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Molecular Mimicry Between Mycobacterial Antigens And Host Myelin Basic Protein

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Hidden challenges
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Introduction

- **Elevated level of antibodies against**
  - **Cytoskeletal proteins (Kroumpouzos *et al*, 1993),**
  - **Rheumatoid factor (Patchelai *et al,* 1973),**
  - **Nuclear factor (Miller *et al,* 1987),**
  - **Phospholipid (Arvieux *et al,* 2002),**
  - **Neutrophil cytoplasm (Medina *et al,* 1998),**
  - **Mitochondria (Gilburd *et al,* 1994).**

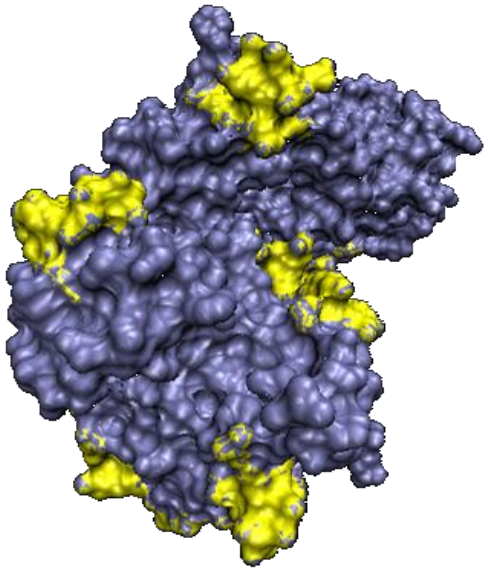
- **Anti *M.leprae* monoclonal antibodies cross-react with human nerve as well as skin components. (Naafs *et al*, 1990; van Den Akker *et al*, 1992).**

- **In leprosy patients the manifestations are mainly exhibited in skin and nerves.**

- **MBP is associated directly or indirectly with neuro-degeneration in leprosy patients (Eutis-Turf *et al*, 1986; Antunes *et al*, 2006).**

Objectives

- To find out the level of auto-antibodies against myelin basic protein in leprosy patients
- To identify and characterize the mimicking epitopes of mycobacterial antigens and MBP.
- Experimental induction and transfer of autoimmune response in naïve female BALB/c mice.



Study Subjects:

- Clinically diagnosed 124 leprosy patients, (based on cardinal features) attending the out patient department (OPD) of NJIL and OMD, ICMR, Agra were chosen for study. Informed consent was taken from the patients and healthy controls.

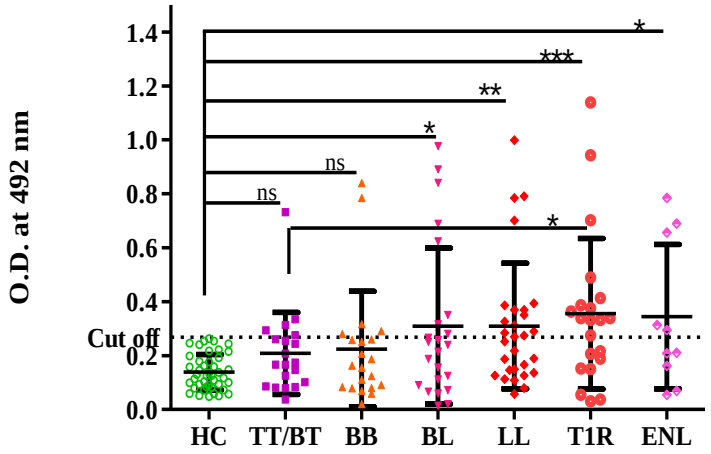
- Experimental Animals
  - Out bred female New Zealand white rabbits
  - inbred strains of female BALB/c mice

- The study was approved by Institutional Animal Ethical Committee and all the animal experiments were done in accordance with the guidelines of Animal Research Ethics Board at our institute.

ELISA :

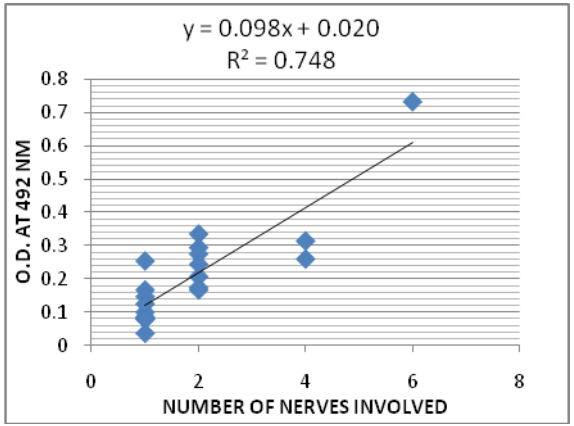
- Level of anti-MBP antibodies was measured by ELISA.

Level of auto-antibodies against MBP in leprosy patients

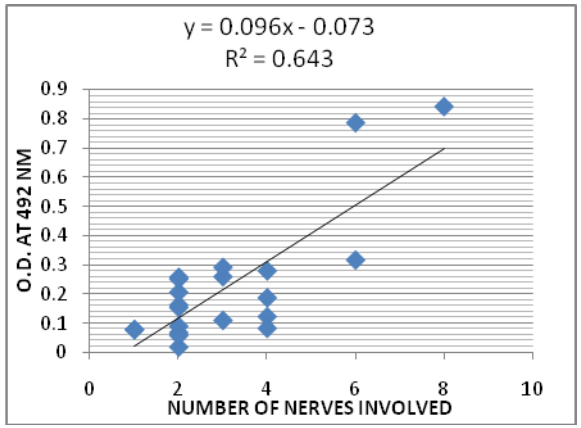


Subjects	HC	Leprosy patients				
	HC	TT/BT	BB	BL	LL	T1R	ENL
Total number of individuals	43	20	21	23	29	21	10
Number of positive	0	5	5	7	12	13	5
Number of negative	43	15	16	16	17	8	5
Percentage positivity	0%	25%*	23.8%	30.4%	41.37%	61.9%*	50%

