Supplementary Material/Methods

**Detection of anti-peptide antibody in VL patients employing ELISA**

The presence of *Leishmania* antigen specific antibody in various test samples was determined by sandwich ELISA method. For determining most optimum antigen concentration, titration was performed using increasing concentration of antigen (peptide). The sera as well as antibody was used employing serial dilutions of specific antibody. Antibody concentration was employed at 1: 50 to 1: 52,000 dilutions. The peptide antigen (P27, P23 or cocktail) was dispensed to each well at the concentration of 0.5, 1, and 1.5μg of the peptide (or its cocktail). Briefly, a known volume of peptide solution (100μl) corresponding to 0.5μg (peptides or their cocktail) in bicarbonate buffer (pH 9.6) was coated in each well of 96-well ELISA plate (Nunc). The peptide coated plate was incubated overnight at 4°C. After incubation, the plate was thoroughly washed with Tween-PBS (0.05 % Tween-20) and blocked for 2 h with 5% BSA. After stipulated incubation, the plate was washed three times with Tween-PBS. A known volume (100μl) of serum (1:100 dilution in PBS-T) belonging to healthy (endemic + non-endemic), VL-BT and other disease sera, incubated for 2 h at 37°C, followed by three washing with Tween-PBS. After extensive washing, the plate was extensively washed with Tween-PBS, followed by incubation with HRP-tagged anti-human secondary antibody (1:5000, in PBS-T) for 1 h. The plate was again washed with Tween –PBS to remove non-interacting secondary antibody. Next, 100μl of substrate (TMB) was dispensed to each well followed by incubation for 30 min in dark. The color reaction was arrested by adding 50μl of stop solution (5% H2SO4 solution). Absorbance of the colored complex was determined at 450 nm.

**Development, isolation, and characterization of peptide specific polyclonal antibodies in rabbit**

A known amount of P27 antigen (150μg) was injected in rabbit with Freund’s complete adjuvant (Sigma). A booster dose of 100μg was administered on day 14 post first immunization. After the second booster dose (Day 21) the immunized rabbit were bled on day 04 post last booster to isolate polyclonal antibody. The peptide P27 was run on 15% SDS-PAGE and immunoblotting was done using rabbit anti-P27 antibody as primary antibody and anti-rabbit antibody as secondary antibody. Isolation of synthetic peptide fragment specific antibody from polyclonal serum was performed in two steps: Protein immobilization and affinity purification.
following protocols recommended in N-hydroxy-succinimide column purification method (NHS; Thermo Scientific Pierce) [1].

For dot blot assay, a known amount (0.5μg) of SLA and synthetic peptide (P27) in PBS was loaded as a dot on NCP blot and placed in ELISA plate for overnight. SLA and PBS were used as a positive and negative control. Blocking was done with 5% BSA in PBS, for 2 h followed by three washing with TBS-T. TBS-T with 0.2% BSA and 0.05% Tween-20 was used as the wash buffer. The experiment was performed in three different sets. In first set, PBS, SLA, and P27 were incubated with affinity purified (P27) specific antibody, in another set sera from healthy control and in the third set; anti-P27 rabbit sera, was used as primary antibody. The primary antibody and antigen were incubated for two hours followed by three washing with TBS-T. HRP-tagged IgG (anti-human or anti-rabbit secondary antibody; Merck Biosciences) was incubated for 1 h followed by three washing with TBS-T. DAB was used as substrate for detection of the blot.

**Immune complex isolation**

The CICs consisting of antigen and antibody complex was precipitated in buffer containing 5% Polyethylene glycol (PEG, Merck, India) and 0.1M Sodium borate (Sigma), pH 8.5 for overnight at 4˚C [2]. The precipitated CIC was pelleted by centrifugation at 6000×g for 45 minutes in cooling centrifuge. The pellet was further washed for three times at 6000×g in 2.5% PEG containing borate buffer (0.1 M) at 4˚C for three times, dissolved in Dulbecco’s Phosphate Buffered Saline (Sigma), pH 7.2 and stored at -80˚C in aliquots for further use. The protein content of isolated CICs was estimated with protein estimation kit (Merck Biosciences) based on Lowry’s method.

**Acid dissociation of immune complex and isolation of antibody using Staphylococcal protein A agarose**

Acid dissociation of antigen and antibody present in CICs was achieved using glycine-HCl buffer at pH 2.5–2.8 following method as described by Gupta and Tan [3]. Further, it was incubated with equilibrated protein A-agarose (Sigma), at 25 °C on shaker incubator for 45 minutes. The mixture was centrifuged at 1500×g for 10 minutes. The antigen free from antibody was obtained from the supernatant due to adsorption of antibody fraction on swollen
protein A agarose. Supernatant was dialyzed using Micro Dispo Dialyzer TM apparatus, (Pall Life Sciences, India) against three changes of 300 ml with PBS for 24 hours at 4°C. Immunoglobulin bound protein A agarose (present in pellet) was washed twice with PBS at 840×g for 15 minutes. Washed pellet was incubated with 2 ml (or twice with the volume of pellet) of 3.5 M MgCl₂ for 15 minutes at 25°C followed by centrifugation at 500×g for 10 minutes. Supernatant containing antibody was dialyzed and washed as described above. Protein concentration was evaluated by BCA method.

