & Agrewala. Crit Rev Microbiol. 2014

What ever may be the reason for BCG failure!

✓ But the fact remains that more people have been vaccinated with BCG than any other vaccine.

✓ Still tuberculosis continues to kill some 3 million people annually.

✓ 2 billion people worldwide are infected with *M. tuberculosis*.

Thus, the protective efficacy of the BCG vaccine remains doubtful!

Any vaccine that require minimum antigen processing will work in TB-endemic population

Peptides can overcome the problem of antigen processing.

Since they can directly bind to MHC molecules and require minimum antigen processing.

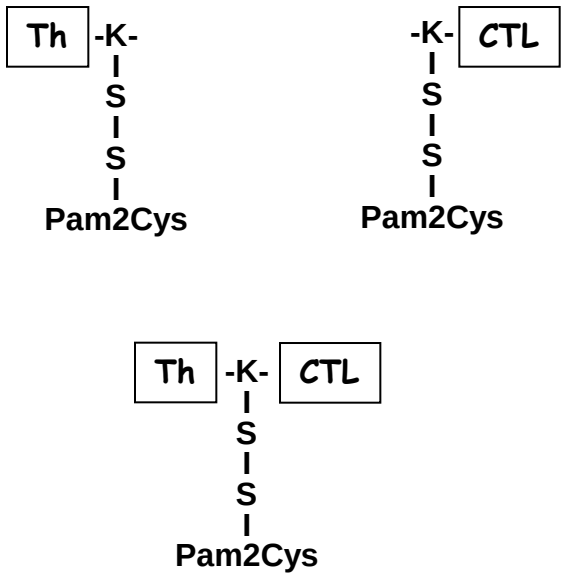
Can peptide based vaccine be successful choice in TB-endemic regions?

PEPTIDE VACCINES

- ✓ Comprise of synthetic peptides that represent immunodominant epitopes
- ✓ T cells and B cells epitope can be precisely selected
- ✓ Epitopes are accurately defined; can avoid autoreactive portions in the antigen
- ✓ Requires no or minimum processing

- X Fails to elicit immune response in genetically diverse human population
- X Requires adjuvant

Th epitope 1: LFAAFPSFAGLRPT FDTRLM
Th epitope 2: SEFAYGSFVRTVSLPVGADE



Schematic representation of the epitope-based vaccine candidate. The vaccine will contain Th and CTL epitopes. In all cases the T-helper (Th) epitope occupies N-terminal position and is separated from the cytotoxic T cell epitope (CTL) epitope by a single lysine (K) residue. Where lipid is attached, this is done through ε-amino group of the lysine residue such that the self-adjuvant lipid, linked through two serine residues (S), forms a branch between the Th and CTL epitope

Gowthaman & Agrewala. Trends Mol Medicine 2012

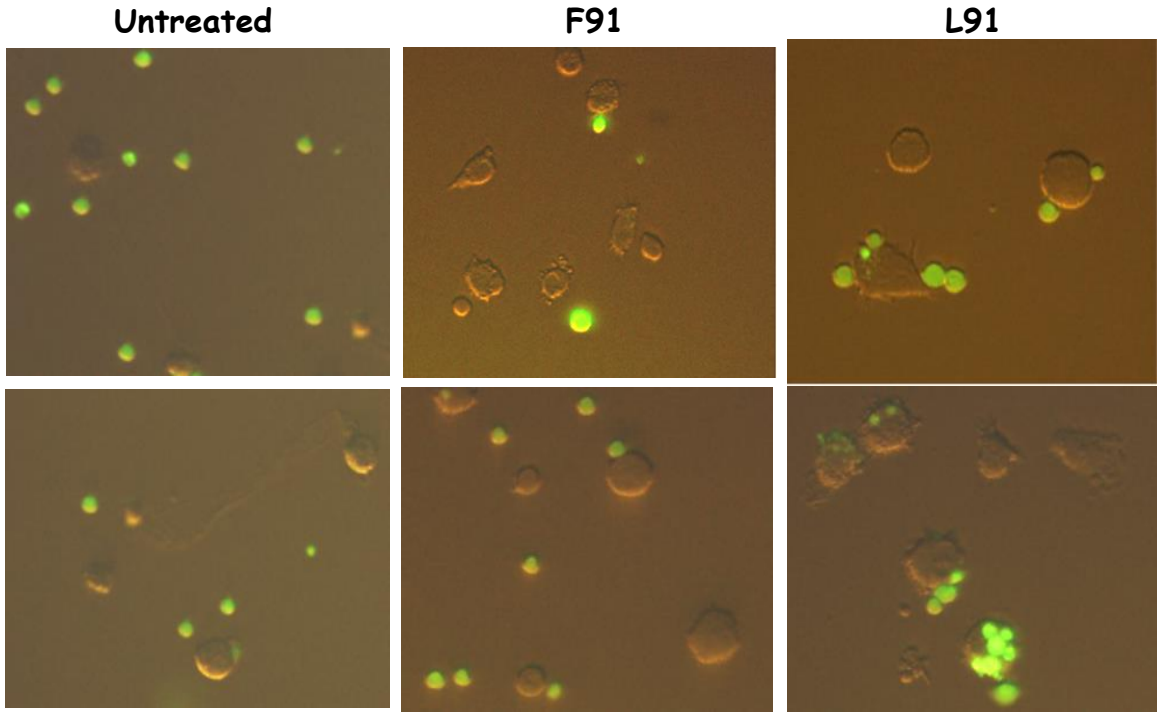
Why peptide vaccine may be better than BCG?

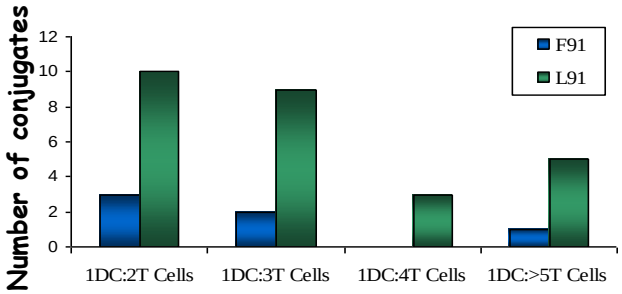
- Can overcome problem of antigen processing due to EM/Mtb/BCG, since no processing is required.
- No fear of preformed immune response generated due to exposure to BCG/EM/Mtb.
- Selective epitopes, can avoid autoreactive/suppressive portions in the antigen.

Whether the construct influences the maturation of DC?

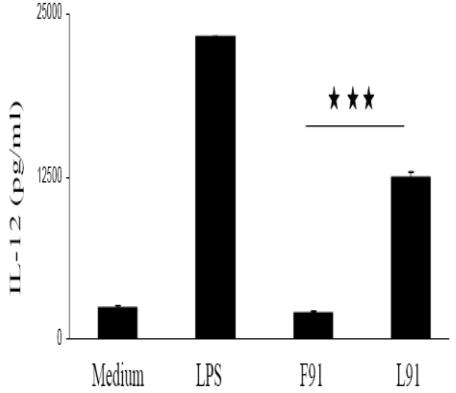
F91: free peptide

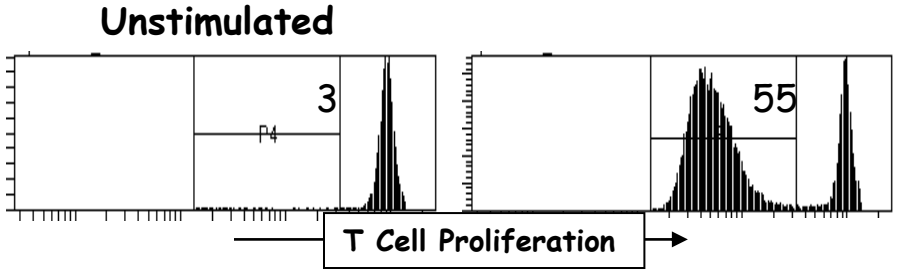
L91: lipidated peptide



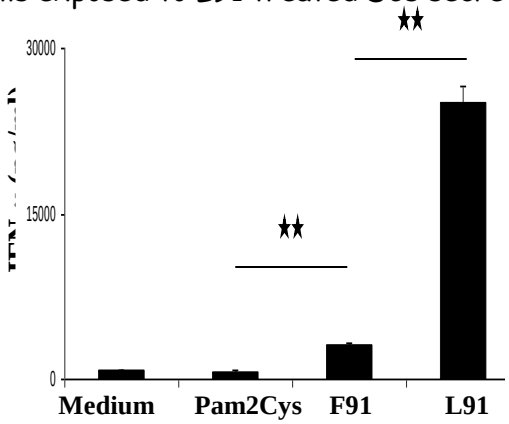


L91 augments IL-12 production by DCs



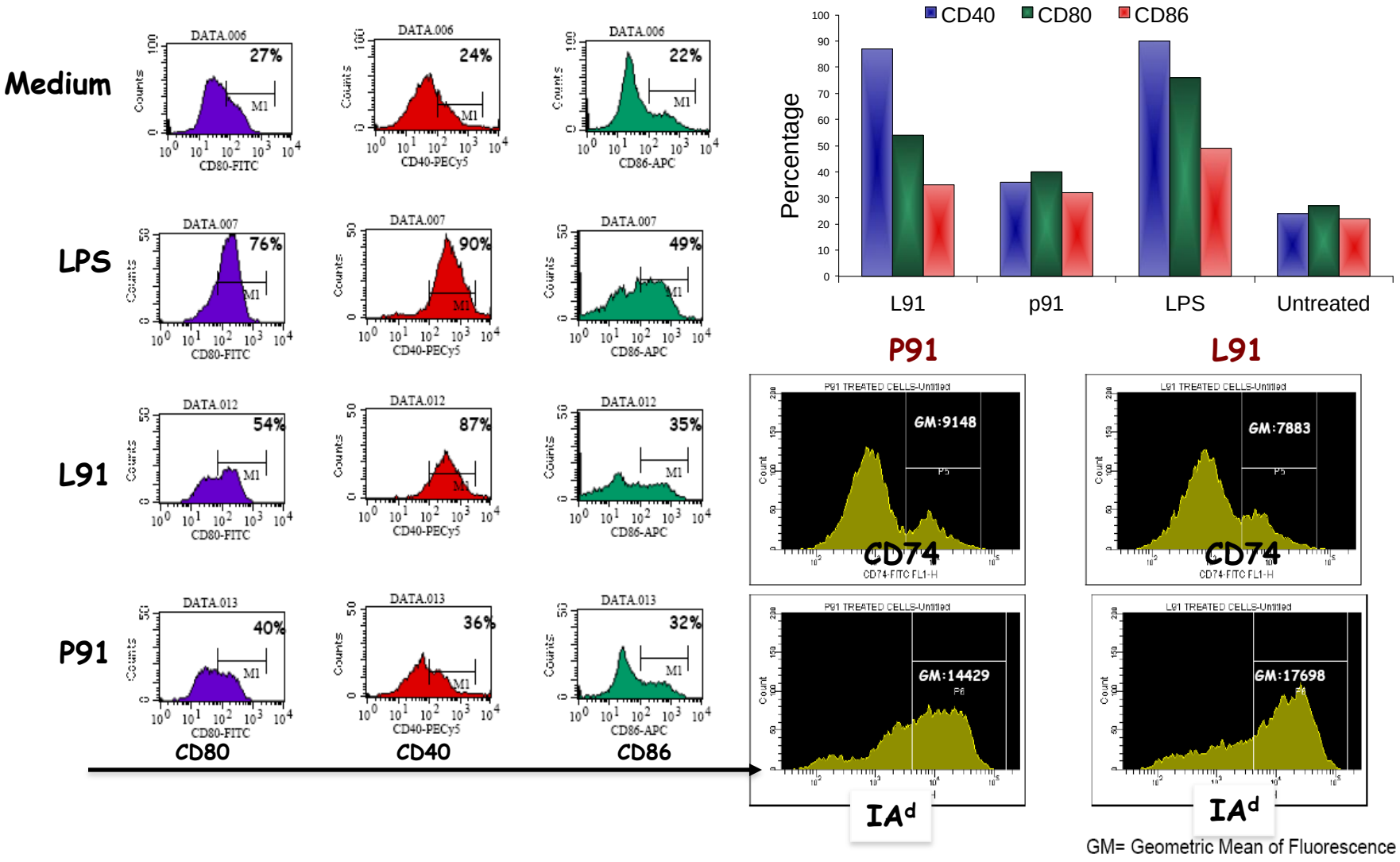


T cells exposed to L91 treated DCs secreted more IFN-γ



Gowthaman & Agrewala. J Infect Dis. 2011

L91 Enhances Activation and Maturation of DCs



Whether the peptides works across MHC barriers?