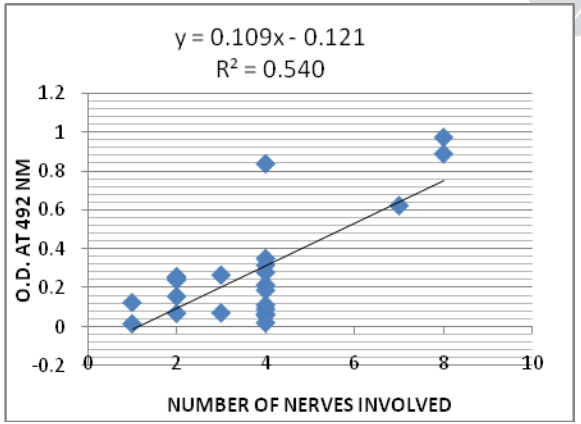
TT/BT



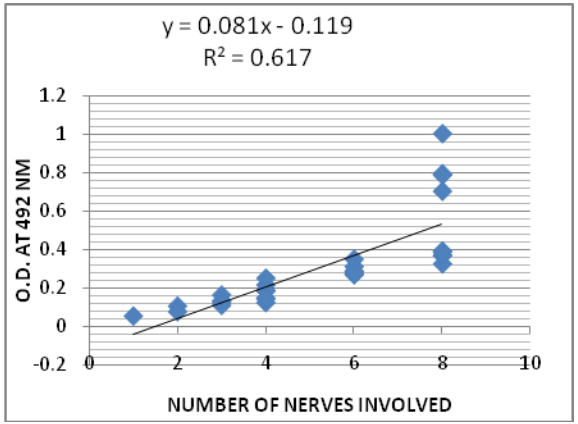
BB



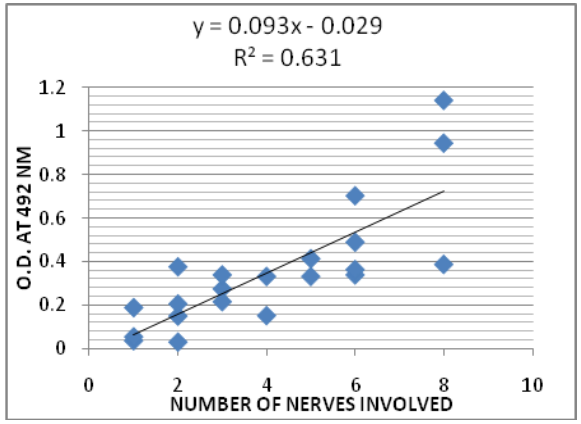
BL



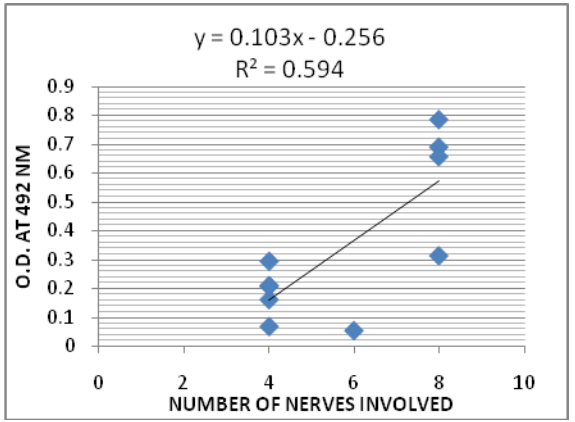
LL



T1R

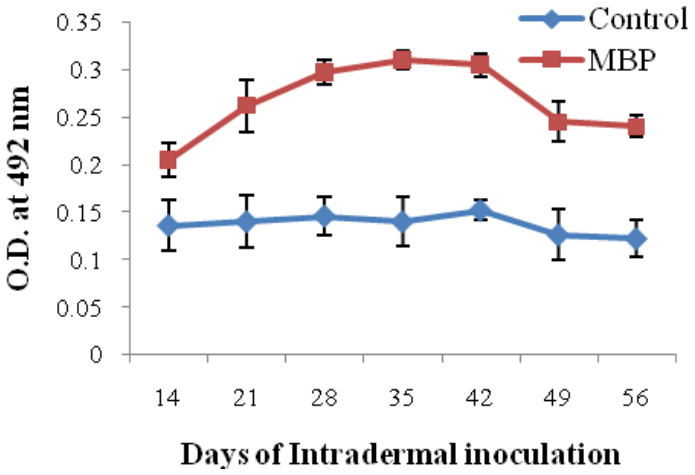


ENL



Hyper-immunization of rabbit

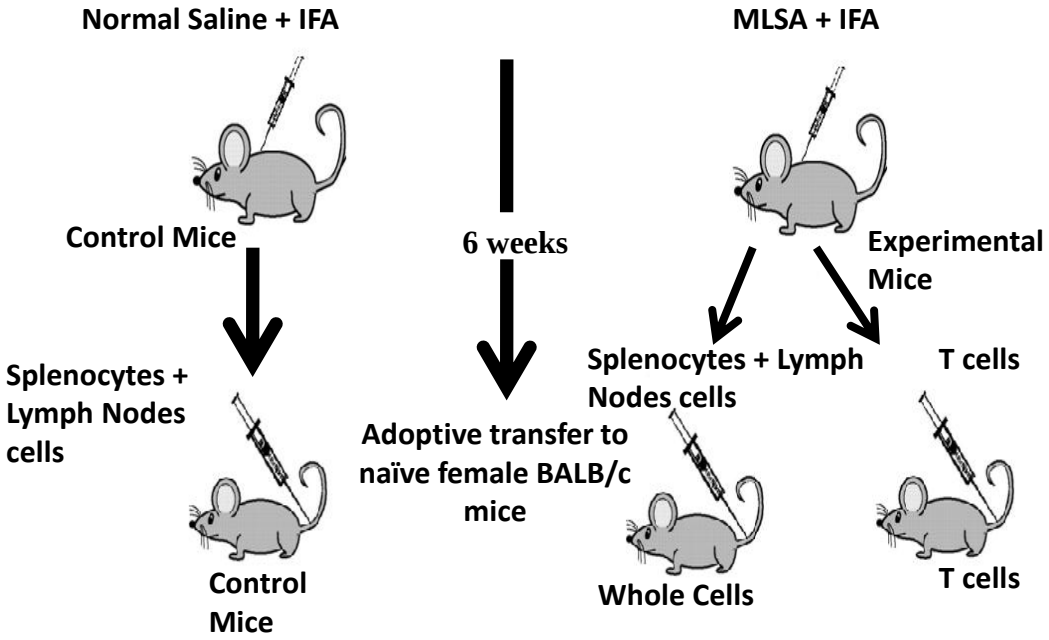
- Hyper-immunised rabbit sera were produced against *M. leprae* sonicated antigens and human MBP.



