Autoimmunity to Tropomyosin-Specific Peptides Induced by *Mycobacterium leprae* in Leprosy Patients: Identification of Mimicking Proteins

Itu Singh1†, Asha Ram Yadav2, Keshar Kunja Mohanty1, Kiran Katoch3, Prashant Sharma2, Vinay Kumar Pathak4, Deepa Bisht2, Umesh D. Gupta5 and Utpal Sengupta1*

1Department of Immunology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India, 2Department of Biochemistry, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India, 3Clinical Division, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India, 4Stanley Browne Laboratory, The Leprosy Mission Trust India, TLM Community Hospital, Delhi, India, 5Animal Experimentation Laboratory, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India

Background: It has been shown earlier that there is a rise in the levels of autoantibodies and T cell response to cytoskeletal proteins in leprosy. Our group recently demonstrated a rise in both T and B cell responses to keratin and myelin basic protein in all types of leprosy patients and their associations in type 1 reaction (T1R) group of leprosy.

Objectives: In this study, we investigated the association of levels of autoantibodies and lymphoproliferation against myosin in leprosy patients across the spectrum and tried to find out the mimicking proteins or epitopes between host protein and protein/s of *Mycobacterium leprae*.

Methodology: One hundred and sixty-nine leprosy patients and 55 healthy controls (HC) were enrolled in the present study. Levels of anti-myosin antibodies and T-cell responses against myosin were measured by ELISA and lymphoproliferation assay, respectively. Using 2-D gel electrophoresis, western blot and MALDI-TOF/TOF antibody-reactive spots were identified. Three-dimensional structure of mimicking proteins was modeled by online server. B cell epitopes of the proteins were predicted by BCPREDS server 1.0 followed by identification of mimicking epitopes. Mice of inbred BALB/c strain were hyperimmunized with *M. leprae* soluble antigen (MLSA) and splenocytes and lymph node cells of these animals were adoptively transferred to naïve mice.

Results: Highest level of anti-myosin antibodies was noted in sera of T1R leprosy patients. We observed significantly higher levels of lymphoproliferative response (*p* < 0.05) with myosin in all types of leprosy patients compared to HC. Further, hyperimmunization of inbred BALB/c strain of female mice and rabbit with MLSA revealed that both hyperimmunized rabbit and mice evoked heightened levels of antibodies against myosin and this autoimmune response could be adoptively transferred from hyperimmunized to naïve mice. Tropomyosin was found to be mimicking with ATP-dependent Clp protease ATP-binding subunit of *M. leprae*. We found four mimicking epitopes between these sequences.
Introduction

Infectious agents of the environment are known to play a role in induction of an imbalance in the homeostatic mechanism of the host leading to an autoimmune disease (1). Hansen’s disease (leprosy) is a chronic granulomatous disease caused by *Mycobacterium leprae* (*M. leprae*). *M. leprae* is an obligatory intracellular bacterium. The three cardinal signs used for diagnosis of leprosy are the presence of anesthetic skin lesion(s), enlarged peripheral nerve(s) and presence of acid-fast bacilli in the skin smear (2).

Upon entry into the host, *M. leprae* is selectively phagocytosed by non-professional phagocytic cells (MHC class II negative Schwann cells) in the peripheral nerve and grow taking advantage of immunologically privileged site (3, 4). In an endemic population, about 95 (5) to 99% (6) of infected individuals do not develop any overt disease. However, it has been found out to be very infectious in household contacts of lepromatous leprosy (LL) due to repeated exposure to *M. leprae* infection (7). The host immune response is responsible for disease manifestation and progression of leprosy.

Infection may initiate a continuous antigenic stimulus and may breakdown the tolerance of the host through several non-specific mechanisms leading to autoimmunity (8). Infection with *M. leprae* evokes considerable changes in the humoral immune system, which involves aberrant responses, often associated with autoimmune syndrome. Presence of some antigenic structures of *M. leprae* that can be immunogenic and are cross-reactive to self-proteins might be responsible for the growth of *M. leprae* in lepromatous type of leprosy (9) wherein T cell-mediated immunity to *M. leprae* is virtually absent. On the contrary, in tuberculoid leprosy and during type 1 reaction (T1R), these similarities may lead to a heightened T-cell response and extensive granuloma formation while *M. leprae* is not observed in the host tissues (9). Our group also reported the sharing of mimicking B cell epitopes between *M. leprae* and the cytotokeratin-10 (10) and myelin basic protein (11) of host.