Immunization
L21, P21, L91, P91



[BALB/c, C57BL/6, C3He, FVB]

Booster 7-14d

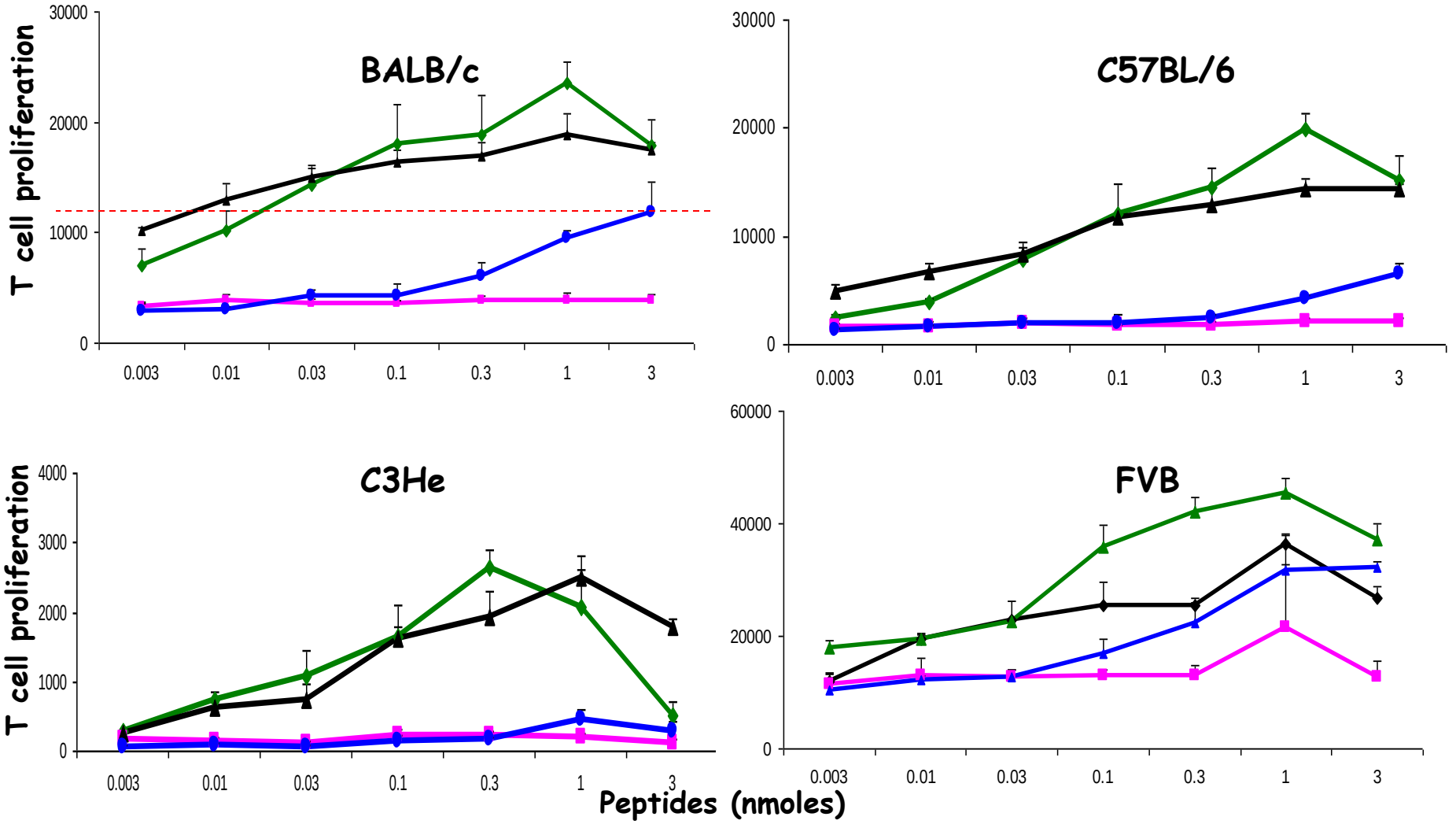
Sacrificed 240 days

In vitro challenge with peptides

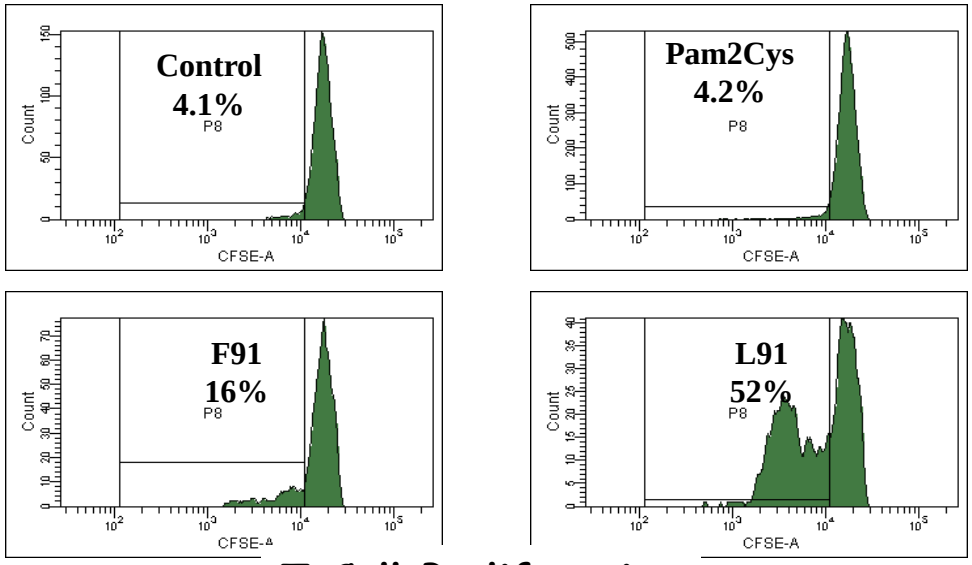
T cell response

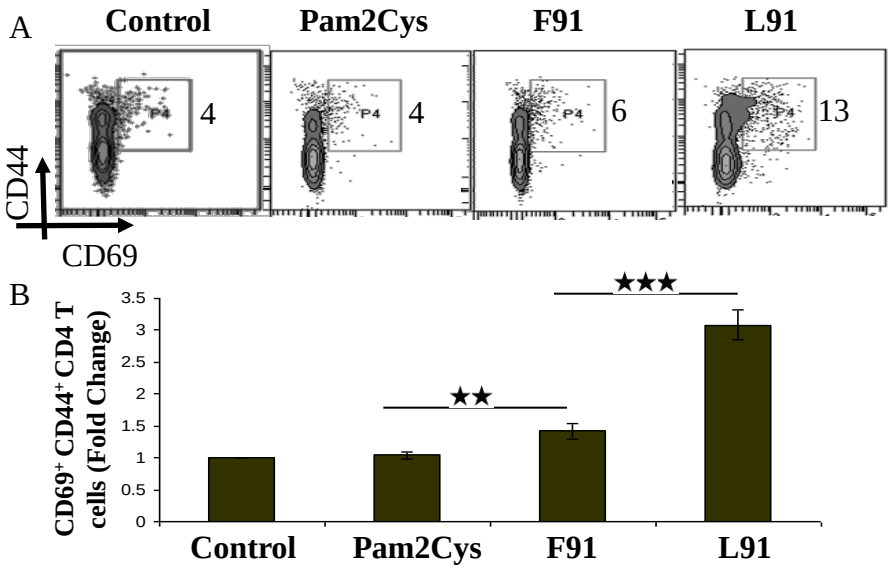
Free peptides were emulsified in IFA.

Peptide works across MHC barriers



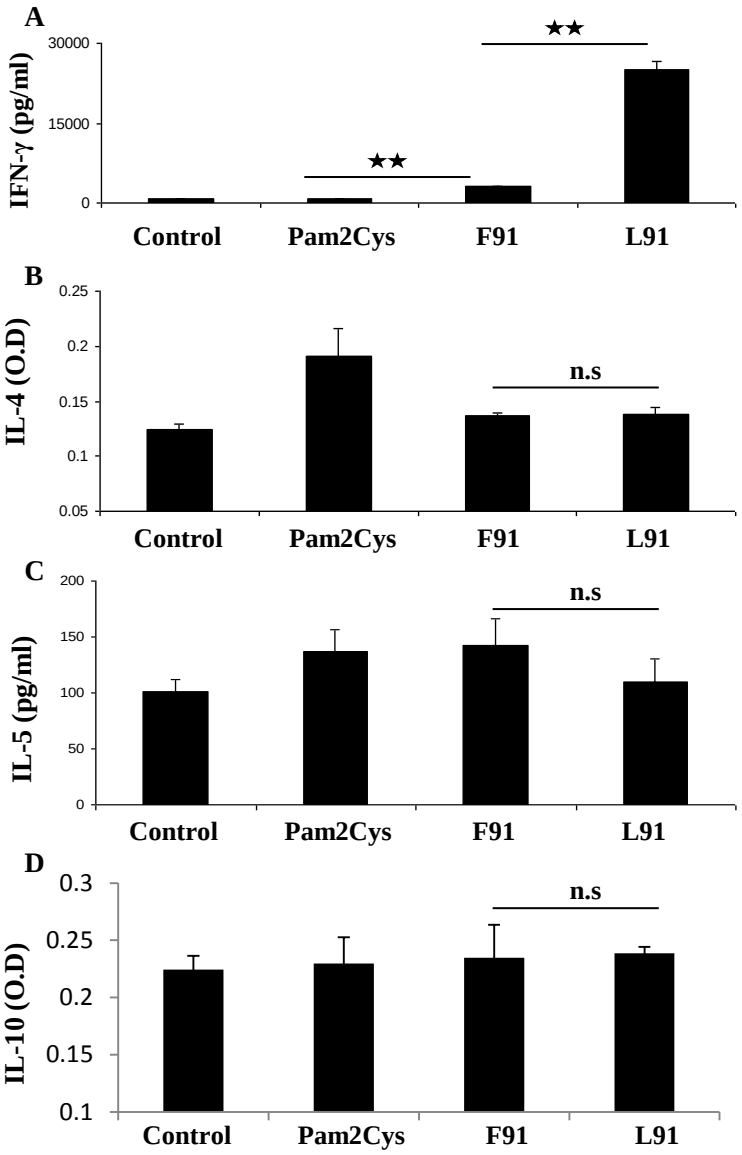
L91 induces proliferation and activation of CD4 T cells