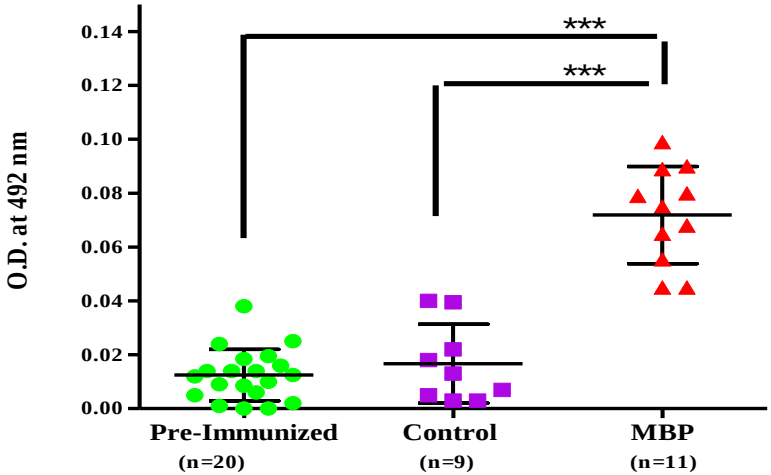
- The highest level of Anti-MBP was observed in rabbit sera at 35th day of immunization with *M. leprae* sonicated antigen (MLSA).

Experimental induction of autoimmune response:

- Female syngeneic strains of BALB/c mice were inoculated with cell extract of *M. leprae* at the interval of 7 days.
- After getting high level of autoantibody splenocytes and cells from lymph nodes were adoptively transferred to naïve mice.



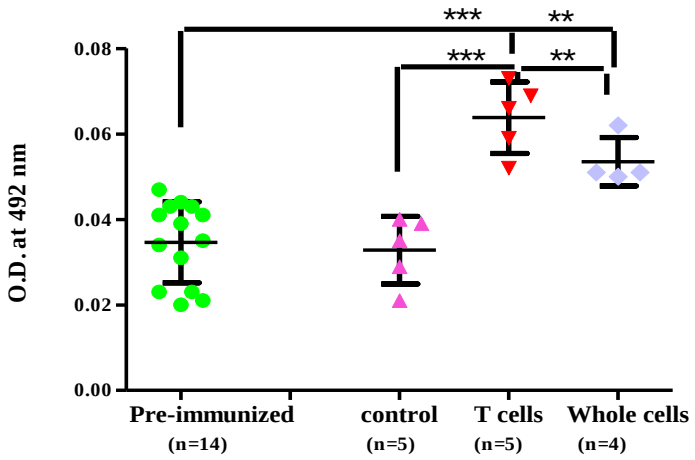
Level of anti-MBP antibodies in female BALB/c mice hyper-immunized with MLSA



(*** p<0.0001)

- Significantly higher level of anti-MBP antibodies in mice hyper-immunized with MLSA

Level of auto-antibodies against MBP in adoptively transferred naïve mice from mice hyper-immunized with MLSA



- Significantly higher level of anti-MBP antibodies has been found in whole cells as well as only T cells transferred mice in comparison to control mice.

SDS-PAGE and Western blot:

- MBP and whole cell extract of *M. leprae* were resolved by SDS-PAGE, using 10% gel (Laemmli *et al*,1970).
- Resolved proteins were electrophoretically transferred to nitrocellulose membrane.
- Blotted with sera from leprosy patients as well as with anti *M leprae* rabbit sera or anti-MBP rabbit sera.

2-D gel electrophoresis and Western blot:

- Both the proteins were further resolved by 2D gel electrophoresis and blotted with anti *M leprae* rabbit sera or anti MBP rabbit sera.

Reactivity of anti *M. leprae* rabbit sera with MBP (SDS-PAGE & 2-D Gel Electrophoresis)

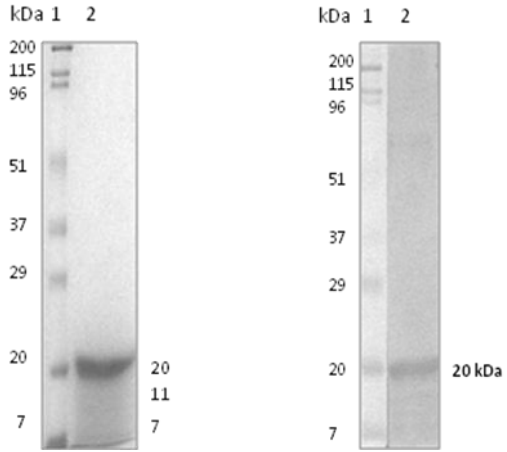


Fig: SDS-PAGE and Western blot pattern of reactivity of anti-MLSA rabbit sera with MBP