In leprosy patients, impairments of nerve and muscle functions are very common. More than 20% of leprosy patients have been shown to have motor deficits and paralysis of muscles (12). Further, *M. leprae* has been shown to be present between the striated muscle fibers of both tuberculoid and lepromatous patients (13–15). *M. leprae* was also shown to be present in smooth muscle fibers of skin, lips, and nipples in LL (16). Degenerative changes in muscle identified as “Leprous myositis” have also been reported (17, 18). Based on the above literature, we hypothesized that muscle weakness in leprosy patients might be due to presence of anti-myosin antibodies, and therefore, auto-reaction might play a role in muscle damage leading to loss of muscle functions in leprosy patients. Hence, we searched for the presence of mimicking protein/s between host myosin and *M. leprae*.

**Conclusion:** These data suggest that these mimicking proteins tropomyosin and ATP-dependent Clp protease ATP-binding subunit of *M. leprae* or more precisely mimicking epitopes (four B cell epitopes) might be responsible for extensive tissue damage during type 1 reaction in leprosy.

**Keywords:** leprosy, myosin, epitopes, mimicking proteins, autoimmunity, tropomyosin

**MATERIALS AND METHODS**

**Antigens**

Non-irradiated *M. leprae* bacilli derived from armadillo was obtained from Colorado State University, Fort Collins, CO, USA (WHO Contract Number NIH-No 1-AI-25469, Leprosy Research Support). *M. leprae* soluble antigen (MLSA) was obtained by sonication of cells of *M. leprae* according to published protocol (19). The protein content of MLSA was assessed by Bradford method (20). Myosin protein from porcine muscle (Cat. No. M0273) was acquired from Sigma-Aldrich Pvt. Ltd., USA.

**Study Subjects**

**Human Subjects**

A total of 169 leprosy patients were enrolled from the Outpatient department of National JALMA Institute for leprosy and Other Mycobacterial Diseases (NJIL&OMD) (ICMR), Agra for the study. Patients were categorized based on Ridley and Jopling scale (21) and were grouped as borderline tuberculoid (BT) (*n* = 30), borderline borderline (BB) (*n* = 23), borderline lepromatous (BL) (*n* = 39), LL (*n* = 32), BT patients with TIR (*n* = 25) and BL/LL with type 2 reaction or erythema nodosum leprosum (ENL) (*n* = 20). Healthy students and staff of the institution with no evidence for leprosy and any other disease were taken as healthy controls (HC) (*n* = 55) in the study.

This study was approved by Institute Human Ethics Committee, and all the subjects were enrolled after giving a written consent to participate in the study.

**Animals**

Outbred female New Zealand white rabbits and female mice of inbred BALB/c strain were obtained from the Central Drug Research Institute (CSIR), Lucknow. All the animals were kept in specific pathogen-free conditions in the Department of Animal Experimentations, NJIL&OMD, Agra, India. Present study was approved by Institute Animal Ethical Committee, and we followed the guidelines laid down by Animal Research Ethics Board at our institute.

**Animal Experimentations**

**Hyperimmunization of Rabbit**

Rabbits (*n* = 3 in each group) were hyperimmunized with protein concentration of 250 μg of MLSA emulsified with Freund’s incomplete adjuvant (IFA) and 250 μg of porcine myosin to produce polyclonal antibodies against these proteins. Control group of rabbits (*n* = 3) was administered with normal saline emulsified with IFA. All the animals were boosted weekly with the same dose of antigens up to eighth week.
Hyperimmunization of Mice
Mice (n = 15) were hyperimmunized with 25 µg of MLSA and control group mice (n = 10) were inoculated with normal saline as described earlier (10).