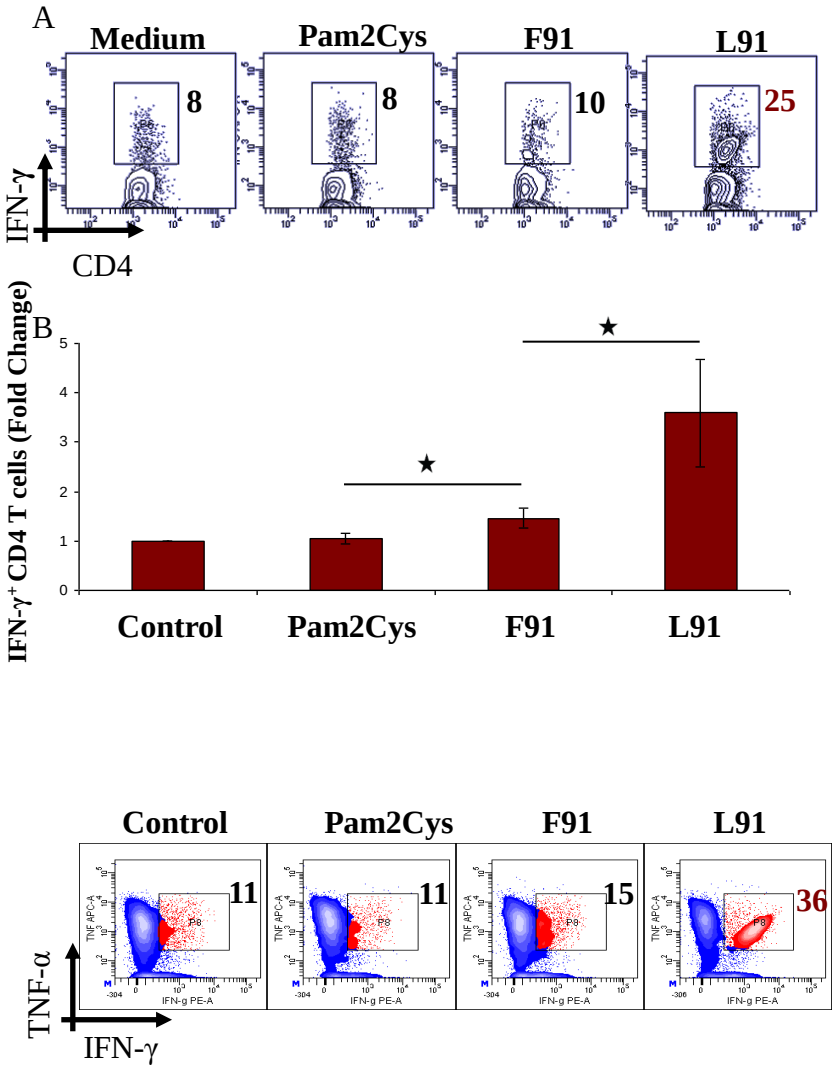




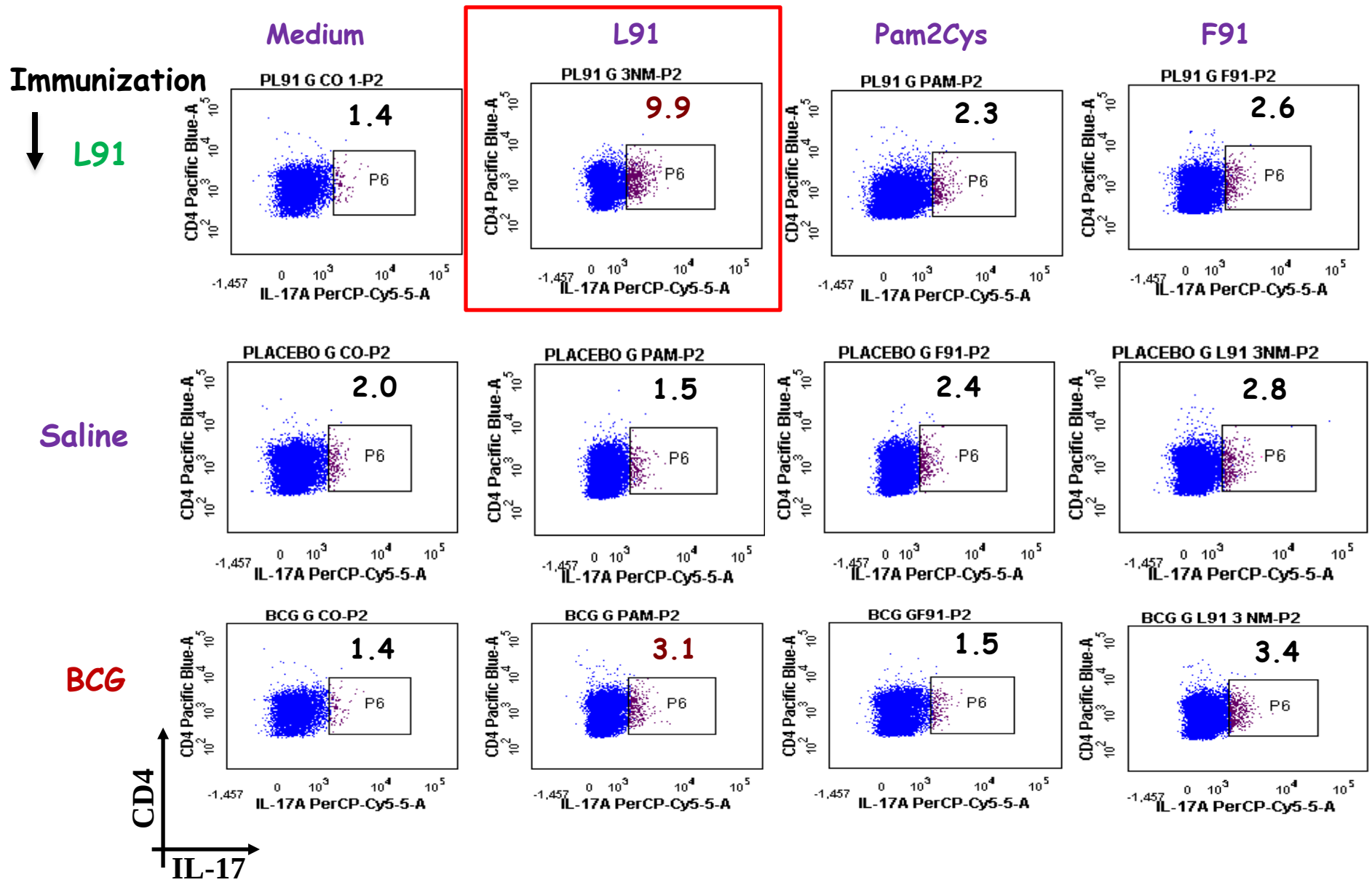
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Immunization with L91 elicits mainly secretion of IFN-γ

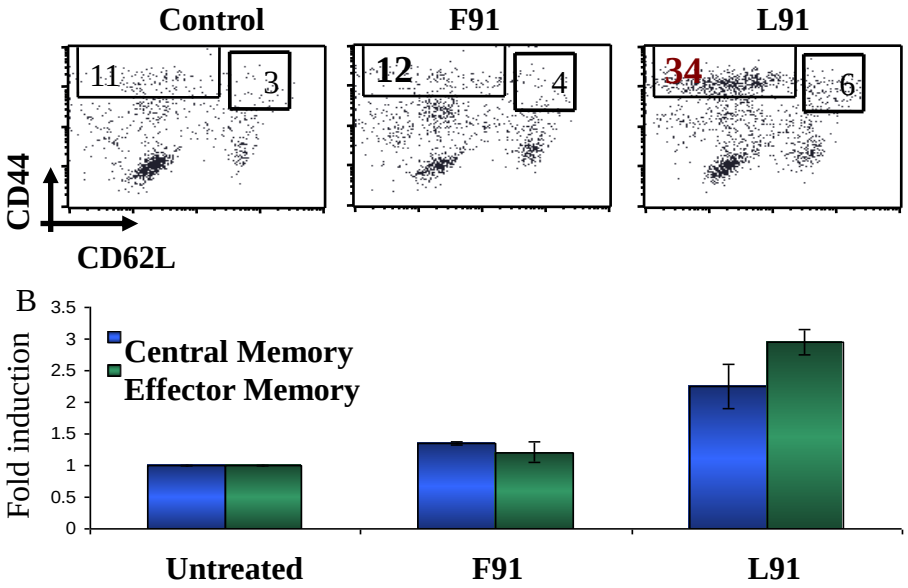


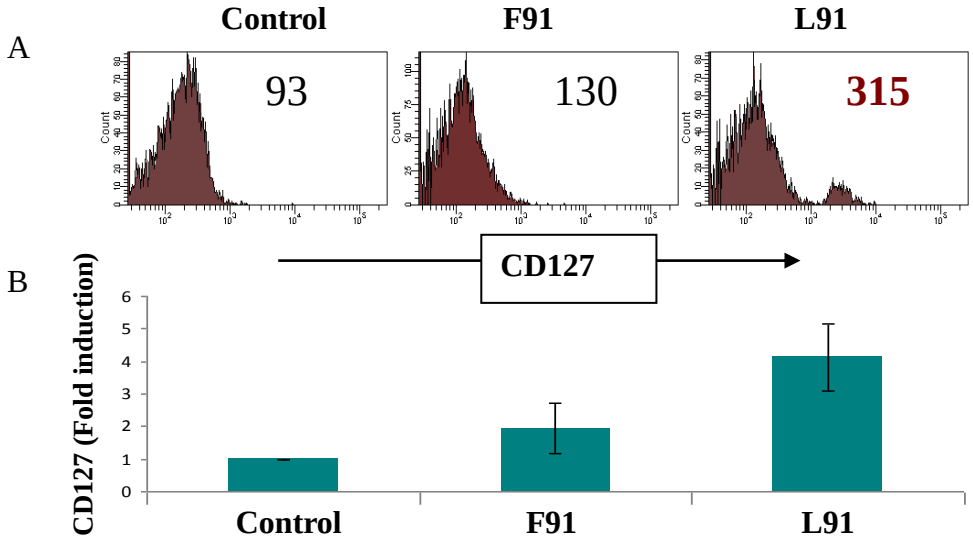


L91 immunization evokes IL-17 secretion



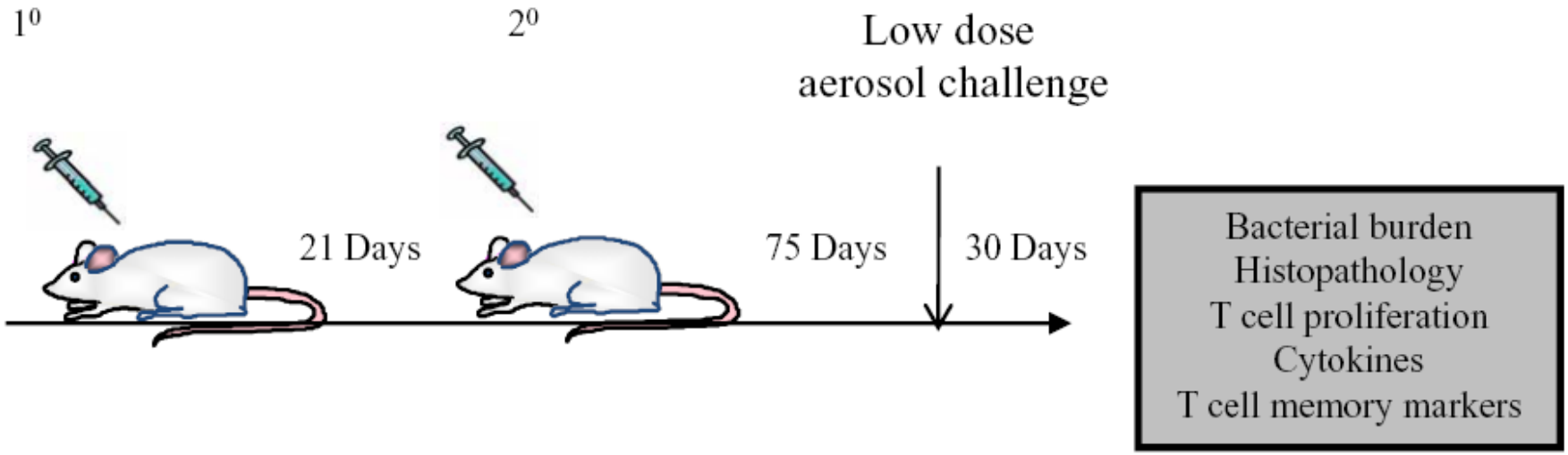
L91 immunization engenders T cell memory