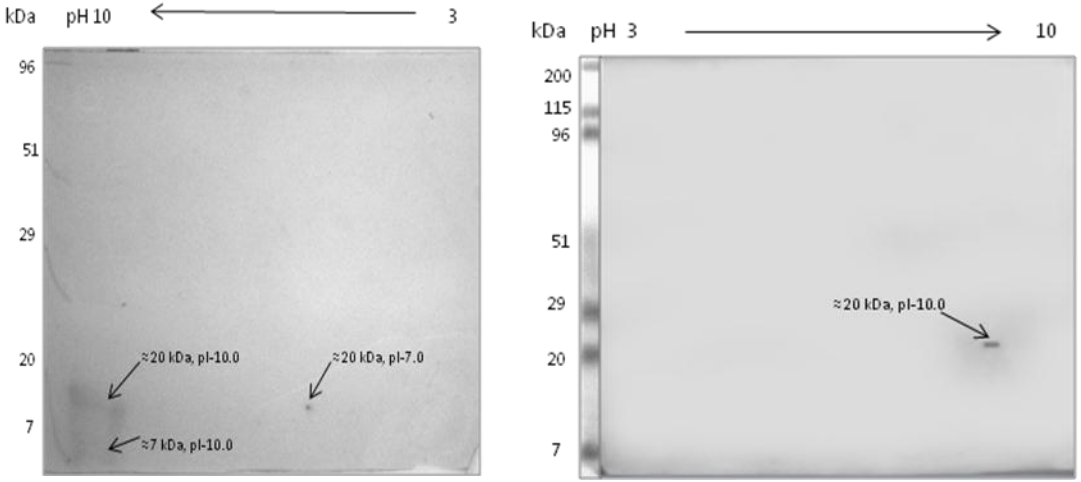


Fig: 2-DGE &Western blot pattern of reactivity of anti-MLSA rabbit sera with MBP

Reactivity of anti MBP rabbit sera with MLSA (SDS-PAGE & 2-D gel electrophoresis)

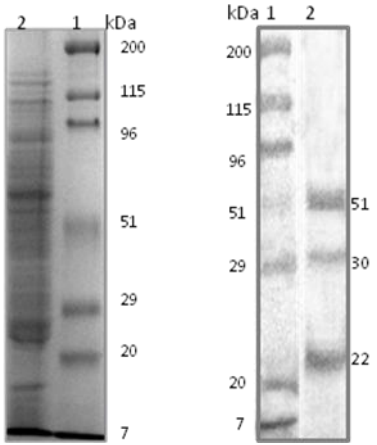


Fig: SDS-PAGE & Western blotting pattern of reactivity of anti-MBP rabbit sera with MLSA

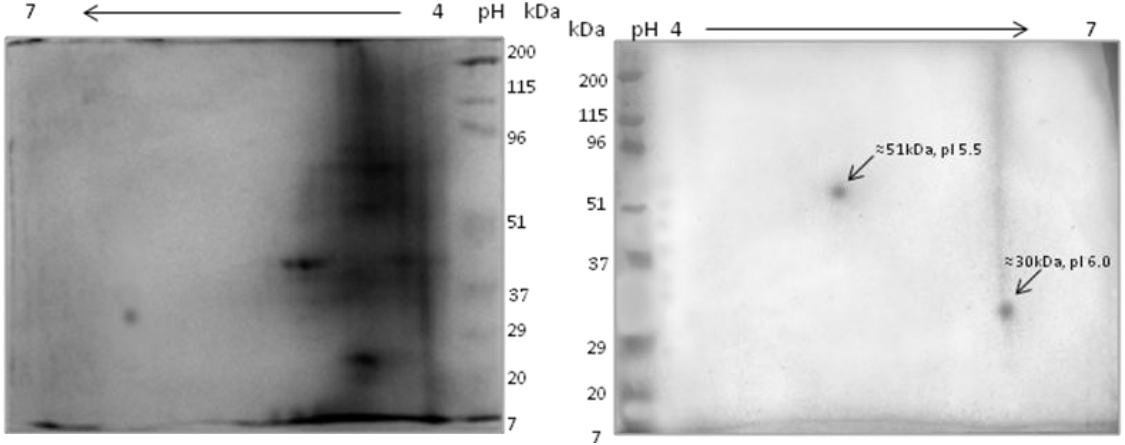


Fig: 2-D gel electrophoresis & Western blotting pattern of reactivity of anti MBP rabbit sera with MLSA

MALDI-TOF/TOF:

- MALDI analysis was done after picking up the reactive spots of MBP and cell extract of *M. leprae* from 2-D gel and it was digested with the trypsin enzyme, and resulting peptide fragments were analyzed using MALDI-TOF MS.

- Mass spectrum generated by the MALDI-TOF MS was submitted to protein data base (i.e. Mascot software) for matching and detection of protein.