Adoptive Transfer
Cells from hyperimmunized mice were adoptively transferred to naïve female mice as reported earlier by Singh et al. (10). Briefly, adoptive transfer was done in control group (n = 5) by intravenous (i.v.) inoculation into the tail vein of suspensions of splenocytes and lymph nodes cells obtained from control mice. Similarly, experimental group (n = 5) were inoculated with immune cells acquired from MLSA-hyperimmunized group. Third group (n = 5) was inoculated intravenously with T cells separated by nylon wool (22) taken from MLSA-hyperimmunized group.

Assessment of Anti-Myosin Antibodies by ELISA
Human Sera
ELISA was done for porcine myosin (Cat. No. M0273, Sigma-Aldrich Pvt. Ltd., USA) -reactive antibodies according to previously described protocol (11) with some changes. Porcine myosin (5 µg/ml) was coated into 96-well ELISA plate (flat bottom, Nunc Amaxisorp, Denmark). ELISA was done according to previously published protocol (11) The absorbance was taken at 492 nM using Spectramax-M2 Reader (Molecular Devices, USA). The cutoff OD was calculated by adding average OD obtained in HC summed up with the value of twice SD.

Experimental Animals
ELISA protocol used for sera from experimental animals was same as described above under human sera except some minor changes in reagents.

Rabbit
Peroxidase conjugated anti-rabbit IgG (Sigma- Aldrich, USA) was used as secondary antibody.

Mice
Dilution of plasma was 50-fold, and secondary antibody was anti-mouse IgG peroxidase (Sigma- Aldrich, USA).

Effect of Myosin on Lymphoproliferation Assay
Lymphoproliferation assay was done as per the protocol described previously with some changes (10). Briefly, peripheral blood mononuclear cells were cultured in RPMI 1640 with 5% FBS in triplicate in presence of 10 µg/ml porcine myosin in Nunc-tissue culture plates (Denmark) and incubated in CO2 incubator for 5 days (Forma Scientific Inc., USA) at 37°C with 5% CO2 in air. Positive control culture was done with phytohemagglutinin. Cells were pulsed with 1 µCi/well of [3H] thymidine after 5 days and incubated further for 18 h. Skatron cell harvester was used for harvesting the cells. Liquid scintillation counting (LKB Wallac, Finland) was used to determine the radioactivity incorporated into DNA. Stimulation index (S.I.) was calculated by using following formula:

S.I. = Counts per minute (CPM) of stimulated cells / CPM of unstimulated cells,

S.I. > 2 was taken as significant stimulation.

Identification of Cross-Reactive Proteins Between Porcine Myosin and MLSA
Characterization of Cross-Reactive Proteins
Two-dimensional PAGE, isoelectric focusing, was carried out using the protocol described by Gorg et al., 2000 (23). Protein samples (100 µg of porcine myosin/MLSA) were loaded on IPG strips (Bio-Rad Laboratories, USA) of pH 3–10 for myosin, pH 4–7 for MLSA and length 7 cm. Proteins were separated in second dimension using 10% SDS-PAGE and transferred to nitrocellulose membrane (NCM) (24). Blotted NCM was blocked with 3% BSA (Sigma, USA) for 1 h, then incubated with pooled leprosy patients’ sera (1:50) while NCM of separated proteins of MLSA was incubated with Myosin-hyperimmunized rabbit sera (1:50). These NCMs were incubated overnight at 4°C followed by three times washing with PBS containing 0.05% Tween-20 and incubated with peroxidase conjugated anti-rabbit IgG (1: 10,000) (Sigma-Aldrich, USA) for 1 h. Later, visualization of antigen antibody reactivity was done by color development with diaminobenzidine (Sigma, USA) solution. Capturing of image was done by Chemidoc (Bio-Rad Laboratories, USA).

MALDI-TOF Analysis
In-gel digestion with trypsin (25) was done according to previously published protocol (10). Mass spectra of digested peptides were analyzed using Mascot Wizard program (Matrix Science, Ltd., London, United Kingdom1). Peptide mass fingerprint of cross-reactive protein of porcine myosin with pooled leprosy patients’ sera was submitted to Mascot search engine and search parameters used for the identification were peptide mass tolerance ±30 ppm, peptide charge state 1+, and maximum missed cleavages 1. However, search parameters used for the identification of the cross-reactive protein of MLSA by MS/MS ion search was peptide mass tolerance ±100 ppm, fragment mass tolerance ±0.5 Da, maximum missed cleavages 1.