**Antigen capture ELISA using CICs antigen**

Double antibody sandwich ELISA was performed according to the protocol of Sengupta and his co-workers, 2002 [3]. The 96-well polystyrene ELISA plate (Nunc) was coated with rabbit anti-P₂₇ antibody (isolated through NHS column) at a concentration of 1µg/ well in carbonate-bicarbonate buffer with pH 9.6 and left for overnight at 4°C. The plate was aspirated and blocked for 2 hours in 5% BSA, followed by washing three times with PBS-T. Further, 100µl of CICs antigen (1 mg/ml stock) was incubated with rabbit antibody (pre-absorbed on well) for two hrs. After stipulated incubation, the plate was extensively washed with PBS-Tween. Next, the plate was incubated for 2 hrs with 100µl serum of the patients with VL-BT, VL-AT, HE, HNE and other diseases background at 37°C. On completion of the stipulated incubation period, the plate was extensively washed and further incubated for 1 hour with HRP tagged anti-human secondary antibody (1:5000, in PBS-T). The plate was again extensively washed, followed by addition of 100 µl of the substrate (TMB) for 30 minutes. Lastly 50 µl of stop solution was added. Finally, the absorbance was taken at RT in dark at 450 nm.

**Reverse Transcription Polymerase Chain Reaction (RT-PCR)**

Reverse transcription was executed using 1µg of total RNA employing cDNA synthesis kit (Roche, USA) according to the manufacturer's instruction. The synthesized cDNA was amplified by PCR for specified genes such as IL-12, IL-10, IFN-γ, TNF-α, inducible nitric oxide synthase (iNOS) and GAPDH etc [4]. GAPDH was used as loading control. The PCR mixture (25µl) contains 0.5µM of forward and reverse primer, 0.5mM of each dNTP, 2 mM MgCl₂, 0.5µg of synthesized cDNA and 1µl polymerase. Details regarding sequence of PCR primers, annealing temperature and size of PCR products have been shown in Table 3. The
PCR was done for 28 cycles where each cycle had denaturation at 95°C for 30 sec, annealing (ranging from 55-62°C) for 30 sec and extension at 72°C for 45 sec. Sample was preheated at 95 °C for 3 min before PCR. The PCR product was run on 1.5% agarose gel, stained with ethidium bromide (0.5μg/ml), and quantified using the gel documentation system and associated Gene-tool software (Syngene, USA).

**References**

1. Jamal F, Shivam P, Kumari S, Singh MK, Sardar AH, Pushpanjali, et al. Identification of *Leishmania donovani* antigen in circulating immune complexes of visceral leishmaniasis subjects for diagnosis. PloS One (2017)12(8): e0182474. doi: 10.1371/journal.pone.0182474.

2. Sengupta K, Ghosh PK, Ganguly S, Das P, Maitra TK, Jalan KN. Characterization of Entamoeba histolytica antigens in circulating immune complexes in sera of patients with amoebiasis. Jour of Health, Popul and Nutr. 2002:215–22.

3. Gupta RC, Tan EM. Isolation of circulating immune complexes by conglutinin and separation of antigen from dissociated complexes by immobilized protein A. Clin and exp immunol. 1981; 46(1):9.

4. Synthesis, characterization, and mechanistic studies of a gold nanoparticle–amphotericin B covalent conjugate with enhanced antileishmanial efficacy and reduced cytotoxicity. International journal of nanomedicine. 2019: 6073-6101.
**SUPPLEMENTARY TABLE**

**TABLE S1:** Showing sample size for ELISA

| Diseases                      | Visceral Leishmaniasis (VL) untreated subject (VL-BT) | Healthy Non-endemic (HNE) | Healthy Endemic (HE) | VL subject after treatment (VL-AT) | VL subject after treatment follow-up (VL-AT) | Viral Flu | Leprosy | Tuberculosis | Malaria | Asthma |
|-------------------------------|------------------------------------------------------|---------------------------|----------------------|-----------------------------------|---------------------------------------------|-----------|---------|--------------|---------|--------|
| Sample Sizes                  | 25                                                   | 11                        | 11                   | 11                                | 11                                          | 11        | 11      | 11            | 11      | 11     |

