Molecular Mimicry Between Mycobacterial Antigens And Host Myelin Basic Protein

Itu Singh¹,²,
K.K.Mohanty²,
A.R. Yadav²,
K. Katoch²,
U.D. Gupta²,
D. Bisht²,
S.C. Vedithi¹,
U. Sengupta¹,²

¹Stanley Browne Laboratory, The Leprosy Mission Community Hospital, Shahdara, Delhi – 110093

²National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL&OMD) (ICMR), Tajganj, Agra – 282001
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

<table>
<thead>
<tr>
<th>Subjects</th>
<th>HC</th>
<th>TT/BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>T1R</th>
<th>ENL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number of individuals</strong></td>
<td>43</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>29</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td><strong>Number of positive</strong></td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>12</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td><strong>Number of negative</strong></td>
<td>43</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Percentage positivity</td>
<td>0%</td>
<td>25%*</td>
<td>23.8%</td>
<td>30.4%</td>
<td>41.37%</td>
<td>61.9%*</td>
<td>50%</td>
</tr>
</tbody>
</table>
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

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

(*** p<0.0001)
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.
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 HARHGFPRH RDTGILDLSG RFFGSRGAP
51 KRGSGKDHHS AARTHYGSL PQAKHGMRQ DENPVHFFK NIVTPRTPPP
101 SQGKRGRGLSL SRFWGAEGQ KPGFFYGGRA SDYKSAHKGL KGHDAGQTLS
151 KIFKLGGGRDS RSGSPMARR
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 pl value: 11.41
Number of mass values searched: 67
Number of mass values matched: 8
Sequence Coverage: 23%
Matched peptides shown in **Bold Red**

<table>
<thead>
<tr>
<th>Mass Value (m/z)</th>
<th>Intensity [a.u.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1475.924</td>
<td>0.0</td>
</tr>
<tr>
<td>1179.743</td>
<td>0.2</td>
</tr>
<tr>
<td>1357.870</td>
<td>0.4</td>
</tr>
<tr>
<td>1383.846</td>
<td>0.6</td>
</tr>
<tr>
<td>1707.946</td>
<td>0.8</td>
</tr>
<tr>
<td>2023.082</td>
<td>1.0</td>
</tr>
<tr>
<td>2111.320</td>
<td>5 x 10^5</td>
</tr>
<tr>
<td>2399.216</td>
<td>1003.681</td>
</tr>
<tr>
<td>2705.361</td>
<td>1839.096</td>
</tr>
<tr>
<td>2211.302</td>
<td>1626.898</td>
</tr>
</tbody>
</table>

1 MAIRKYKPTT SGRRGASVSD FTDITRTKPE KALMRSLHGH GGRNVHGRT
51 TRHKGGHHR AYRLIDFRRN DTDGVNAKVA HIEYDPNRTA NIAALLHFLDG
101 KKYRILAPQG LSQGDVVESG ANADIKPGNN LPLRNIFAGT LIHAVELRPG
151 GGAKLARSAG SSIQLLGKES SYASLRMPSG EIRRVDVRCR ATVGEVNGAE
201 QANINWGBKAG RMRWKGKRPS VRVVVNNPVD HPHGGEENGKT SGGRHPVSPW
251 GKFEGRTRKP NKSSNKLIVR RRRTGKKHAR
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 pl value: 4.91
Sequence Coverage: 1%
Matched peptides shown in **Bold Red**
Bioinformatics approaches:

• B cell epitopes of both the cross reactive proteins of *M. leprae* and MBP were identified by BCPRED 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 Specificity: 75%
Prediction method: aap
Use overlap filter: yes
-------------------------------
AAP Predictions

<table>
<thead>
<tr>
<th>Position</th>
<th>Epitope</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>VVHFKNIVTPRTPPPSQGK</td>
<td>1</td>
</tr>
<tr>
<td>121</td>
<td>KPGFGYGGGRASDYKSAHKGL</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>GAPKRGSGKGDGHHAARTTHY</td>
<td>1</td>
</tr>
</tbody>
</table>
Submitted sequence: 280 amino acids (50S ribosomal protein L2 – *M. leprae*)  
Epitope length: 20 amino acids  
Classifier Specificity: 75%  
Prediction method: aap  
Use overlap filter: yes  