B Cell Epitope Prediction
BCPREDs server 1.0 was used (aap prediction method) to identify B cell epitopes of the mimicking proteins.2 Predicted B cell epitope length was of 20 amino acids and classifier specificity used was 75% (26).

Three-Dimensional Structure of Identified Protein
Structure of mimicking proteins of M. leprae and porcine myosin was predicted by submitting the sequence to Phyre2 server3 (27). VMD viewer4 was used for analysis of modeled structure (28).

1http://www.matrixscience.com (Accessed: November 11, 2009 and Accessed: September 4, 2017).
2http://ailab.ist.psu.edu/bcpred/predict.html (Accessed: September 6, 2017).
3http://www.sbg.bio.ic.ac.uk/phyre2/html/.
4www.ks.uiuc.edu/Research/vmd/.
Statistical Analysis
Data were analyzed using GraphPad prism software version 5.0 (GraphPad Prism, La Jolla, CA, USA). Cutoff value for ELISA data were expressed as mean ± 2SD and p value < 0.05 was considered as statistically significant. Under the respective figure or table legend specific test used for analysis has been mentioned. PD Quest Software (Bio-Rad, USA) was used to analyze 2-D blot data.

RESULTS

Levels of IgG Antibodies Against Myosin in Leprosy Patients’ Sera
Highest mean OD value was obtained in the sera of T1R (0.416 ± 0.18) that was followed by LL (0.339 ± 0.13), ENL (0.322 ± 0.12), BL (0.302 ± 0.10), BB (0.275 ± 0.08), and TT/BT (0.264 ± 0.08). The mean OD value in sera of T1R patients' group was significantly higher than TT/BT (p < 0.0001), BB (p < 0.0001), LL (p < 0.001), and ENL (p < 0.05) group of patients (Figure 1). The cutoff OD value for myosin was found to be 0.282. Seropositivity of antibodies against myosin in the sera of all types of leprosy patients is shown in Table 1. Highest percent of seropositivity was observed in T1R (75%) followed by LL (56.25%), BL (50%), ENL (46.66%), BB (43.47%), and TT/BT (35%). The seropositivity of patients with T1R was found to be significantly higher than TT/BT (p = 0.02) using Fisher’s exact test.

Lymphoproliferative Response of Leprosy Patients in the Presence of Host Myosin
The highest mean value of S.I. was obtained in T1R (4.06 ± 2.7) group of leprosy patients which was followed by BL/LL (2.71 ± 1.6), TT/BT (2.46 ± 1.3), and ENL (2.02 ± 1.4) patients.

The mean values of S.I. in the presence of myosin were found to be significantly higher in TT/BT (p = 0.005), BL/LL (p = 0.004), T1R (p = 0.004), ENL (p = 0.05) groups of leprosy patients in comparison to HC by using unpaired two-tailed t test (Figure 2).

Levels of IgG Antibodies Against Myosin in MLA-Hyperimmunized Rabbit
Significantly higher levels of anti-myosin antibodies were observed in MLA-hyperimmunized rabbit in comparison to control rabbit (average OD ± SD of MLA hyperimmunized vs control 1.258 ± 0.16 vs 0.158 ± 0.03, p < 0.05). Highest levels of antibody against myosin (Figure 3) were observed at 35th day of immunization with MLA.

IgG Antibody Levels Against Myosin in MLA-Hyperimmunized Mice
It was observed that MLA-hyperimmunized mice induce significantly elevated levels of anti-myosin antibodies in comparison to control mice (p < 0.0001). Mean level of antibodies against myosin was found to be significantly higher than pre-immunized (pre-immunized vs MLA hyperimmunized 0.011 ± 0.009 vs 0.073 ± 0.035, p < 0.0001) and control group (control vs MLA hyperimmunized 0.012 ± 0.012 vs 0.073 ± 0.035, p < 0.0001) at sixth week of inoculation with MLA in female BALB/c mice by using one-way ANOVA (and non-parametric) and Bonferroni’s multiple comparison post-test (Figure 4).