**Statistical data**

*Table S1.1: Descriptive data statistics corresponding to figure 6A and 6B*

**Body weight**

*Day 0*

|         | A | B | C | D | E | F |
|---------|---|---|---|---|---|---|
|         |   |   |   |   |   |   |
| Unvaccinated | 5 | 5 | 5 | 5 | 5 | 5 |
| Adjuvant  | 21.00 | 21.50 | 20.90 | 22.00 | 20.90 | 21.70 |
| Peptide$_{27}$ | 23.50 | 24.22 | 24.60 | 24.30 | 24.20 | 23.50 |
| Peptide$_{23}$ | 5.200 | 2.720 | 3.700 | 2.300 | 3.300 | 1.800 |
| Cocktail  | 22.34 | 22.66 | 22.30 | 22.76 | 22.58 | 22.58 |
| SLA      | 1.081 | 1.053 | 1.543 | 0.9555 | 1.282 | 0.8106 |
### Day 30

|         | I          | J          | K          | L          | M          | N          |
|---------|------------|------------|------------|------------|------------|------------|
|         | Unvaccinated | Adjuvant   | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail   | SLA        |
| Number of values | 5          | 5          | 5          | 5          | 5          | 5          |
| Minimum | 18.10      | 16.60      | 23.00      | 21.70      | 26.00      | 22.00      |
| Maximum | 23.78      | 21.90      | 27.60      | 25.80      | 29.70      | 25.70      |
| Range   | 5.860      | 5.300      | 4.600      | 4.100      | 3.700      | 3.700      |
| Mean    | 20.45      | 19.64      | 25.16      | 23.64      | 28.30      | 24.27      |
| Std. Deviation | 2.231      | 2.152      | 1.710      | 1.498      | 1.543      | 1.506      |
| Std. Error of Mean | 0.9976     | 0.9626     | 0.7646     | 0.6698     | 0.6899     | 0.6733     |

### Day 63

|         | Q          | R          | S          | T          | U          | V          |
|---------|------------|------------|------------|------------|------------|------------|
|         | Unvaccinated | Adjuvant   | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail   | SLA        |
| Number of values | 5          | 5          | 5          | 5          | 5          | 5          |
| Minimum | 15.76      | 16.60      | 23.60      | 21.70      | 27.60      | 21.70      |
| Maximum | 21.00      | 21.40      | 27.80      | 28.80      | 32.80      | 25.40      |
| Range   | 5.240      | 4.800      | 4.000      | 6.900      | 5.000      | 3.700      |
| Mean    | 18.05      | 18.44      | 25.90      | 25.12      | 29.90      | 23.47      |
| Std. Deviation | 2.012      | 1.804      | 1.488      | 2.796      | 1.970      | 1.615      |
| Std. Error of Mean | 0.8998     | 0.8066     | 0.6656     | 1.250      | 0.8809     | 0.7221     |

### Spleen weight

### Day 0

|         | A          | B          | C          | D          | E          | F          |
|---------|------------|------------|------------|------------|------------|------------|
|         | Unvaccinated | Adjuvant   | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail   | SLA        |
| Number of values | 5          | 5          | 5          | 5          | 5          | 5          |
| Minimum | 67.80      | 69.90      | 59.80      | 59.98      | 59.90      | 55.90      |
| Maximum | 83.70      | 81.80      | 81.97      | 81.80      | 79.56      | 81.00      |
| Range   | 15.90      | 11.70      | 22.17      | 21.82      | 19.66      | 25.10      |
| Mean    | 74.80      | 74.18      | 72.85      | 72.66      | 70.47      | 73.12      |
| Std. Deviation | 6.757      | 4.504      | 9.116      | 8.135      | 9.299      | 11.10      |
| Std. Error of Mean | 3.022      | 2.014      | 4.077      | 3.638      | 4.159      | 4.963      |
### Table S1. 2: data corresponding to Figure 7 (A-E)

**Data corresponding to IFN-γ (Figure 7A)**

#### Day 0

| Group | Unvaccinated | Adjuvant | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail | SLA       |
|-------|--------------|----------|----------------|----------------|----------|-----------|
| A     | 192.000000   | 189.000000 | 311.0000       | 276.000000    | 367.000000 | 212.000000 |
| B     | 145.000000   | 156.000000 | 365.0000       | 189.000000    | 412.000000 | 189.000000 |
| C     | 198.000000   | 177.000000 | 279.0000       | 145.000000    | 389.000000 | 268.000000 |
| D     | 112.000000   | 198.000000 | 342.0000       | 311.000000    | 198.000000 | 301.000000 |
| E     | 98.000000    | 68.000000  | 134.0000       | 289.000000    | 279.000000 | 198.000000 |
### Day 30

|       | Group I | Group J | Group K | Group L | Group M | Group N |
|-------|---------|---------|---------|---------|---------|---------|
|       | Unvaccinated | Adjuvant | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail | SLA     |
| 1     | 118.000 | 158.000000 | 250.0000 | 176.000000 | 418.000000 | 235.000 |
| 2     | 161.000 | 130.000000 | 325.0000 | 23.000000  | 440.000000 | 260.000 |
| 3     | 113.000 | 112.000000 | 279.0000 | 322.000000 | 266.000000 | 254.000 |
| 4     | 99.000  | 129.000000 | 309.0000 | 260.000000 | 358.000000 | 345.000 |
| 5     | 126.000 | 127.000000 | 367.0000 | 285.000000 | 389.000000 | 321.000 |