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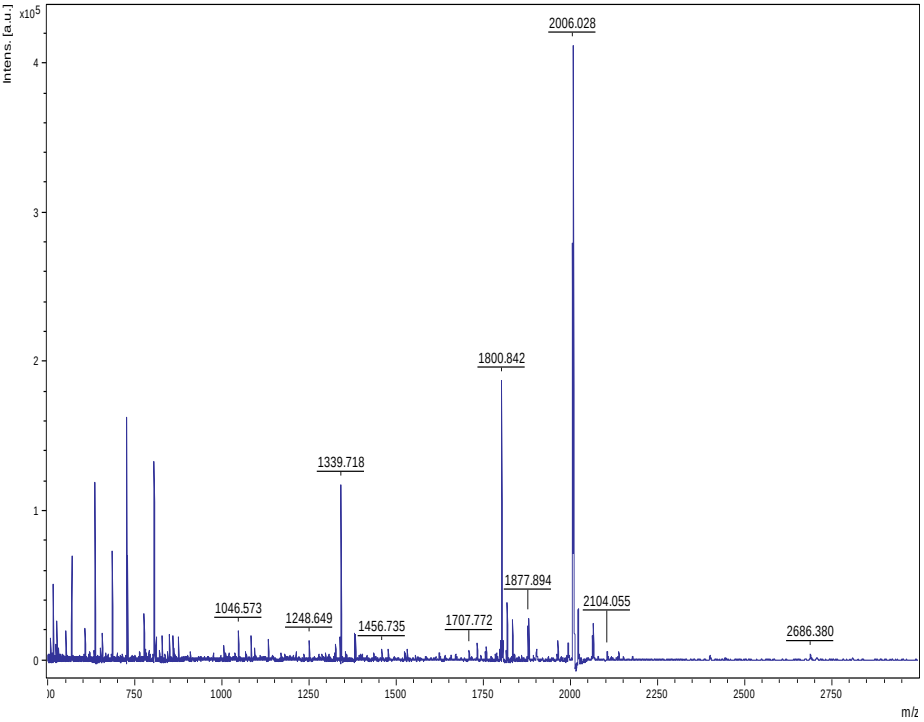
Mass spectrum & Mascot search result of cross-reactive protein of MBP



Mascot Search Result
Protein View

Match to: **MBP_BOVIN** Score: **104** Expect: **2e-006**
Myelin basic protein (MBP) (Myelin A1 protein) (20 kDa microtubule-stabilizing protein) - Bos taurus (Bovine)
Nominal mass (M_r): **18312**; Calculated pI value: **11.28**
Number of mass values searched: **30**
Number of mass values matched: **8**
Sequence Coverage: **46%**

Matched peptides shown in **Bold Red**

```
  1 AAQKRPSQRS KYLASASTMD HARHGFLPRH RDTGILDSLG RFFGSDRGAP
 51 KRGSGKDGHH AARTTHYGSL PQKAQGHRPQ DENPVVHFFK NIVTPRTPPP
101 SQGKGRGLSL SRFSWGAEGQ KPGFGYGGRA SDYKSAHKGL KGHDAQGTLS
151 KIFKLGGRDS RSGSPMARR
```

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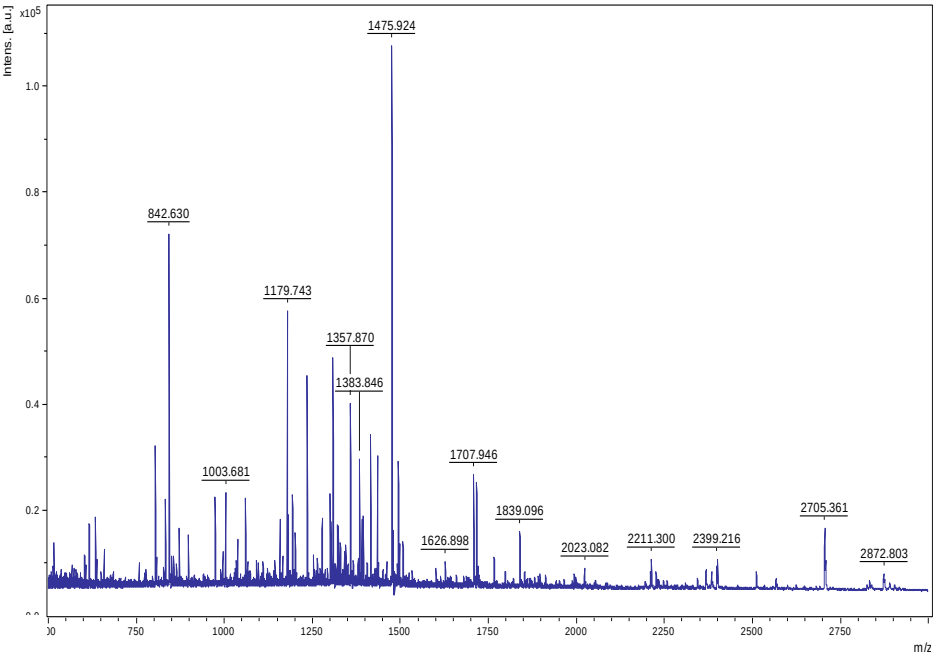
Mass spectrum & Mascot search result of cross-reactive protein of MLSA (50S ribosomal protein L2 – *M. leprae*)



Mascot Search Result
Protein View

Match to: **RL2_MYCLE** Score: **55** Expect: **0.021**
50S ribosomal protein L2 – *Mycobacterium leprae*
Nominal mass (M_r): **30593**; Calculated pI value: **11.41**
Number of mass values searched: **67**
Number of mass values matched: **8**
Sequence Coverage: **23%**
Matched peptides shown in **Bold Red**

```
  1  MAIRKYKPTT SGRRGASVSD FTDITRTKPE KALMRSLHGH GGRNVHGRIT
 51  TRHKGGGHKR AYRLIDFRRN DTDGVNAKVA HIEYDPNRTA NIALLHFLDG
101  KKRYILAPQG LSQGDVVESG ANADIKPGNN LPLRNIPAGT LIHAVELRPG
151  GGAKLARSAG SSIQLLGKES SYASLRMPSG EIRRVDVRCR ATVGEVGNAE
201  QANINWGKAG RMRWKGKRPS VRGVVMNPVD HPHGGGEGKT SGGRHPVSPW
251  GKPEGRTRKP NKSSNKLIVR RRRTGKKHAR
```