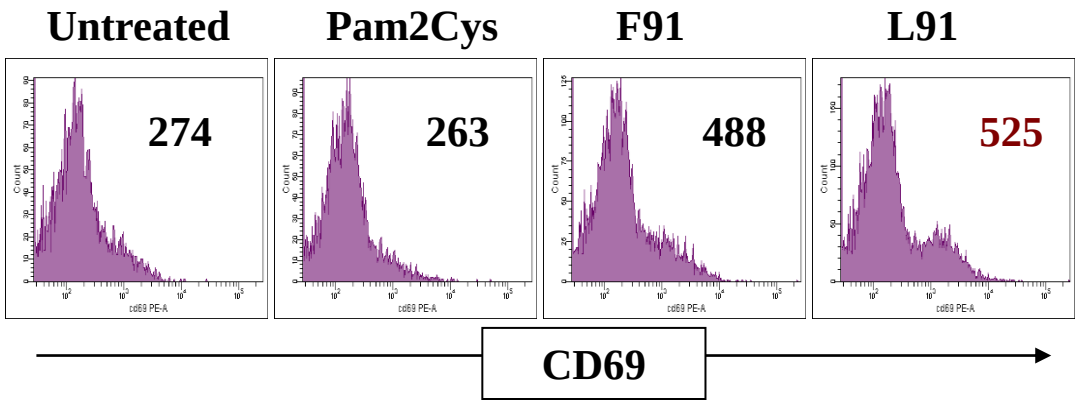
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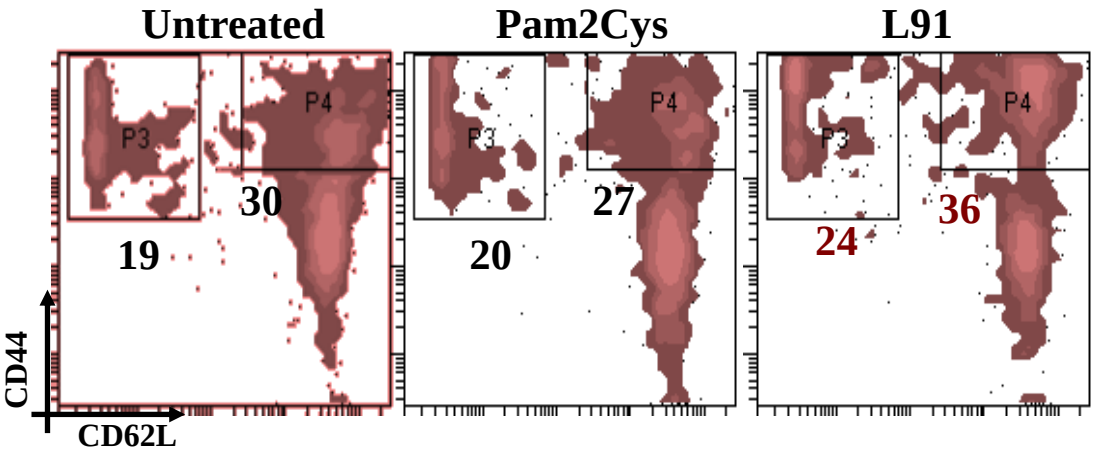
L91 Vaccination Protects Mice Against *Mtb*



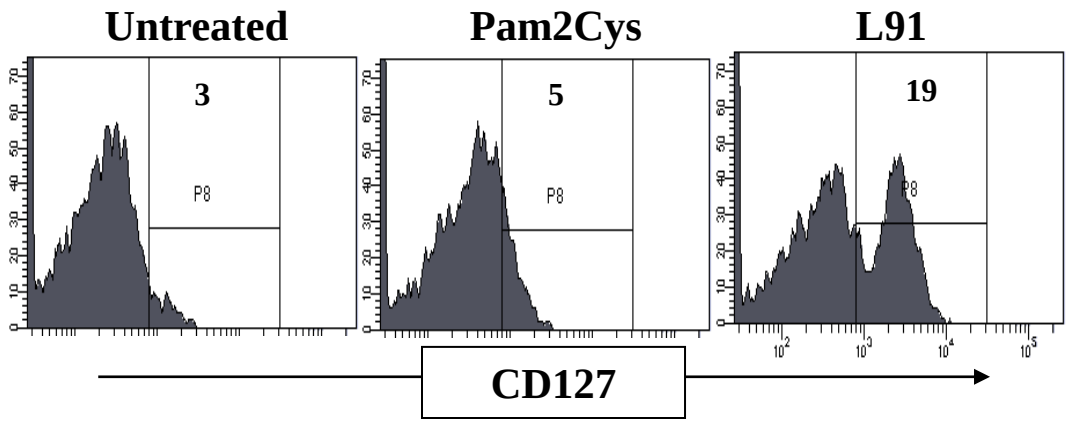
Experimental design for protection studies in mice. Different groups of BALB/c mice were immunized with L91 or controls (F91, LH, BCG and placebo). Peptides were given at a dose of 20 nmol (primary immunization) and 10 nmol (booster). BCG (10^6 CFU/animal) was given as control. Mice were rested for 75 days. Animals were challenged with a low dose aerosol of *M. tb* H37*Rv* (~100 CFU/mouse) 75 days post immunization. Thirty days after infection, animals were sacrificed and studied for immune response and pathology.

L91 evokes enduring memory CD4 T cell response in *Mtb* infected mice



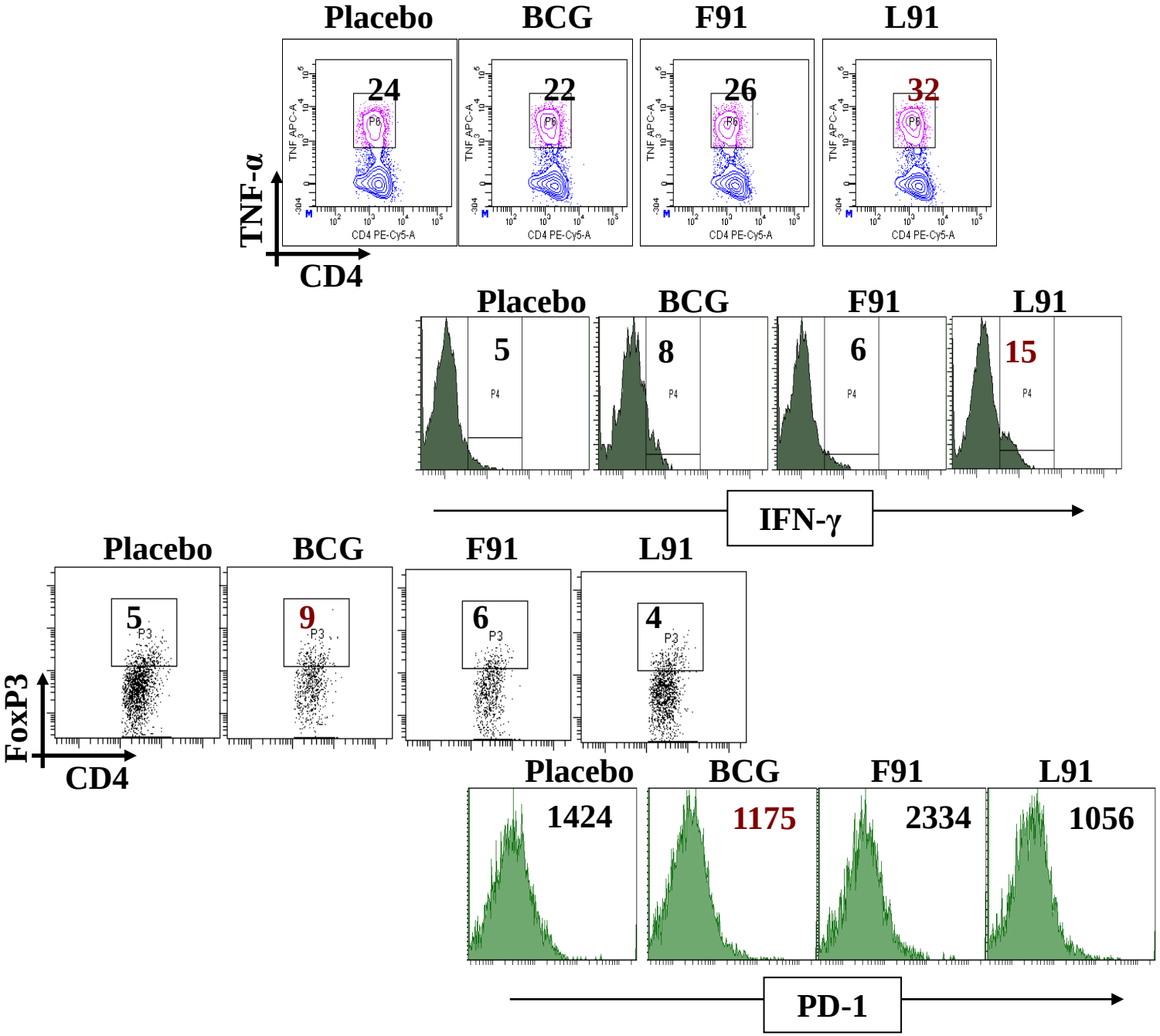


Central: $CD44^{hi}/CD6L^{hi}$
Effector: $CD44^{hi}/CD62L^{lo}$

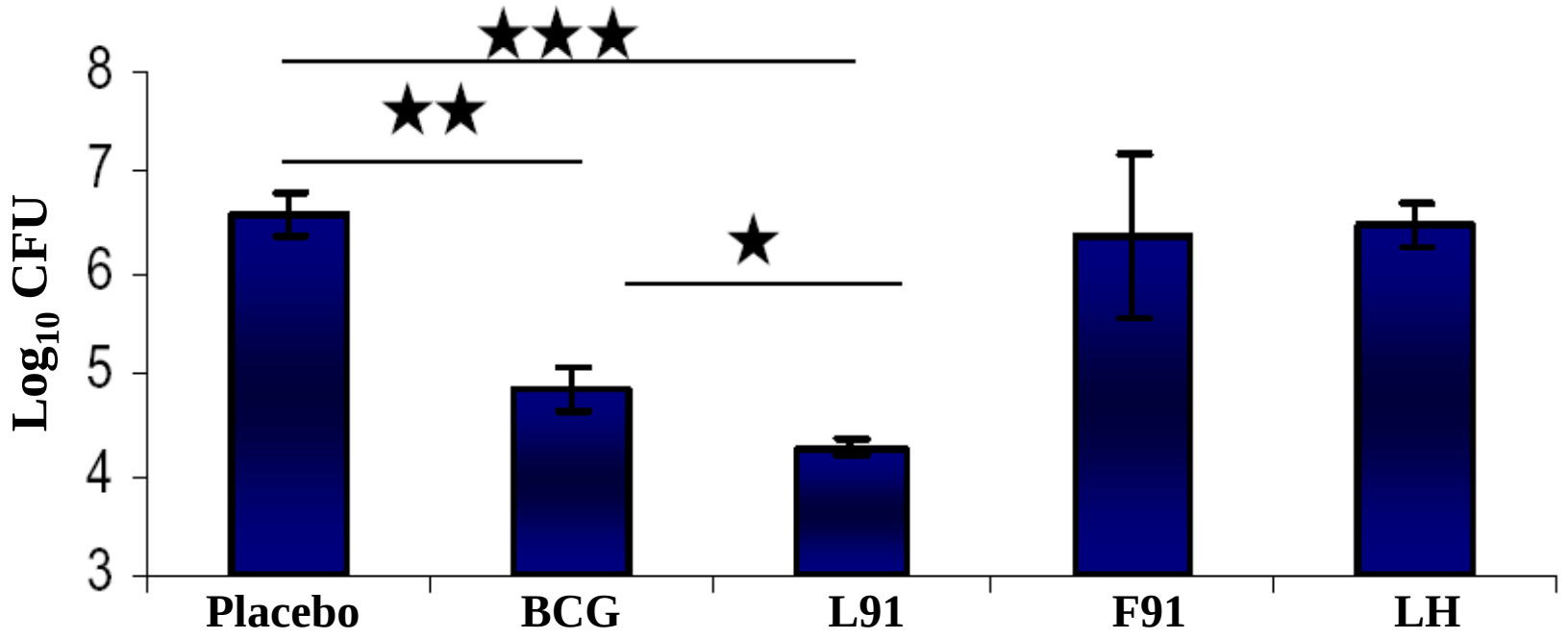


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L91 immunization induces better lung immunity than BCG