### Day 63

|       | Group Q | Group R | Group S | Group T | Group U | Group V |
|-------|---------|---------|---------|---------|---------|---------|
|       | Unvaccinated | Adjuvant | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail | SLA     |
| 1     | 112.000 | 155.000000 | 245.0000 | 178.000000 | 412.000000 | 333.000 |
| 2     | 165.000 | 67.000000  | 423.0000 | 287.000000 | 444.000000 | 345.000 |
| 3     | 78.000  | 78.000000  | 278.0000 | 342.000000 | 368.000000 | 156.000 |
| 4     | 78.000  | 132.000000 | 411.0000 | 400.000000 | 485.000000 | 252.000 |
| 5     | 135.000 | 121.000000 | 371.0000 | 289.000000 | 391.000000 | 414.000 |

**Data corresponding to cytokine TNF-α (as depicted in Figure 7B)**

### Day 0

|       | Group A | Group B | Group C | Group D | Group E | Group F |
|-------|---------|---------|---------|---------|---------|---------|
|       | Unvaccinated | Adjuvant | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail | SLA     |
| 1     | 98.000000 | 89.000000 | 125.0000 | 111.000000 | 111.000000 | 87.000000 |
| 2     | 47.000000 | 51.000000 | 187.0000 | 98.000000  | 167.000000 | 145.000000 |
| 3     | 71.000000 | 91.900000 | 76.0000  | 100.000000 | 264.000000 | 109.000000 |
| 4     | 112.000000 | 103.000000 | 186.0000 | 78.000000  | 156.000000 | 136.000000 |
| 5     | 51.000000 | 45.000000  | 103.0000 | 141.000000 | 77.000000  | 52.000000  |
**Day 30**

|                | Group I | Group J | Group K | Group L | Group M | Group N |
|----------------|---------|---------|---------|---------|---------|---------|
| Unvaccinated   | 62.000  | 64.00000| 175.000 | 136.0000 | 229.0000 | 88.000  |
| Adjuvant       | 68.000  | 58.00000| 159.000 | 145.0000 | 215.0000 | 150.000 |
| Peptide\text{27} | 76.000  | 89.00000| 186.000 | 113.0000 | 197.0000 | 153.000 |
| Peptide\text{23} | 70.000  | 65.00000| 155.000 | 112.0000 | 198.0000 | 171.000 |
| SLA            | 62.000  | 68.00000| 141.000 | 135.0000 | 121.0000 | 93.000  |

**Day 63**

|                | Group Q | Group R | Group S | Group T | Group U | Group V |
|----------------|---------|---------|---------|---------|---------|---------|
| Unvaccinated   | 101.000 | 34.00000| 255.000 | 132.0000 | 232.0000 | 81.000  |
| Adjuvant       | 38.000  | 66.00000| 189.000 | 165.0000 | 312.0000 | 254.000 |
| Peptide\text{27} | 78.000  | 81.00000| 176.000 | 213.0000 | 198.0000 | 156.000 |
| Peptide\text{23} | 68.000  | 45.00000| 165.000 | 98.00000 | 287.0000 | 165.000 |
| SLA            | 32.000  | 101.00000| 111.000 | 139.0000 | 111.0000 | 99.000  |

**Date corresponding to cytokine IL-12 (as depicted in Figure 7C)**

**Day 0**

|                | Group A | Group B | Group C | Group D | Group E | Group F |
|----------------|---------|---------|---------|---------|---------|---------|
| Unvaccinated   | 40.00000| 51.00000| 98.00000| 85.00000| 100.00000| 112.00000|
| Adjuvant       | 55.60000| 49.00000| 141.00000| 125.00000| 151.00000| 69.000000|
| Peptide\text{27} | 83.00000| 71.00000| 124.00000| 110.00000| 144.00000| 98.000000|
| Peptide\text{23} | 67.00000| 98.000000| 156.00000| 145.00000| 160.00000| 78.000000|
| SLA            | 72.00000| 51.00000| 165.00000| 135.00000| 155.00000| 87.000000|
Day 30