<table>
<thead>
<tr>
<th>Position</th>
<th>Epitope</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>247</td>
<td>VSPWGKPEGRTRKPNKSSNK</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>AIRKYKPTTSGRRGASVSDF</td>
<td>1</td>
</tr>
<tr>
<td>109</td>
<td>QGLSQGDVVESGANADIKPG</td>
<td>1</td>
</tr>
<tr>
<td>134</td>
<td>RNIPAGTLHAVELRPGGGA</td>
<td>1</td>
</tr>
<tr>
<td>200</td>
<td>EQANINWGBKGRMRWKGKRP</td>
<td>1</td>
</tr>
<tr>
<td>224</td>
<td>VVMNPVDHPHGGGEGKTSGG</td>
<td>1</td>
</tr>
<tr>
<td>68</td>
<td>RRNDTDGVNAKVAHIEYDPN</td>
<td>1</td>
</tr>
<tr>
<td>41</td>
<td>GGRNVHGRITTRHKGGGHKR</td>
<td>0.077</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Position</th>
<th>Epitope</th>
<th>Score</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>MVSKSLRPLPVAKEMSEES</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>384</td>
<td>NTLSAPTIVKDFPETTPLT</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>LLAEGRDPYPVIAERTHTLA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>431</td>
<td>ELNDPVVQRERFGQQVRAAA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>VRAAYPDLATDSATDDIVGI</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>RQLQMPDDSVYIDGEWETI</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>465</td>
<td>AALEYAMPPCTGTGMDIRL</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>DVDLGDIVYHVGNISSRSG</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>252</td>
<td>FELNRVFRNEGADSTHSPEF</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>FARNSGKLCFATLQDGDGTN</td>
<td>0.922</td>
<td></td>
</tr>
<tr>
<td>186</td>
<td>VIRAIATLERRGFEVTPE</td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>409</td>
<td>IPGVTEKDLYRLRGVELATG</td>
<td>0.818</td>
<td></td>
</tr>
<tr>
<td>50Sleprae</td>
<td>MAIRKYKPTTSGGRASVSDFTDITRTKPEKALMRSLHGGGRNVHGRIITTRHKGGHKRM</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>MBP2</td>
<td>-----------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>MBP3</td>
<td>-----------------------------------------------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>50Sleprae</td>
<td>AYRLIDFRRNRTDVNAKVAHIYDPNRTANIALHLFDGKKRYILAPQGLSQGDVVESG</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>MBP2</td>
<td>D---YSAHKGL∼</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>MBP3</td>
<td>AARTTHY</td>
<td>MBP3</td>
<td></td>
</tr>
<tr>
<td>50Sleprae</td>
<td>ANADIKPNGNLPLRNIPAGTLIHAVELRPGGGAKLARSAGSSIOQLGKESSYASLRMPG</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>MBP2</td>
<td>∼</td>
<td>MBP2</td>
<td></td>
</tr>
<tr>
<td>MBP3</td>
<td>∼</td>
<td>MBP3</td>
<td></td>
</tr>
<tr>
<td>50Sleprae</td>
<td>EIRRVDVRCATVGEVNAEQANINWGKAGRMWKGKRPVRGVMNPVDHPHGGEGKT</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>MBP2</td>
<td>∼</td>
<td>MBP2</td>
<td></td>
</tr>
<tr>
<td>MBP3</td>
<td>∼</td>
<td>MBP3</td>
<td></td>
</tr>
<tr>
<td>50Sleprae</td>
<td>SGGRHPVSPGWKPEGTRKPNSSNKLIVRRRTGKHKAR</td>
<td>MBP1</td>
<td></td>
</tr>
<tr>
<td>MBP2</td>
<td>∼</td>
<td>MBP2</td>
<td></td>
</tr>
<tr>
<td>MBP3</td>
<td>∼</td>
<td>MBP3</td>
<td></td>
</tr>
</tbody>
</table>

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**

Mimicking B cell epitopes

1. GGRNVHG  
2. NPVDHPHGGE
Lysyl
MNADPLETDAALPEQFRIRRDKRARLLAEGRDYPVVAIERHTLAEVRAAYPDALTDSAT
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------

Lysyl
DDIVGIAGRVFARNSGKLCFATLQDGDTNLQVMSLNKVGSETLDAWKVDVLDGDIVY
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------