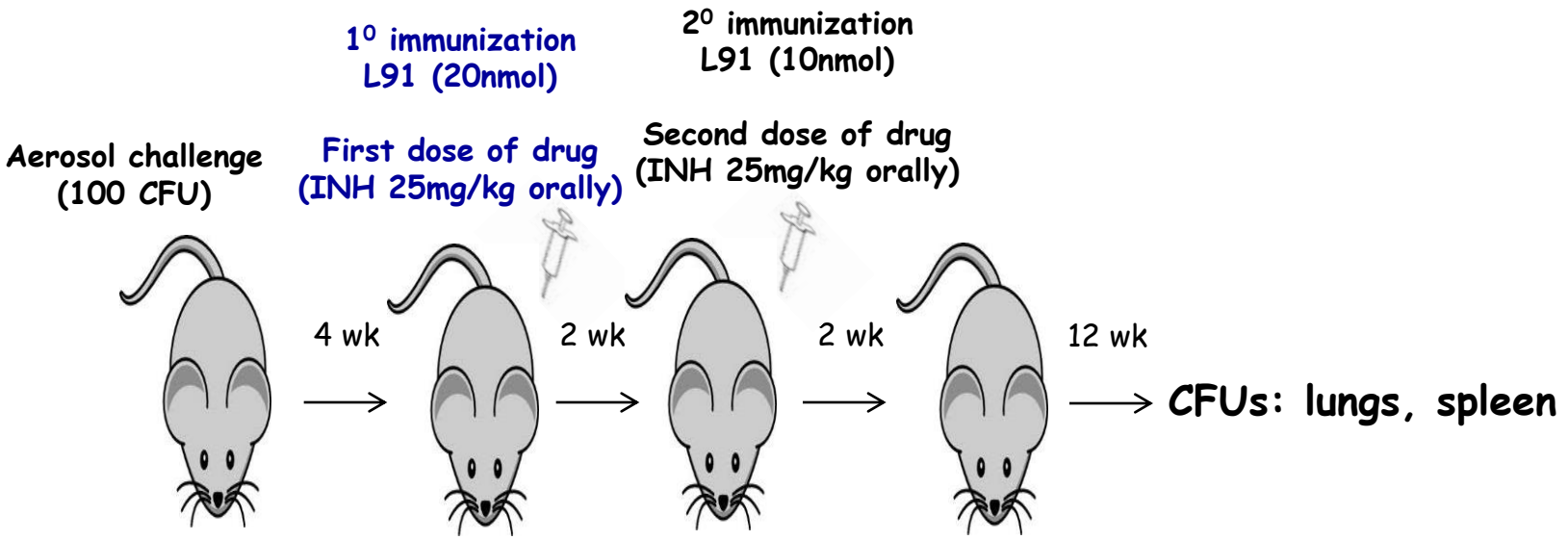


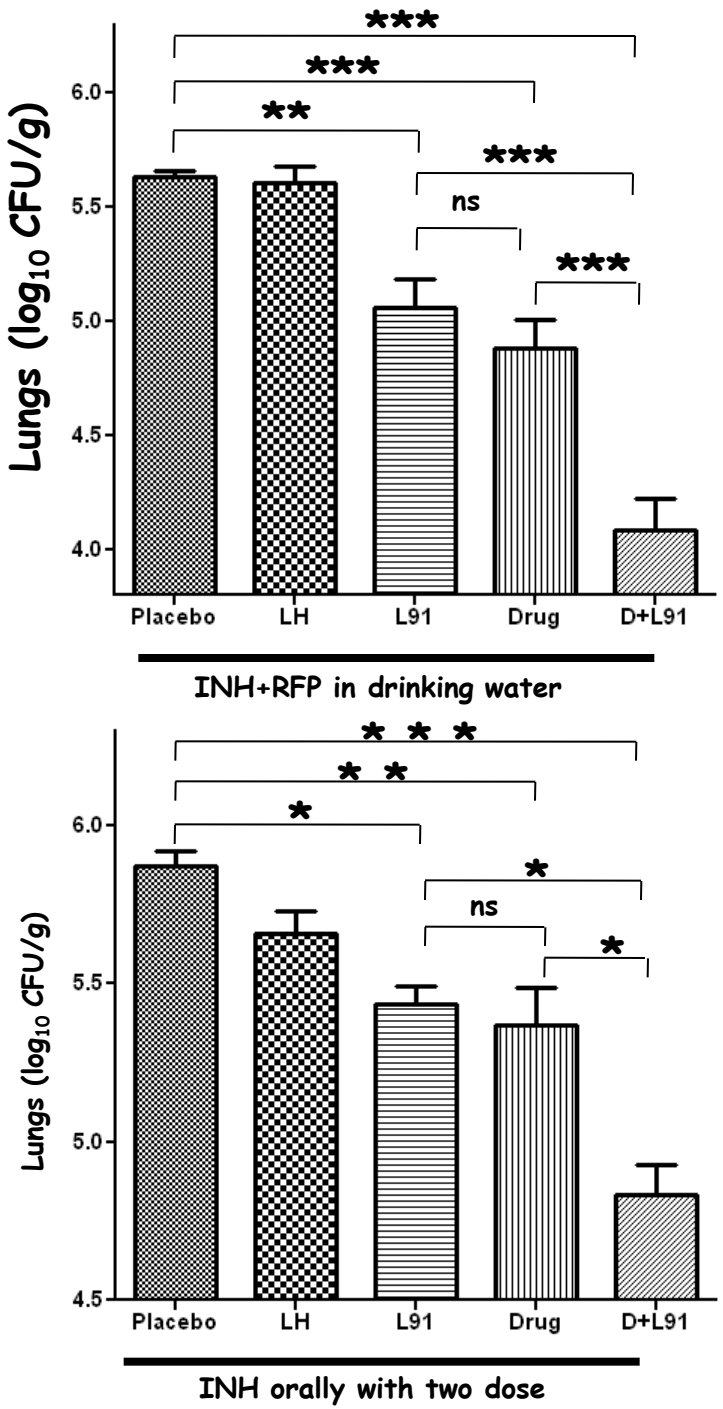
L91 immunization engenders better protection than BCG in mice

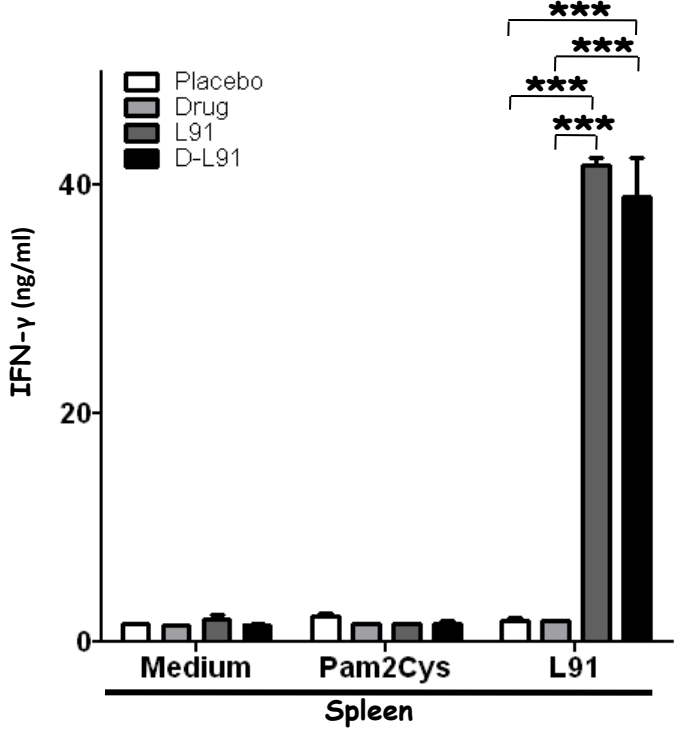


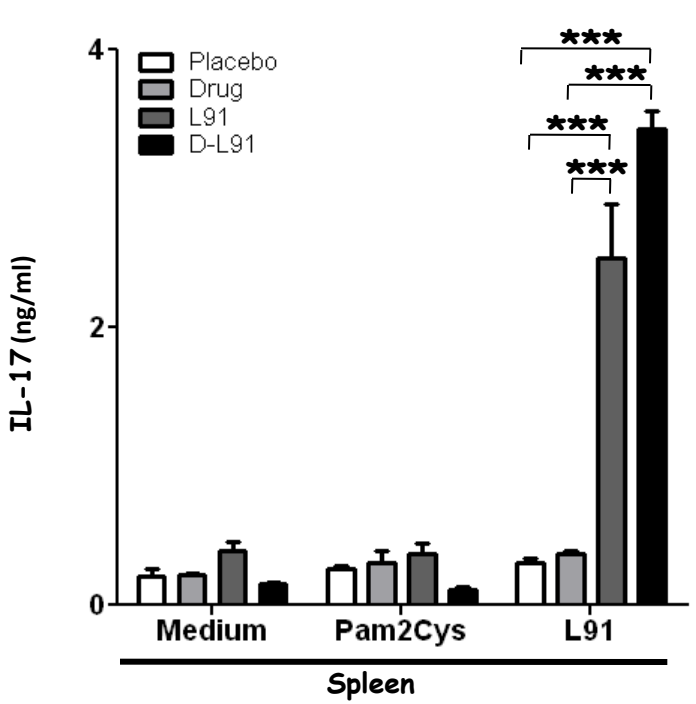
L91 immunization engenders better protection than BCG in mice. Mtb load in lungs was enumerated by CFU plating. Results are depicted as bar graphs with mean ± SD (log_{10} value). Data shown are from 3 independent experiments. '*' indicates $p<0.05$, '**' $p<0.01$, '***' $p<0.001$.

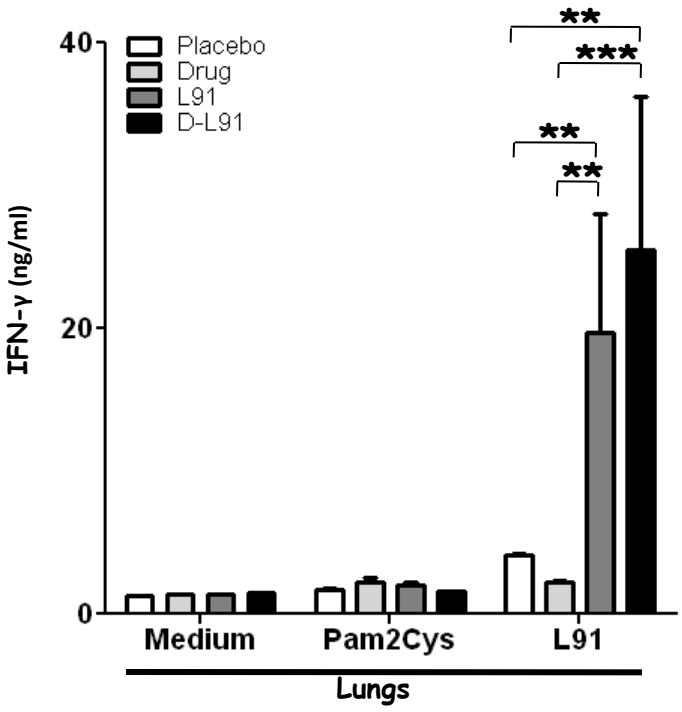
A novel therapeutic strategy to reinforce the potency of drugs to kill *Mycobacterium tuberculosis* by concurrently bolstering host immunity by promiscuous peptide conjugated to TLR-2 agonist