|        | Group I | Group J | Group K | Group L | Group M | Group N |
|--------|---------|---------|---------|---------|---------|---------|
| Unvaccinated | Adjuvant | Peptide\textsubscript{27} | Peptide\textsubscript{23} | Cocktail | SLA     |
| 1      | 59.000  | 54.00000| 150.000 | 132.0000 | 212.0000 | 81.000  |
| 2      | 40.000  | 56.00000| 159.000 | 135.0000 | 212.0000 | 114.000 |
| 3      | 36.000  | 71.00000| 156.000 | 113.0000 | 198.0000 | 126.000 |
| 4      | 48.000  | 45.00000| 165.000 | 142.0000 | 117.0000 | 125.000 |
| 5      | 52.000  | 68.00000| 141.000 | 129.0000 | 111.0000 | 101.000 |

Day 63

|        | Group Q | Group R | Group S | Group T | Group U | Group V |
|--------|---------|---------|---------|---------|---------|---------|
| Unvaccinated | Adjuvant | Peptide\textsubscript{27} | Peptide\textsubscript{23} | Cocktail | SLA     |
| 1      | 34.000  | 51.00000| 221.000 | 211.0000 | 241.0000 | 112.000 |
| 2      | 41.000  | 41.00000| 153.000 | 123.0000 | 163.0000 | 109.000 |
| 3      | 55.000  | 31.00000| 145.000 | 115.0000 | 175.0000 | 132.000 |
| 4      | 34.000  | 51.00000| 178.000 | 138.0000 | 188.0000 | 123.000 |
| 5      | 39.000  | 37.00000| 199.000 | 159.0000 | 250.0000 | 99.000  |

*Data corresponding to cytokine IL-17 (as depicted in Figure 7D)*

Day 0

|        | Group A | Group B | Group C | Group D | Group E | Group F |
|--------|---------|---------|---------|---------|---------|---------|
| Unvaccinated | Adjuvant | Peptide\textsubscript{27} | Peptide\textsubscript{23} | Cocktail | SLA     |
| 1      | 234.0000 | 255.0000 | 399.000 | 412.0000 | 455.0000 | 245.0000 |
| 2      | 178.0000 | 156.0000 | 534.000 | 189.0000 | 412.0000 | 189.0000 |
| 3      | 198.0000 | 177.0000 | 279.000 | 145.0000 | 555.0000 | 268.0000 |
| 4      | 112.0000 | 211.0000 | 342.000 | 311.0000 | 198.0000 | 301.0000 |
| 5      | 98.0000  | 68.0000  | 134.000 | 289.0000 | 367.0000 | 344.0000 |
### Day 30

|     | Group I       | Group J      | Group K      | Group L      | Group M     | Group N     |
|-----|---------------|--------------|--------------|--------------|-------------|-------------|
|     | Unvaccinated  | Adjuvant     | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail    | SLA         |
| 1   | 130.000       | 144.000000   | 454.000000   | 257.000000   | 531.000000 | 400.000     |
| 2   | 117.000       | 140.000000   | 324.000000   | 285.000000   | 448.000000 | 350.000     |
| 3   | 128.800       | 125.000000   | 378.000000   | 343.000000   | 491.000000 | 173.000     |
| 4   | 135.000       | 151.000000   | 412.000000   | 240.000000   | 481.000000 | 262.000     |
| 5   | 130.000       | 136.000000   | 377.000000   | 291.000000   | 389.000000 | 425.000     |

### Day 63

|     | Group Q       | Group R      | Group S      | Group T      | Group U     | Group V     |
|-----|---------------|--------------|--------------|--------------|-------------|-------------|
|     | Unvaccinated  | Adjuvant     | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail    | SLA         |
| 1   | 111.000       | 143.000000   | 555.000000   | 356.000000   | 634.000000 | 398.000     |
| 2   | 187.000       | 77.000000    | 423.000000   | 287.000000   | 444.000000 | 345.000     |
| 3   | 78.000        | 99.000000    | 278.000000   | 342.000000   | 587.000000 | 178.000     |
| 4   | 98.000        | 143.000000   | 411.000000   | 442.000000   | 485.000000 | 252.000     |
| 5   | 135.000       | 132.000000   | 378.000000   | 289.000000   | 391.000000 | 434.000     |

Data corresponding to cytokine IL-10 (as depicted in Figure 7E)