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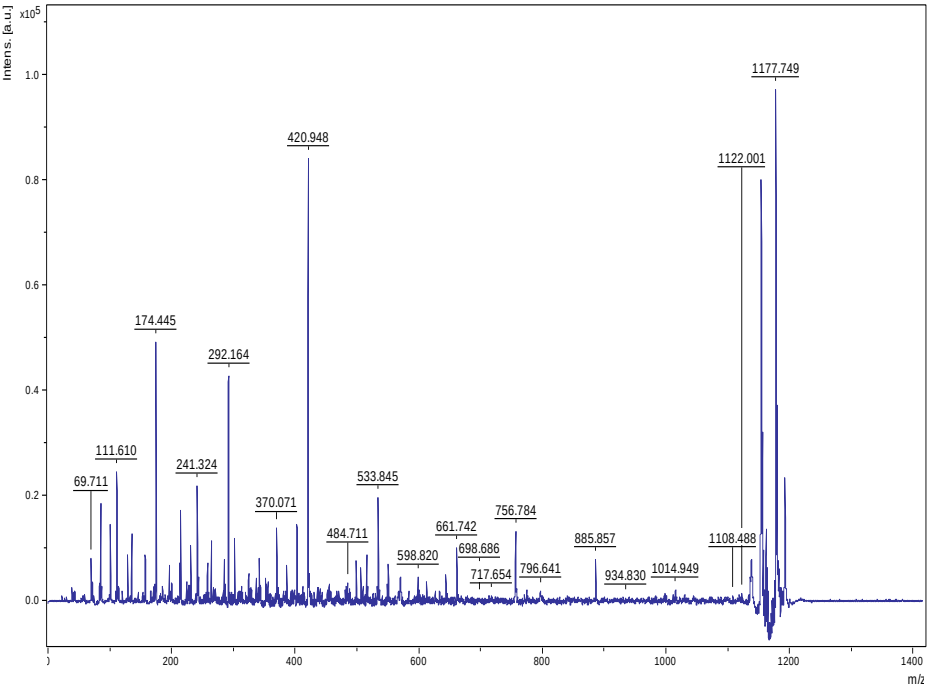
Mass spectrum & Mascot search result of cross-reactive protein of MLSA (Lysyl-tRNA synthetase)



Mascot Search Result
Protein View
Match to: **SYK_MYCLE** Score: **16** Expect: **1.8e+002**
Lysyl-tRNA synthetase (EC 6.1.1.6) (Lysine--tRNA ligase) (LysRS) - Mycobacterium leprae
Found in search of DATA.TXT
Nominal mass (M_r): **56614**; Calculated pI value: **4.91**
Sequence Coverage: **1%**
Matched peptides shown in **Bold Red**

```
  1 MNADPLETDA ALPEQFRIRR DKRARLLAEG RDPYPVAIER THTLAEVRAA
 51 YPDLATDSAT DDIVGIAGRV IFARNSGKLC FATLQDGDGT NLQVMISLNK
101 VGSETLDAWK VDVDLGDIVY VHGNVISSRS GELSVLADSW QMVSKSLRPL
151 PVAHKEMSEE SRVRQRYVDL IVCPQVRIVA HQRIAVIRAI RTALERRGFL
201 EVETPMLQTL AGGAAARPFV THSNALDIDL YLRIAPELFL KRCIVGGFDK
251 VFELNRVFRN EGADSTHSPE FSMLETYQTY GTYDDSAVVT REIIQEVADE
301 AIGTRQLQMP DDSVYDIDGE WETIQMYPSL SAVLGEEITP QTSVDRLRAI
351 ADRLGRGIGP EILDKPSYGH GRLVEQLWEY TVGNTLSAPT FVKDFPVETT
401 PLTRQHRSIP GVTEKWDLYL RGVELATGYS ELNDPVVQRE RFGQQVRAAA
451 AEEDEAMALD EEFLAALEYA MPPCTGTGMG IDRLLMSLTG LSIRETVLFP
501 IVRPHSN
```

Bioinformatics approaches:

- B cell epitopes of both the cross reactive proteins of *M. leprae* and MBP were identified by BCPREDS Server 1.0.
- B cell epitopes of both the proteins were matched to find out the similarity between them.
- Cross reactive proteins of MBP and *M. leprae*, that was identified by MALDI was three dimensionally modeled by CPH server.

BCPREDS Server 1.0

Submitted sequence: 169 amino acids (MBP)
Epitope length: 20 amino acids
Classifier Specificty: 75%
Prediction method: aap
Use overlap filter: yes

AAP Predictions

Position	Epitope	Score
85	VVHFFKNIVTPRTPPPSQGK	1
121	KPGFGYGGRASDYKSAHKGL	1
48	GAPKRGSGKDGHHAARTTHY	1

BCPREDS Server 1.0

Submitted sequence: 280 amino acids (50S ribosomal protein L2 – *M. leprae*)
Epitope length: 20 amino acids
Classifier Specificty: 75%
Prediction method: aap
Use overlap filter: yes

AAP Predictions

Position	Epitope	Score
247	VSPWGKPEGRTRKPNKSSNK	1
2	AIRKYKPTTSGRRGASVSDF	1
109	QGLSQGDVVESGANADIKPG	1
134	RNIPAGTLIHAVELRPGGGA	1
200	EQANINWGKAGRMRWKGKRP	1
224	VVMNPVDHPHGGGEGKTSGG	1
68	RRNDTDGVNAKVAHIEYDPN	1
41	GGRNVHGRITTRHKGGGHKR	0.077

BCPREDS Server 1.0

Submitted sequence: 507 amino acids (Lysyl-tRNA synthetase – *M. leprae*)
Epitope length: 20 amino acids
Classifier Specificty: 75%
Prediction method: aap
Use overlap filter: yes