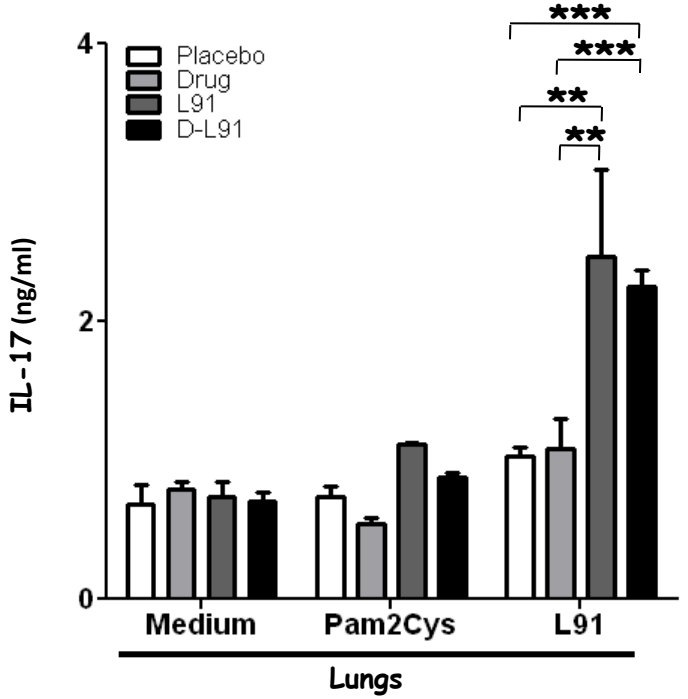


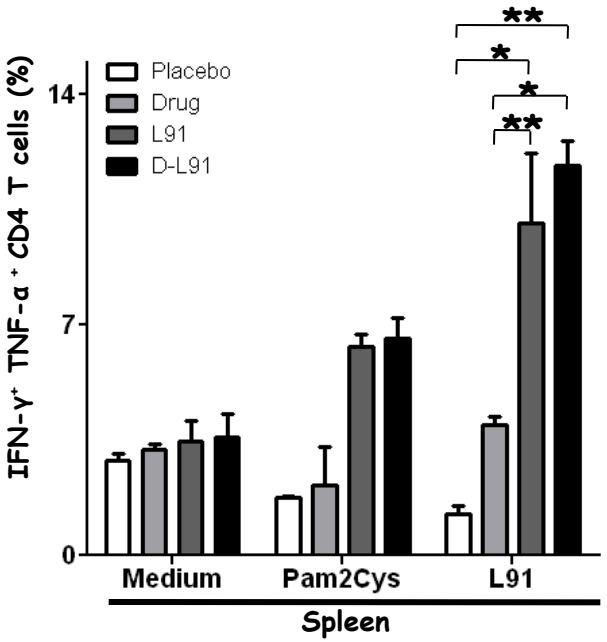

***
***
**
***
ns
***
Lungs (log10 CFU/g)
6.0
5.5
5.0
4.5
4.0
Placebo
LH
L91
Drug
D+L91
INH+RFP in drinking water
* * *
* *
*
*
ns
*
Lungs (log10 CFU/g)
6.0
5.5
5.0
4.5
Placebo
LH
L91
Drug
D+L91
INH orally with two dose

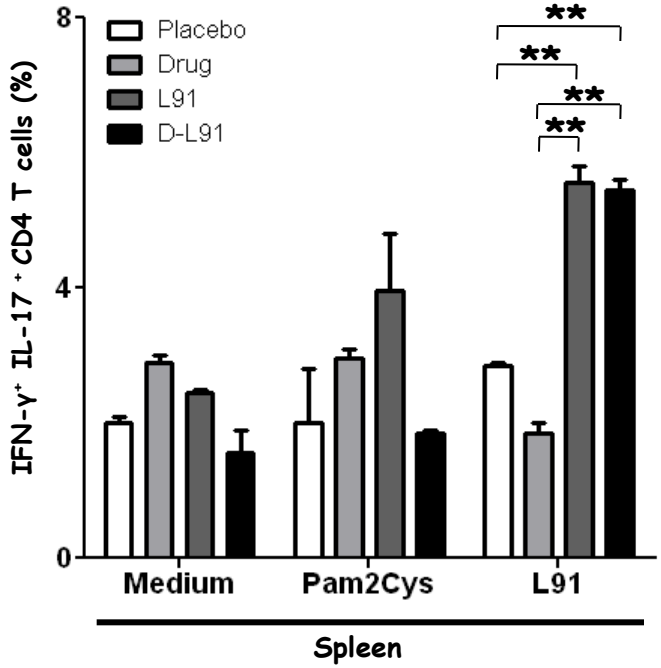

Placebo
Drug
L91
D-L91
IFN-γ (ng/ml)
0
20
40
***
***
***
***
Medium
Pam2Cys
L91
Spleen

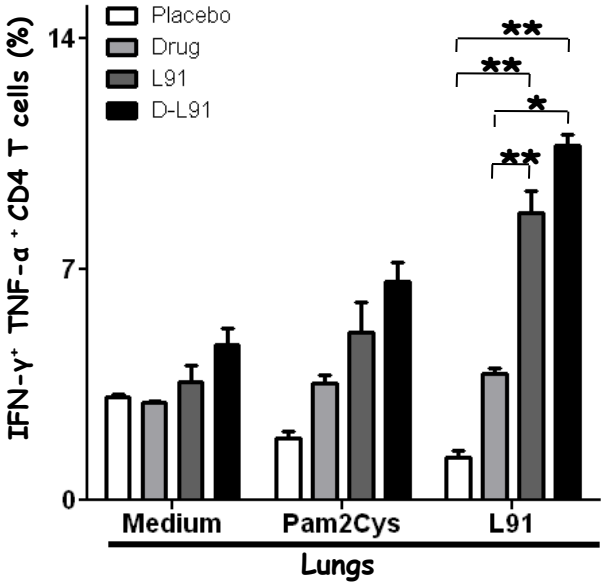

Placebo
Drug
L91
D-L91
IL-17 (ng/ml)
0
2
4
***
***
***
***
Medium
Pam2Cys
L91
Spleen

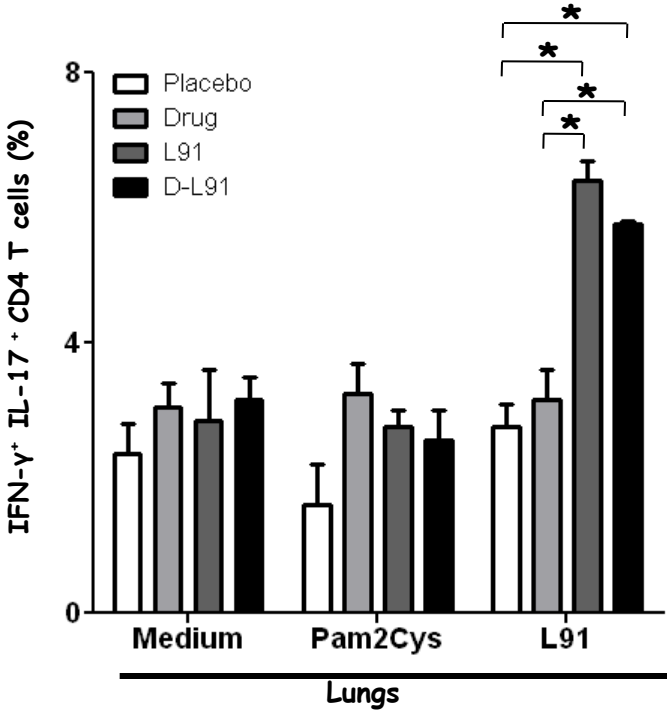

Placebo
Drug
L91
D-L91
IFN-γ (ng/ml)
0
20
40
**
***
**
**
Medium
Pam2Cys
L91
Lungs


Placebo
Drug
L91
D-L91
IL-17 (ng/ml)
0
2
4
***
***
**
**
Medium
Pam2Cys
L91
Lungs

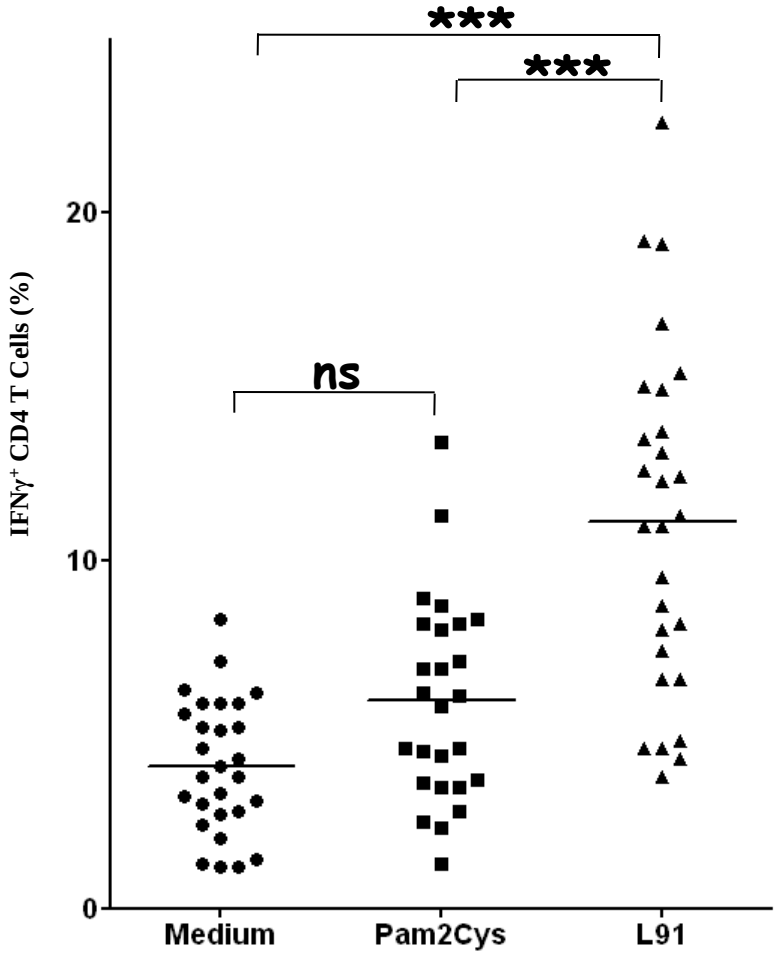

IFN-γ+ TNF-α+ CD4 T cells (%)
Placebo
Drug
L91
D-L91
14
7
0
Medium
Pam2Cys
L91
Spleen

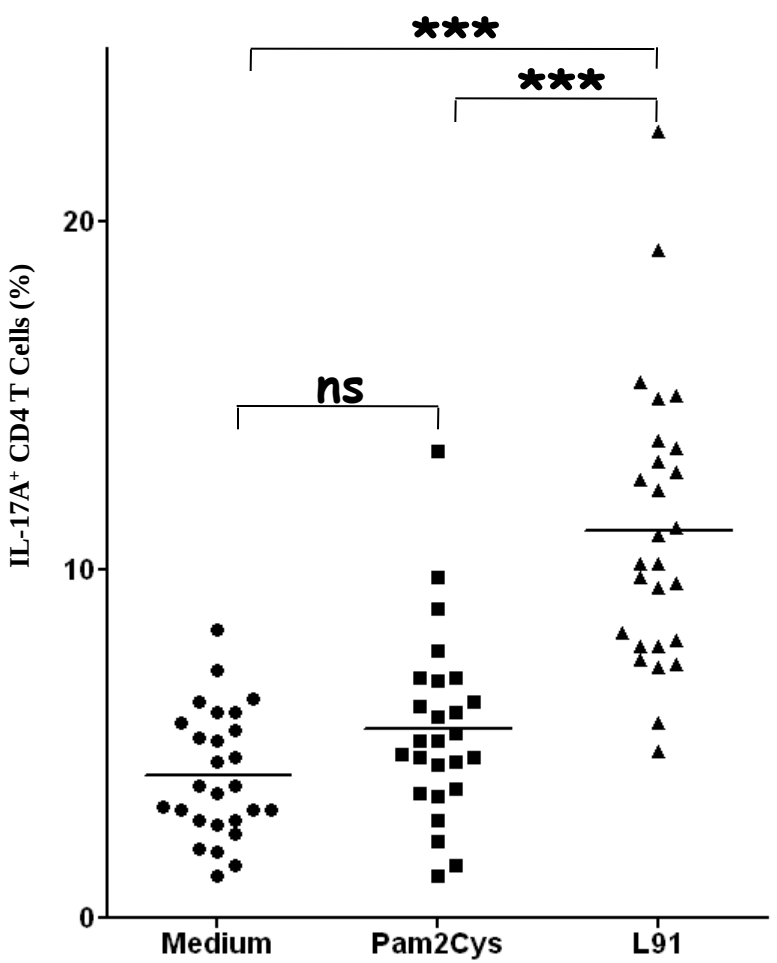

IFN-γ+ IL-17+ CD4 T cells (%)
Placebo
Drug
L91
D-L91
8
4
0
Medium
Pam2Cys
L91
Spleen