### Day 0

|     | Group A       | Group B      | Group C      | Group D      | Group E     | Group F     |
|-----|---------------|--------------|--------------|--------------|-------------|-------------|
|     | Unvaccinated  | Adjuvant     | Peptide$_{27}$ | Peptide$_{23}$ | Cocktail    | SLA         |
| 1   | 154.000000   | 245.000000   | 198.000      | 123.000000   | 78.000000   | 213.000000  |
| 2   | 123.000000   | 144.000000   | 98.000       | 153.000000   | 104.000000  | 145.000000  |
| 3   | 189.000000   | 154.000000   | 123.000      | 234.000000   | 98.000000   | 108.000000  |
| 4   | 255.000000   | 265.000000   | 78.000       | 110.000000   | 143.000000  | 98.000000   |
| 5   | 276.000000   | 176.000000   | 132.000      | 94.000000    | 56.000000   | 155.000000  |
### Day 30

| Group | Unvaccinated | Adjuvant | Peptide\textsubscript{27} | Peptide\textsubscript{23} | Cocktail | SLA |
|-------|--------------|----------|---------------------------|---------------------------|----------|-----|
| 1     | 260.000      | 243.00000| 55.00000                  | 156.00000                 | 45.00000 | 108.000 |
| 2     | 317.000      | 285.00000| 123.00000                 | 100.00000                 | 85.00000 | 113.000 |
| 3     | 268.800      | 270.00000| 78.00000                  | 110.00000                 | 110.0000 | 96.000 |
| 4     | 335.000      | 360.00000| 111.00000                 | 136.00000                 | 55.00000 | 252.000 |
| 5     | 256.000      | 236.00000| 178.00000                 | 98.00000                  | 91.00000 | 86.000 |

### Day 63

| Group | Unvaccinated | Adjuvant | Peptide\textsubscript{27} | Peptide\textsubscript{23} | Cocktail | SLA |
|-------|--------------|----------|---------------------------|---------------------------|----------|-----|
| 1     | 412.000      | 455.00000| 67.00000                  | 198.00000                 | 45.00000 | 154.000 |
| 2     | 255.000      | 323.00000| 112.00000                 | 145.00000                 | 76.00000 | 122.000 |
| 3     | 353.000      | 298.00000| 76.00000                  | 76.00000                  | 132.0000 | 175.000 |
| 4     | 398.000      | 376.00000| 176.00000                 | 59.00000                  | 48.00000 | 43.000 |
| 5     | 298.000      | 165.00000| 65.00000                  | 111.00000                 | 75.00000 | 99.000 |
**Table S1.3:**

Data corresponding to cytokine IgG1 isotype (as depicted in Figure 5A)

**IgG1 (Figure 5A)**

|   | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
|   | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide |
| 1 | Number of values | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| 2 | Minimum | 0.0260 | 0.1200 | 0.080000 | 0.1000 | 0.1800 | 0.2000 | 0.1000 | 0.1000 | 0.2000 |
| 3 | Maximum | 0.0570 | 0.5700 | 0.7000 | 0.0220 | 0.0500 | 0.7000 | 0.1220 | 0.7000 | 0.9500 |
| 4 | Range | 0.0210 | 0.4500 | 0.6200 | 0.7220 | 0.5180 | 0.5000 | 0.7220 | 0.5200 | 0.7500 |
| 5 | Mean | 0.3368 | 0.3196 | 0.3269 | 0.4435 | 0.3989 | 0.4453 | 0.4383 | 0.4115 | 0.4858 |
| 6 | Std. Deviation | 0.1945 | 0.1596 | 0.2221 | 0.2518 | 0.1556 | 0.1939 | 0.2494 | 0.1986 | 0.2444 |
| 7 | Std. Error of Mean | 0.0501 | 0.0407 | 0.0412 | 0.0720 | 0.0440 | 0.0598 | 0.0640 | 0.0469 | 0.0766 |

Date corresponding to cytokine IgG2a isotype (as depicted in Figure 5B)

|   | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
|   | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide |
| 1 | Number of values | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| 2 | Minimum | 0.1400 | 0.1400 | 0.2500 | 0.1800 | 0.0877 | 0.4309 | 0.2010 | 0.1100 | 0.4200 |
| 3 | Maximum | 1.395 | 1.395 | 1.395 | 1.395 | 1.395 | 1.395 | 1.395 | 1.395 | 1.395 |
| 4 | Range | 1.210 | 0.9100 | 1.250 | 1.342 | 1.172 | 1.340 | 1.422 | 1.250 | 1.430 |
| 5 | Mean | 0.5482 | 0.4392 | 0.6500 | 0.7837 | 0.5976 | 1.126 | 0.9144 | 0.3052 | 1.189 |
| 6 | Std. Deviation | 0.3951 | 0.1352 | 0.1972 | 0.4666 | 0.3719 | 0.4714 | 0.4150 | 0.3042 | 0.5032 |
| 7 | Std. Error of Mean | 0.1037 | 0.0069 | 0.0114 | 0.0268 | 0.0374 | 0.1536 | 0.1250 | 0.1136 | 0.1444 |