AAP Predictions

Position	Epitope	Score
142	MVSKSLRPLPVAHKEMSEES	1
384	NTLSAPTFVKDFPVETTPLT	1
26	LLAEGRDPYPVAIERTHTLA	1
431	ELNDPVVQRERFGQQVRAAA	1
47	VRAAYPDLATDSATDDIVGI	1
305	RQLQMPDDSVYDIDGEWETI	1
465	AALEYAMPPCTGTGMGIDRL	1
112	DVDLGDIVYVHGNVISSRSG	1
252	FELNRVFRNEGADSTHSPEF	1
72	FARNSGKLCFATLQDGDGTN	0.922
186	VIRAIRTALERRGFLEVETP	0.834
409	IPGVTEKWDLYLRGVELATG	0.818

```
50Sleprae      MAIRKYKPTTSGRRGASVSDFTDITRTKPEKALMRSLHGHGGRNVHGRITTRHKGGGHKR
MBP1           ------------------------------------------------------------
MBP2           ----------------------------------KPGFGYGGRAS---------------
MBP3           -------------------------GAP-------KRGSGKDGHH---------------
50Sleprae      AYRLIDFRRNDTDGVNAKVAHIEYDPNRTANIALLHFLDGKKRYILAPQGLSQGDVVESG
MBP1           ----------------------------------VVHFFKNIV-------------------
MBP2           D-----------------------------------------YKSAHKGL----------
MBP3           AARTTHY-------------------------------------------------------
50Sleprae      ANADIKPGNNLPLRNIPAGTLIHAVELRPGGGAKLARSAGSSIQLLGKESSYASLRMPSG
MBP1           ------------------------------------------------------------
MBP2           ------------------------------------------------------------
MBP3           ------------------------------------------------------------
50Sleprae      EIRRVDVRCRATVGEVGNAEQANINWGKAGRMRWKGKRPSVRGVVMNPVDHPHGGGEGKT
MBP1           ---------------------------------------------TPRTPPPSQGK---
MBP2           ------------------------------------------------------------
MBP3           ------------------------------------------------------------
50Sleprae      SGGRHPVSPWGKPEGRTRKPNKSSNKLIVRRRRTGKKHAR
MBP1           ----------------------------------------
MBP2           ----------------------------------------
MBP3           ----------------------------------------
```

Mimicking B cell epitopes

1. GGRNVHG

2. NPVDHPHGGGE

Blue colored fonts are B cell epitopes of 50S ribosomal protein L2 – *M. leprae*

Green colored fonts are similar B cell epitopes of MBP with the sequence of 50S ribosomal protein L2 – *M. leprae*

Red colored underlined fonts are similar B cell epitopes of both the proteins.

Similarity between the predicted B cell epitopes of MBP and 50S ribosomal protein L2 of *Mycobacterium leprae*

```
Lysyl       MNADPLETDAALPEQFRIRRDKRARLLAEGRDPYPVAIERTHTLAEVRAAYPDLATDSAT
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       DDIVGIAGRVIFARNSGKLCFATLQDGDGTNLQVMISLNKVGSETLDAWKVDVDLGDIVY
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       VHGNVISSRSGELSVLADSWQMVSKSLRPLPVAHKEMSEESRVRQRYVDLIVCPQVRIVA
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       HQRIAVIRAIRTALERRGFLEVETPMLQTLAGGAAARPFVTHSNALDIDLYLRIAPELFL
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       KRCIVGGFDKVFELNRVFRNEGADSTHSPEFSMLETYQTYGTYDDSAVVTREIIQEVADE
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       AIGTRQLQMPDDSVYDIDGEWETIQMYPSLSAVLGEEITPQTSVDRLRAIADRLGRGIGP
MBP1        ------------------------------------------------------------
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
```

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Contd…

```
Lysyl       EILDKPSYGHGRLV---EQLWEYTVGNTLSAPTFVKDFPVETTPLTRQHRSIPGVTEKWD
MBP1        ------------------------------VVHFFKNIVTPRTP----------------
MBP2        ----KPGFGYGGRA---SDYKSAHKGL---------------------------------
MBP3        ---GAPKRGSGKDGHHAARTTHY-------------------------------------
Lysyl       LYLRGVELATGYSELNDPVVQRERFGQQVRAAAAEEDEAMALDEEFLAALEYAMPPCTGT
MBP1        ------------------------------------------------------PPSQGK
MBP2        ------------------------------------------------------------
MBP3        ------------------------------------------------------------
Lysyl       GMGIDRLLMSLTGLSIRETVLFPIVRPHSN
MBP1        ------------------------------
MBP2        ------------------------------
MBP3        ------------------------------
```

Mimicking B cell epitopes

1. APTFVKDFPVETTP
2. PPCTGT

Blue colored fonts are B cell epitopes of Lysyl tRNA synthetase – *M. leprae*

Green colored fonts are similar B cell epitopes of MBP with the sequence of Lysyl tRNA synthetase – *M. leprae*

Red colored underlined fonts are similar B cell epitopes of both the proteins.

Similarity between the predicted B cell epitopes of MBP and Lysyl tRNA synthetase of *Mycobacterium leprae*

Mimicking B cell epitopes

B cell epitopes of 50S ribosomal protein L2 – *M. leprae*	B cell epitopes of MBP - Host	Mimicking B cell epitopes
VSPWGKPEGRTRKPNKSSNK	VVHFFKNIVTPRTPPPSQGK	GGRNVHG
AIRKYKPTTSGRRGASVSDF	KPGFGYGGRASDYKSAHKGL	NPVDHPHGGGE
QGLSQGDVVESGANADIKPG	GAPKRGSGKDGHHAARTTHY	
RNIPAGTLIHAVELRPGGGA		
EQANINWGKAGRMRWKGKRP		
VVMNPVDHPHGGGEGKTSGG		
RRNDTDGVNAKVAHIEYDPN		
GGRNVHGRITTRHKGGGHKR		