Lysyl
VHGNISSRSGELSVALSWQMVSKLRLPLPVAHKEMSEESRVRQRYVDLIVCPQVRIVA
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------

Lysyl
HQRIAVIRAIRTALEGFLVETPMLQTLAGAAARPVTHSNALDLRLRIAPELFL
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------

Lysyl
KRCIVGGFKVRELNVRFRNEGADTHSPEFSLETYQTGYDDSVTREIIQEVAD
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------

Lysyl
AIGTRQLQMPDDSVYDIDGEWETIQMPYLSAVLGEITPQTSVRLRAIDRLGRGIGP
MBP1
------------------------------------------------------------
MBP2
------------------------------------------------------------
MBP3
------------------------------------------------------------
### Similarity between the predicted B cell epitopes of MBP and Lysyl tRNA synthetase of *Mycobacterium leprae*

<table>
<thead>
<tr>
<th>Lysyl</th>
<th>MBP1</th>
<th>MBP2</th>
<th>MBP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EILDKPSYGHGRLV--EQLWEYTGVNTLSAPTFVKDFPVETTPLTRQHRSPGVTEKWD</td>
<td>VVHFKNIVTPRT</td>
<td>KPGFGYGGRA---SDYKSAHKG</td>
<td>GAPKRGSKGDHHAARTTHY</td>
</tr>
<tr>
<td>MBP1</td>
<td>MBP2</td>
<td>MBP3</td>
<td>Lysyl</td>
</tr>
<tr>
<td>VVHFKNIVTPRT</td>
<td>KPGFGYGGRA---SDYKSAHKG</td>
<td>GAPKRGSKGDHHAARTTHY</td>
<td>GMGIDRLMSLTGLSIRETVLPVIPRPHSN</td>
</tr>
</tbody>
</table>

**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.**

**Mimicking B cell epitopes**

1. APTFVKDFPVETTP
2. PPCTGT
## Mimicking B cell epitopes

<table>
<thead>
<tr>
<th>B cell epitopes of 50S ribosomal protein L2 – <em>M. leprae</em></th>
<th>B cell epitopes of MBP - Host</th>
<th>Mimicking B cell epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSPWGBKPEGRTKPNKSSNK</td>
<td>VVHFKNIVTPRTPPPSQGK</td>
<td>GGRNVHG</td>
</tr>
<tr>
<td>AIRKYKPTTSGRRGASVSDF</td>
<td>KPGFGYGGRASDYKSAHKGL</td>
<td>NPVDHPHGGGE</td>
</tr>
<tr>
<td>QGLSQGDDVVESGANADIKPG</td>
<td>GAPKRGSGKDGHHAARTTHY</td>
<td></td>
</tr>
<tr>
<td>RNIPAGTLIHAVELRPGGGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQANINWGKAGRMRWKGKRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VVMNPVDPHGGGGEKTSGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRNDTDGVNAKVAHIEYDPN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGRNVHG</td>
<td>TRHRHGKGGHHR</td>
<td></td>
</tr>
</tbody>
</table>
B cell epitopes of Lysyl-tRNA synthetase – *M. leprae*

- MVSKSLRPLPVAHKEMSEES
- NTLSPAFTFKDFPVEETPTLT
- LLAEGRDPYPVAIERTHTLA
- ELNDPVVQRERFGQQVRAAA
- VRAAYPDLATDSATDDIVGI
- RQLQMPDDSVYIDIGEWETI
- AALEYAMPPCTGTGGMGIDRL
- DVDLGDIVYVHGNVISSRSRG
- FELNRVFRNEGADSTHSPEF
- FARNSGKLCFATLQDGDGTN
- VIRAIERTALERRGFLEVETP
- IPGVTEKWDLYLRGVELATG

B cell epitopes of MBP - Host

- VVHFFKNIVTPRTPPPSQGK
- KPGFGYGGRASDYKSAHKGL
- GAPKRSGSKDGHHAAARTTHY

Mimicking B cell epitopes

- APTFKDFPVEETP
- 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*. 
• 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.


**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.

**Acknowledgment**

Our special thanks to The Leprosy Mission Trust – the host organization and National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR) for its support. ICMR for financial assistance.