Data corresponding to cytokine IgG2a/IgG1 isotype ratio (as depicted in Figure 5C)

|   | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
|   | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide | PB Peptide |
| 1 | Number of values | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| 2 | Minimum | 0.0393 | 0.0393 | 0.0393 | 0.0393 | 0.0393 | 0.0393 | 0.0393 | 0.0393 |
| 3 | Maximum | 8.079 | 8.079 | 8.079 | 8.079 | 8.079 | 8.079 | 8.079 | 8.079 |
| 4 | Range | 8.040 | 8.040 | 8.040 | 8.040 | 8.040 | 8.040 | 8.040 | 8.040 |
| 5 | Mean | 0.3458 | 0.1729 | 0.1729 | 0.1729 | 0.1729 | 0.1729 | 0.1729 | 0.1729 |
| 6 | Std. Deviation | 0.1256 | 0.005233 | 0.005233 | 0.005233 | 0.005233 | 0.005233 | 0.005233 | 0.005233 |
**SUPPLEMENTARY FIGURES AND TABLE**

A

![Supplementary Figure A](image)

**FIGURE S1**: Effect of dose of peptide_{27} on activation of humoral immune response in Balb/C mice. The experimental animals (two animals each group) were immunized with increasing amount (10-30 µg) of peptide administered in combination with completed Freund’s adjuvant. The animals were boosted with same amount of peptide in combination with incomplete Freund’s adjuvant on day 21 post first immunization. Subsequently, the animals were bled five days post administration of booster dose (A): A representative Western blot showing effect of dose of peptide on induction of specific antibodies in the immunized animals. Peptide_{27} was resolved on 15 % SDS-PAGE (showing 2.8 kDa molecular weight) and immunoblotted on NCP. The blot was developed using mouse anti-Peptide_{27} sera isolated from animals that were immunized with 10µg, 20µg and 30µg of the peptide_{27} (with FCA and FIA) respectively as specified in the preceding lines. The anti-mouse antibody was used as a secondary antibody.

B

![Supplementary Figure B](image)
(B): ELISA results showing highest antibody response in the animals immunized with 30 µg of the peptide27. An aliquot of Peptide27 solution (100 µl) at a concentration of 0.5 µg per well was coated in the 96 well ELISA plate. The mouse serum corresponding to Peptide27 specific primary antibody (induced upon immunization with 10µg, 20µg and 30µg of the peptide27) was set at a dilution of 1:500. The ELISA plate was washed with Tween-PBS extensively. Next, HRP tagged anti-mouse secondary antibody (1:5000 dilution) was dispensed in various wells. The absorbance (at 450 nm) of the colored complex formed after incubation with TMB reagent has been plotted in the graph.
### B

**Sequences producing significant alignments**

| Description | GenPrt | Max Score | Total Score | Query Cover | E Value | Per. Ident | Accession |
|-------------|--------|-----------|-------------|-------------|---------|------------|-----------|
| putative tubulin folding cofactor D (Leishmania infantum) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania donovani) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania donovani) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| Tubulin folding cofactor D, Cterminal family protein (Leishmania donovani) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| Tubulin folding cofactor D, Cterminal family protein (Leishmania donovani) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative/GeneDB/mf.2007.20 (Leishmania donovani) | XP_001495031.1 | 88.4 | 88.4 | 100% | 2e-18 | 100.00% | XP_001495031.1 |
| putative tubulin folding cofactor D (Leishmania major strain Friedlin) | XP_001495031.1 | 81.7 | 81.7 | 96% | 4e-18 | 96.15% | XP_001495031.1 |
| putative tubulin folding cofactor D (Leishmania brasilensis MEB/MER/5810) | XP_001495031.1 | 73.2 | 73.2 | 100% | 4e-13 | 85.19% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania tarentolae) | XP_001495031.1 | 70.6 | 70.6 | 100% | 3e-12 | 81.48% | XP_001495031.1 |
| putative tubulin folding cofactor D (Leishmania mexicana MHOM/GT/2001/1150) | XP_001495031.1 | 68.5 | 68.5 | 100% | 2e-11 | 80.77% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania gypsi) | XP_001495031.1 | 68.1 | 68.1 | 100% | 2e-11 | 81.48% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania panamensis) | XP_001495031.1 | 68.1 | 68.1 | 100% | 2e-11 | 81.48% | XP_001495031.1 |