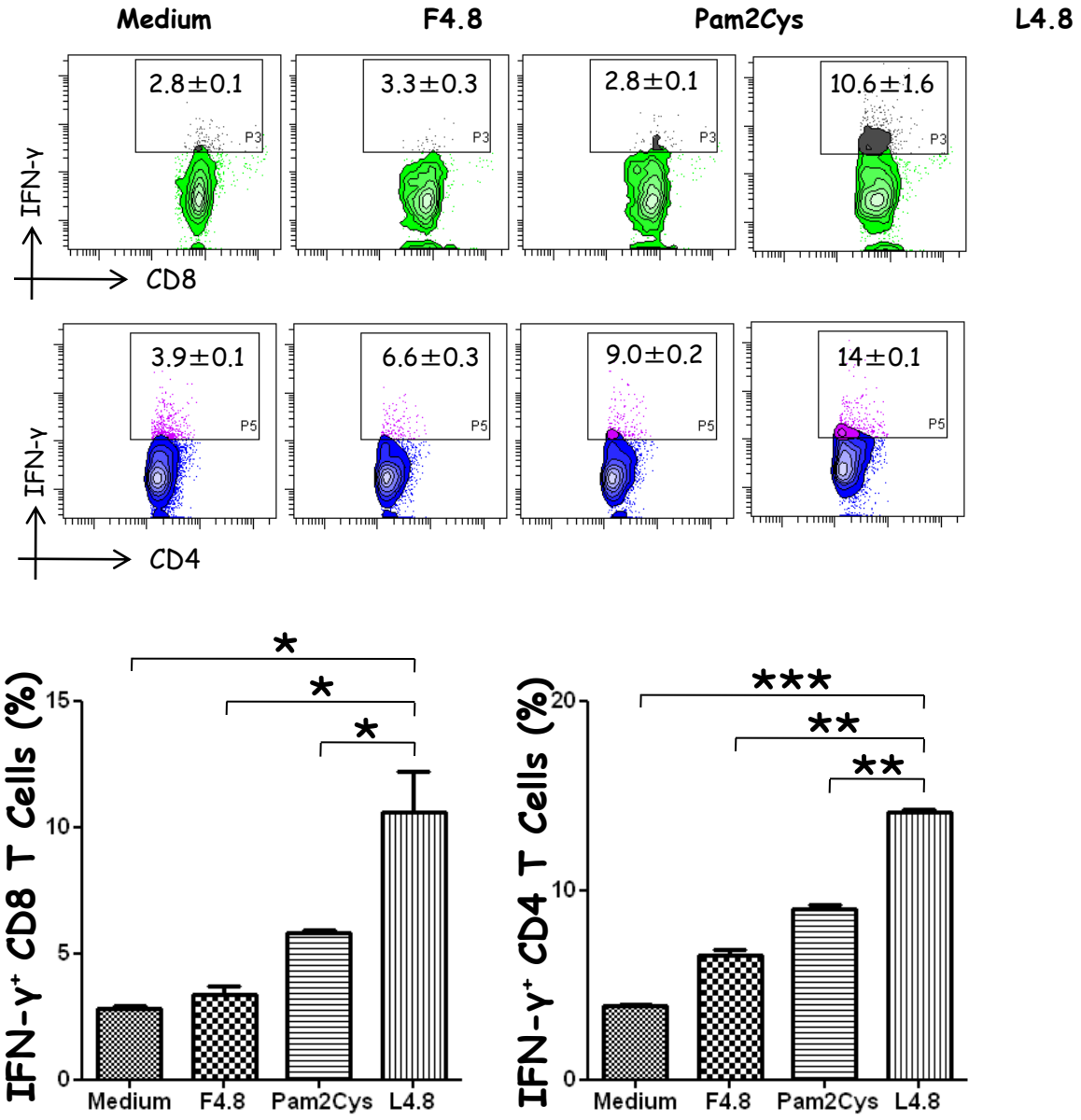

IFN-γ+ TNF-α+ CD4 T cells (%)
Placebo
Drug
L91
D-L91
14
7
0
Medium
Pam2Cys
L91
Lungs


IFN-γ+ IL-17+ CD4 T cells (%)
Placebo
Drug
L91
D-L91
8
4
0
Medium
Pam2Cys
L91
Lungs

PBMCs of TB patients on drug therapy on *in vitro* stimulation with L91 induces secretion of IFN-γ



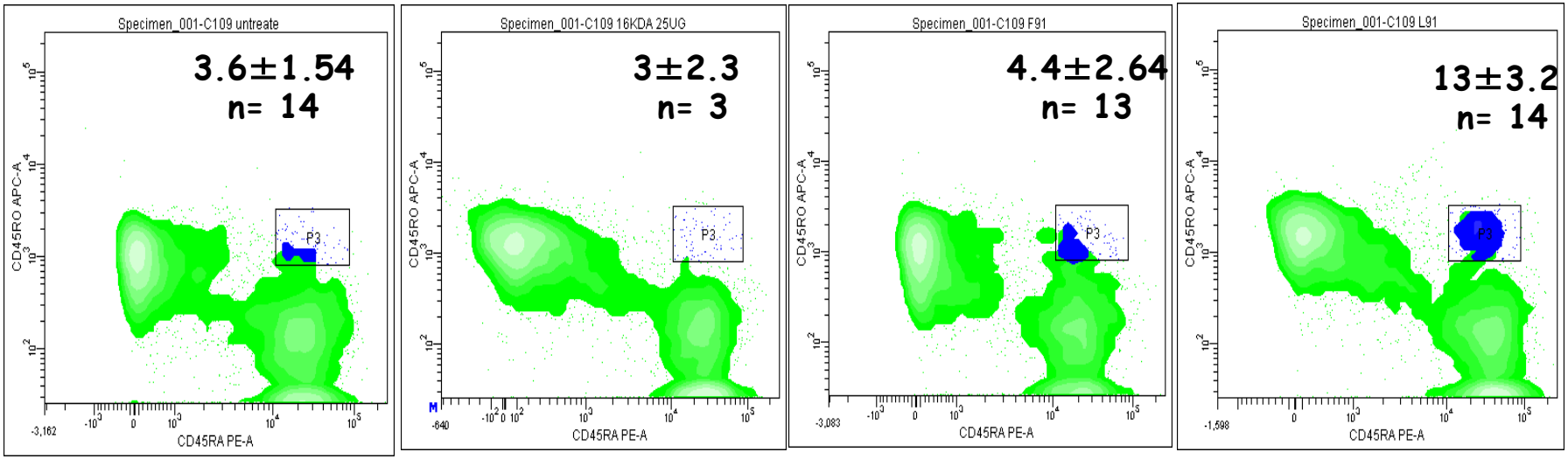



Medium
F4.8
Pam2Cys
L4.8
2.8±0.1
3.3±0.3
2.8±0.1
10.6±1.6
P3
IFN-γ
CD8
3.9±0.1
6.6±0.3
9.0±0.2
14±0.1
P5
IFN-γ
CD4
IFN-γ+ CD8 T Cells (%)
15
10
5
0
*
*
*
Medium
F4.8
Pam2Cys
L4.8
IFN-γ+ CD4 T Cells (%)
20
10
0
***
**
**
Medium
F4.8
Pam2Cys
L4.8

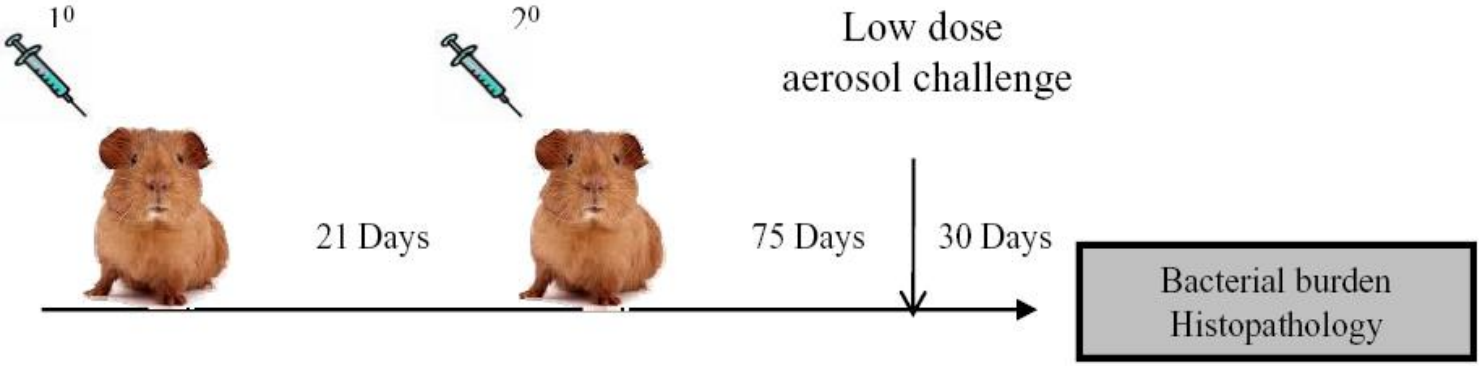
L91 expands central memory pool of CD4 T cells of TB patients