B cell epitopes of Lysyl-tRNA synthetase – *M. leprae*

MVSKSLRPLPVAHKEMSEES

NTLSAPTFVKDFPVETTPLT

LLAEGRDPYPVAIERTHTLA

ELNDPVVQRERFGQQVRAAA

VRAAYPDLATDSATDDIVGI

RQLQMPDDSVYDIDGEWETI

AALEYAMPPCTGTGMGIDRL

DVDLGDIVYVHGNVISSRSG

FELNRVFRNEGADSTHSPEF

FARNSGKLCFATLQDGDGTN

VIRAIRTALERRGFLEVETP

IPGVTEKWDLYLRGVELATG

B cell epitopes of MBP - Host

VVHFFKNIVTPRTPPPSQGK

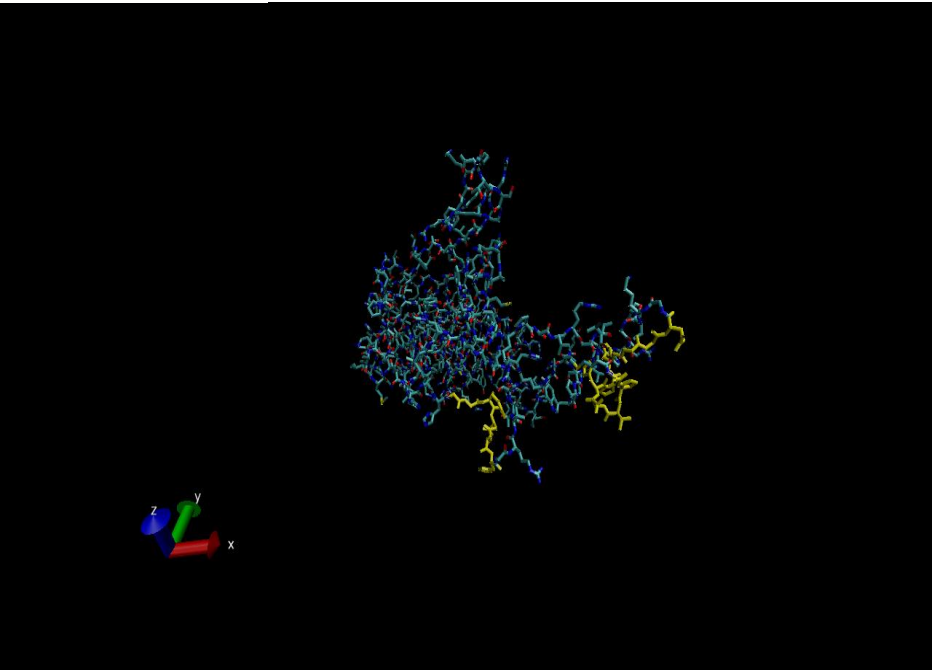
KPGFGYGGRASDYKSAHKGL

GAPKRGSGKDGHHAARTTHY

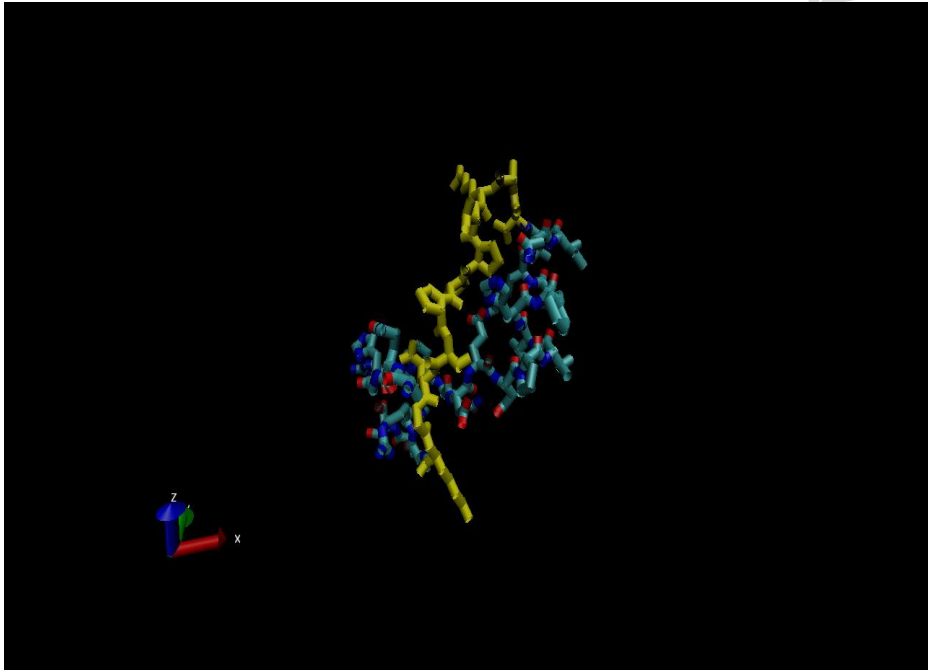
Mimicking B cell epitopes

APTFVKDFPVETTP

PPCTGT



Three dimensional structure of 50S ribosomal protein L2 of *M. leprae*. Yellow area showing the common B cell epitopes of MBP and 50S ribosomal protein L2 of *M. leprae*.



Three dimensional structure of host protein MBP. Yellow area showing the common B cell epitopes of MBP and 50S ribosomal protein L2 of *M. leprae*.

Summary

- **Significantly higher level of autoantibodies against MBP was observed in leprosy patients across the spectrum.**
- **Cross-reactive protein of MBP was in the range of ~20 kDa, at pI 10.**
- **Mimicking protein of MBP is myelin A1 protein while mimicking protein of *M. leprae* is 50S ribosomal protein L2 and Lysyl tRNA synthetase.**
- **Two B cell epitopes of MBP and 50S ribosomal protein and 2 B cell epitopes of MBP and Lysyl tRNA synthetase are similar to each other.**
- **Autoimmunity raised in mice by MLSA can be adoptively transferred.**

References

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Conclusions:

- **Our findings suggested that some B cell epitopes of MBP and 50S ribosomal protein L2/Lysyl-tRNA synthetase of *M. leprae* may be responsible for a heightened autoantibody response in leprosy patients.**

- **Autoimmune response is adoptively transferrable to naïve mice by immune cells.**

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