### C

**Sequences producing significant alignments**

| Description | GenPrt | Max Score | Total Score | Query Cover | E Value | Per. Ident | Accession |
|-------------|--------|-----------|-------------|-------------|---------|------------|-----------|
| putative tubulin folding cofactor D (Leishmania infantum) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania donovani) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania donovani) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| Tubulin folding cofactor D, Cterminal family protein (Leishmania donovani) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| Tubulin folding cofactor D, Cterminal family protein (Leishmania donovani) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative/GeneDB/mf.2007.20 (Leishmania donovani) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| putative tubulin folding cofactor D (Leishmania major strain Friedlin) | XP_001495031.1 | 74.4 | 74.4 | 100% | 2e-14 | 100.00% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania tarentolae) | XP_001495031.1 | 66.0 | 66.0 | 100% | 2e-11 | 91.30% | XP_001495031.1 |
| putative tubulin folding cofactor D (Leishmania mexicana MHOM/GT/2001/1150) | XP_001495031.1 | 58.7 | 58.7 | 0% | 2e-08 | 98.48% | XP_001495031.1 |
| tubulin folding cofactor D, putative (Leishmania braziliensis MHOM/BZ/75/254) | XP_001495031.1 | 53.2 | 53.2 | 95% | 2e-08 | 77.27% | XP_001495031.1 |
| putative tubulin folding cofactor D, putative (Leishmania gypsi) | XP_001495031.1 | 43.5 | 43.5 | 0.006 | 69.57% | XP_001495031.1 |
| putative tubulin folding cofactor D, putative (Leishmania panamensis) | XP_001495031.1 | 43.5 | 43.5 | 0.006 | 69.57% | XP_001495031.1 |
FIGURE S2:

(A) Snapshot of Mass spectrum of the conserved *Leishmania* hypothetical protein.

(B) Snapshot showing pBLAST of peptide sequence ‘KAEVALFRAHLRLVTHVTGEDS’ using non-redundant protein sequence database.

(C) Snapshot showing pBLAST of peptide sequence ‘ARNELYDMLEIDPPAAPAARAMAGESANE’ using non-redundant protein sequence database.

(D) MSA analysis of the epitope with other *Leishmania* species suggested a conserved homology within species analysis performed by Clustal X 2.0.
FIGURE S3 (A): HPLC analysis of synthetic peptide peptide27. (B): HPLC analysis of the peptide23. A single peak validates purity of the two synthesized peptides.
**FIGURE S4**: The cross-reactivity of Peptide$_{27}$ with sera of the patients suffering from leishmaniasis and other infectious diseases as revealed by ELISA. An aliquot of Peptide$_{27}$ (100 µl) at a concentration of 0.5 µg per well was coated using ELISA plate. Primary antibody was set at a concentration of 1:500. HRP tagged anti-human secondary antibody (1:1000 dilution) was added in various wells. The optical density of the colored complex produced after addition of TMB reagent was determined at 450 nm. **(A)** The OD values for the VL subjects was more than two-fold as high as healthy control and patients suffering from other diseases as specified in the figure. The ODs for the VL subject ranges between 0.2 to as high as 1.3. The ODs value for healthy endemic and healthy non-endemic subjects ranges between 0.116 to 0.48 and 0.075 to 0.4 respectively. On the other hand, the ODs value for viral flu, leprosy, tuberculosis, malaria and asthma lies between 0.21 to 0.51, 0.075 to 0.55, 0.047 to 0.6, 0.15 to 0.486, and 0.178 to 0.5 respectively as shown by frequency distribution graph. **(B)** Considering VL-BT subject as an experimental test group while healthy and other diseases subjects as control group, the statistical analysis depicting mean, standard deviation, standard error of mean as (0.80, 0.31), (0.33, 0.118) and (0.06, 0.03) respectively. The lower limits of positivity (cut-off) for the diagnostic antigens was 0.6. The P value was <0.0001. An unpaired $t$-test was found significant with P value <0.0001. **(C)** The ELISA revealed the sensitivity 80%; Se CI95% (69.5 to 88.5) % and specificity 100%; Sp CI (86.2 to 100) % with the area under curve 0.878.
FIGURE S5: Peptide\textsubscript{27} was resolved on 15 % SDS-PAGE showing 2.8 kDa and immunoblotted on NCP. (A) The blot was developed using rabbit anti-Peptide\textsubscript{27} serum as source of primary antibody (raised against 150 µg Peptide\textsubscript{27} with FCA and FIA) and anti-rabbit antibody as secondary antibody. (B) The cross reactivity of SLA and Peptide\textsubscript{27} with affinity purified peptide specific antibody and anti-peptide rabbit sera. The Peptide\textsubscript{27} and SLA were immobilized in the form of a dot on NCP. The PBS was included as a negative control. An affinity purified Peptide\textsubscript{27} specific antibody, anti-Peptide\textsubscript{27} rabbit sera, and sera from healthy subject was considered as test and negative control. The Spot ELISA result revealed that an affinity purified anti-Peptide\textsubscript{27} specific antibody exhibited positive reactivity with Peptide\textsubscript{27} and SLA justifying its immunological relevance.
FIGURE S6: Splenic parasite load (number of amastigotes per 1000 splenocytes) on day 63 post challenge (PC) in groups of animals immunized with various forms of peptide-based vaccines.