IMTECH

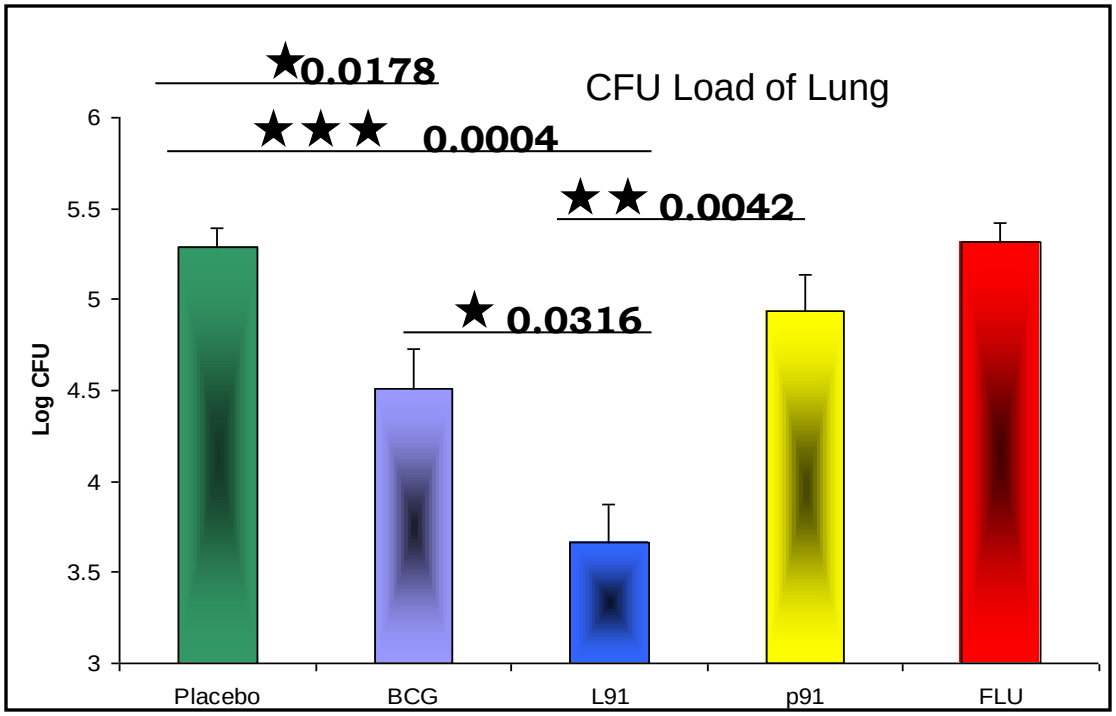
Untreated **16 kDa** **F91** **L91**



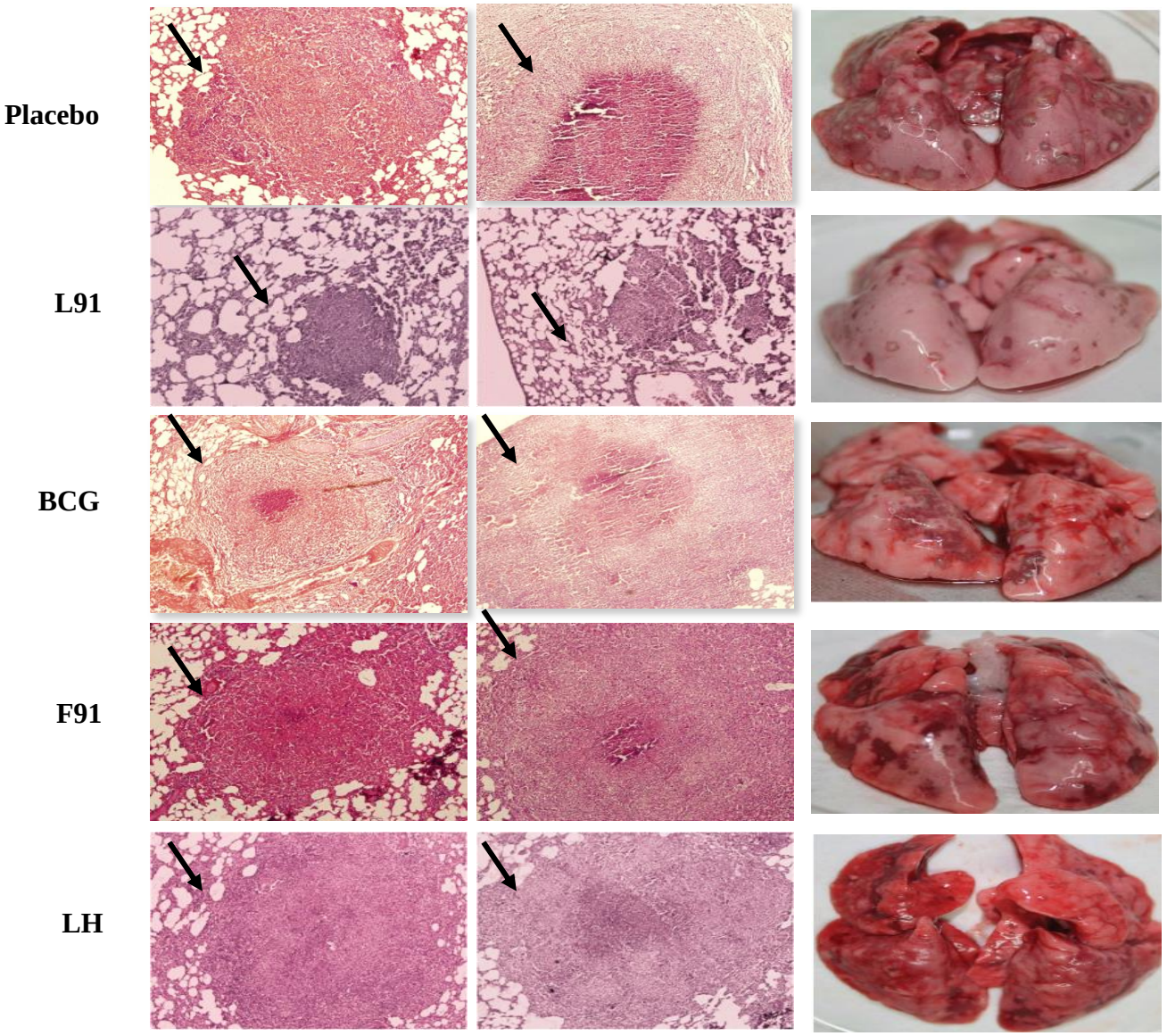
L91 Protects Guinea Pigs from *Mtb* better than BCG



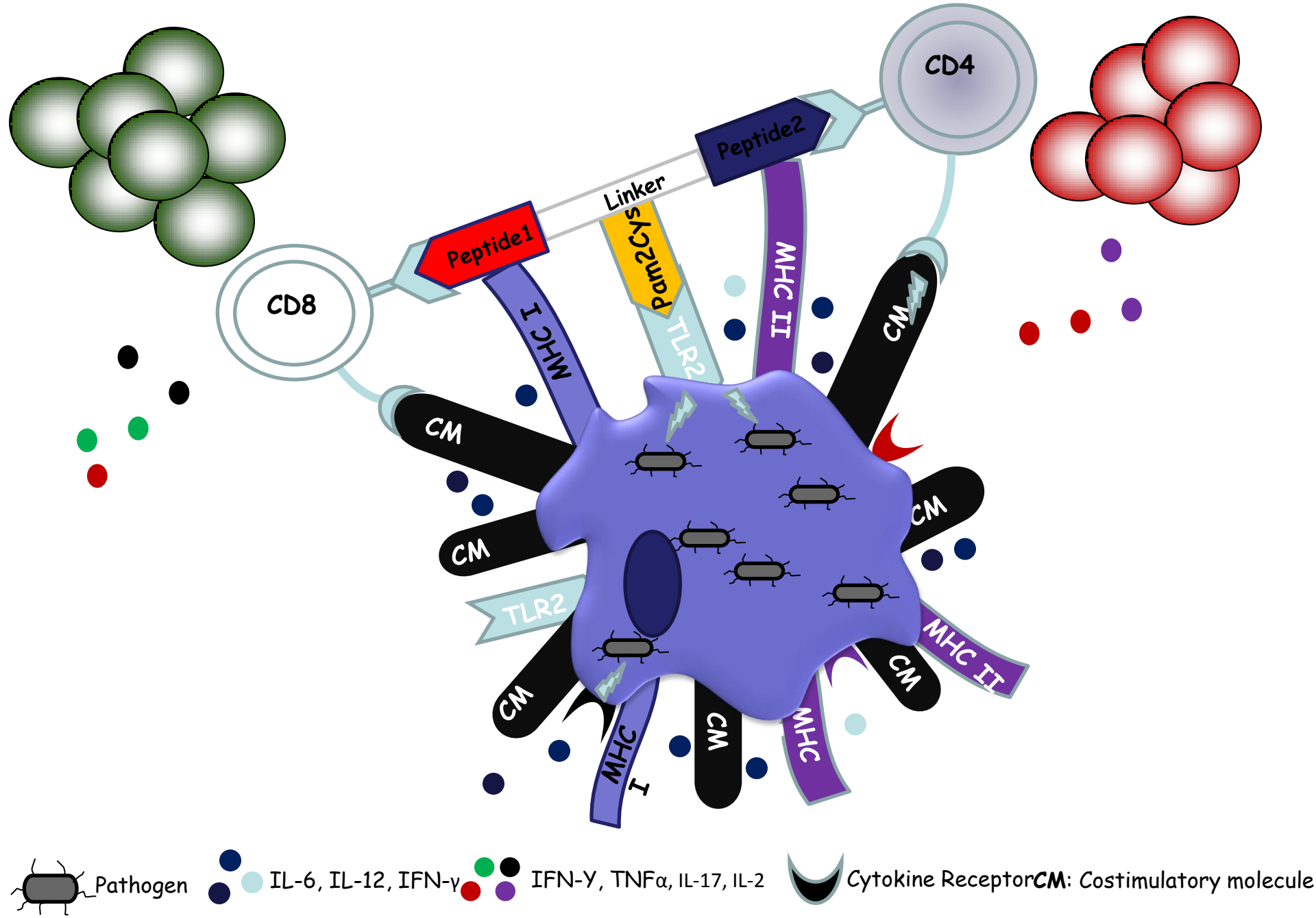
Experimental design for the protection studies in Guinea pigs. Different groups of Duncan-Hartley Guinea pigs were immunized with L91 or controls (F91, LH, BCG and placebo). Peptides were given at a dose of 100 nmol (primary immunization) and 50 nmol (booster). BCG (10^6 CFU/ animal) was given as a control. Animals were rested for 75 days and challenged with a low dose aerosol of *M. tb* H37*Rv* (~30 CFU/animal), 75 days post immunization. Thirty days after infection, animals were sacrificed and studied for bacterial burden and pathology.



L91 immunized exhibit constrained pathology upon *Mtb* challenge in Guinea pigs



L91 immunized Guinea pigs exhibit constrained pathology upon M. tb challenge in Guinea pigs. The protection studies were performed as described in Fig. 3. Representative photomicrographs of formalin fixed H & E stained histopathology lung sections of Guinea pigs (left and center panels). Arrows indicate regions of granulomas or necrosis. Right panel shows photographs of gross pathology in lungs of immunized and infected animals. Data are representative of 2 comparable experiments with a minimum of 5 animals per group.



Gowthaman & Agrewala. Trends Mol Medicine 2012

CONCLUSION

Lipidated peptide vaccine may be a future prophylactic strategy to eradicate TB from TB-endemic zones

Juraj Ivanyi, TB & RI Unit, Hammersmith Hospital, London

David Jackson, University of Melbourne, Australia

Pushpa Gupta and UD Gupta, JALMA, India

AK Janmeja, Government Medical College and Hospital, India

IMTECH, CSIR and DBT for Financial Support


THANKS

Fails to elicit immune response in genetically diverse human population!

Answer: Promiscuous Peptides?

Promiscuous Peptide Selection

We generated 141 over lapping peptides from:

16kDa, 19kDa, 30kDa, 38kDa, 65kDa, CFP-10, ESAT-6

All the individuals generated robust T cell proliferation and predominant secretion of IFN-γ by PBMCs of the PPD⁺ healthy individuals against 16 kDa (Acr1) antigen of *Mtb*.

1 20

MATTLPVQRHPRSLFPEFSELFAAFPSFAGLRPTFDTRLMRLEDEMKEGRYEVRA

ELPGVDPDKDVDIMVRDGQLTIKAERTEQKDFDGRSEFAYGSFVRTVSLPVGADE

DDIKATYDKGILTVSVAVSEGKPTEKHIQI RSTN (141 AAs)

Identification of T-Helper Cell Activating Promiscuous Peptides

Buffy coat of 20 PPD^+ healthy volunteers with no history of TB were selected and tested using promiscuous peptides for:

(i) Peptides binding to HLA-class II molecules

(ii) T cell proliferation

(iii) Secretion of IFN-γ

Peptides of sequence 21-40 and 91-110 showed permissive binding

HLA DRB1	Binding Affinity p21-40	Binding Affinity p91-110
0101	+++	++++
0103	++	++++
0301	+	++
0401	+++	++++
0701	++	++++
1101	+++	++++
1301	++	+++
1501	++++	++++
1601	+++	++++

The HLA type of donors was performed by low resolution PCR with sequence-specific primers (PCR-SSP). All presenting HLA-DR-homozygous L-BCL were chosen to conform at least to the donor's major serological, and usually to molecular, type. The affinity of the presenting MHC class II molecule for peptides p21-40 and p91-110 was determined by a competitive MHC binding assay. ++++ indicates high-affinity binding (IC50<10μM); +++ intermediate affinity (10 μM<IC_{50}<100μM); and ++ indicates low affinity (100–1000μM).

Agrewala et al. 1997, 1998, 1999

Peptides of sequence 21-40 (p21-40) and 91-110 (p91-110) showed T cell proliferation in all the tested individuals.

p91-110 induces mainly secretion of IFN-γ.

Th1 clones (IFN-γ): 90%
Th0 clones (IFN-γ +IL-4): 10%

p21-40 promotes production of IFN-γ and IL-4.

Th0 clones (IFN-γ + IL-4): 75%
Th2 clones (IL-4): 25%

p21-40: LFAAFPSFAGLRPTFDTRLM
p91-110: SEFAYGSFVRTVSLPVGADE

Agrewala et al. 1997, 1998, 1999

Peptides are weak immunogens!

Can conjugation with Pam2Cys increase the immunogenecity of peptides?

Why Pam2Cys?

S-[2,3-is(palmitoyloxy)propyl]cysteine

Ligand for TLR2, induces signaling through it.

Has self-adjuvanting properties when conjugated with peptides.

Induces robust Th1 and Th17 type immune responses.

Long-term protective effect.

Potent immunogenicity can be explained by its ability to mature and activate dendritic cells (suggesting that vaccines containing this lipid moiety may interact directly with DCs to promote immune responses).

Jackson et al. 2004, Zhu et al. 2004, Düesberg et al. 2002, Zeng et al. 2002

Why TLR2?

Incorporating the Pam2Cys into peptide structures effectively triggers the TLR2 and secretion of IL-12 by DCs.

TLR2 is copiously present on DCs, monocytes, and lung epithelia.

Why DCs?

DCs are decisive for efficient immune response.

Only cells, capable of activating *naïve* T cells.

Skews immune response towards Th1 and Th17 cells.

Constitutive expression of MHCs and costimulatory molecules.

Advantage of Vaccine

Totally synthetic

No adjuvant is required

Can activate CD4 and CD8 T cells

Skews immune response to Th1 and Th17 type

Can activate naïve T cells