Adoptive Transfer With Immune Cells in Inbred Strains of Naïve Female BALB/c Mice
It was observed that significantly higher level of anti-myosin antibodies was observed in sera of adoptively transferred mice with nylon wool separated T cells (T cell vs pre-immunized 0.113 ± 0.0017 vs 0.090 ± 0.013, p < 0.001), splenocytes and lymph nodes cells (whole cell vs pre-immunized 0.1152 ± 0.027472 vs

| TABLE 1 | Sero-positivity of anti-myosin antibodies in sera of leprosy patients and healthy controls (HC). |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Subjects | HC | TT/borderline tuberculoid | Borderline borderline | Borderline lepromatous | Lepromatous leprosy | Type 1 reaction | Erythema nodosum leprosum |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Total number of individuals | 45 | 20 | 23 | 26 | 32 | 20 | 15 |
| Number of positive | 3 | 7 | 10 | 13 | 18 | 15 | 7 |
| Number of negative | 42 | 13 | 13 | 13 | 14 | 5 | 8 |
| Percentage positivity (%) | 6.66 | 3.6 | 4.37 | 50 | 56.25 | 75 | 46.66 |

*p value < 0.05
identification of mimicking B-cell epitopes between tropomyosin and probable ATP-dependent Clp protease ATP-binding subunit of *M. leprae*.

It was observed that four B-cell epitopes are mimicking epitopes of tropomyosin of host and probable ATP-dependent Clp protease ATP-binding subunit of *M. leprae*. It was noted that CLPC191–205 with TM41–48 and TM12, CLPC237–248 with TM49–60, CLPC453–465 with TM106–113 and TM23–28 and CLPC51–560 and TM161–178 are putative mimicking epitopes (Figure 7).
TABLE 2 | Cross-reactive proteins identified by MALDI-TOF.

| Cross-reactive Protein                      | Protein identified                                      | Accession number | Mascot Score | Nominal mass | pl     | Sequence coverage (%) |
|---------------------------------------------|---------------------------------------------------------|------------------|--------------|--------------|-------|-----------------------|
| Myosin cross-reacted with pooled leprosy patients’ sera | Tropomyosin alpha striated muscle isoform (Homo sapiens) | AAT68295.1       | 93           | 32,690       | 4.67  | 42                    |
| MLSA cross-reacted with anti-myosin rabbit sera | ATP-dependent Clp protease ATP-binding subunit of Mycobacterium leprae | P24428           | 3            | 93,944       | 5.57  | 1                     |

Three-Dimensional Structure of Tropomyosin and Probable ATP-Dependent Clp Protease ATP-Binding Subunit of M. leprae

Mimicking B-cell epitopes of both the proteins are highlighted on 3-dimensional structure of the proteins. It has been found that four putative mimicking B cell epitopes of CLPC of *M. leprae* and tropomyosin are present on the surface of the proteins (Figures 8 and 9).

DISCUSSION

In this study, we demonstrated the cross-reactivity of tropomyosin with sera of leprosy patients/*M. leprae* components using porcine myosin. The cross-reactivity is found in experimental animals also which are hyperimmunized with MLSA/porcine myosin. These results taken together suggest that common epitopes are shared between *M. leprae* and tropomyosin. Molecular mimicry is defined as epitopes shared between microbial antigens and host self-components (29) which may...
**FIGURE 7** | Multiple sequence alignment of Probable ATP-dependent clp protease ATP-binding subunit (CLPC) of Mycobacterium leprae and B cell epitopes of tropomyosin (TM) of host. Red color—showing predicted B cell epitopes of CLPC of M. leprae. Purple color—showing predicted B cell epitopes of tropomyosin of host. Yellow color—highlighted sequences showing mimicking B cell epitopes of both the proteins.

| CLPC | MFERFTDRARRVVVLAAQEEARMLNHNYIGTEHILLGLIHEGEGVAAKSDLGELISLEAVR |
|-----|---------------------------------------------------------------|
| TM  | SQVEDIQGGQAPSCHFIPPTRAKVLLESLREALQLGHNIGTEHILLGLIREGEVGAAKSDLGELISLEAVR |
|     | AAMESKLDPVIGKEKIEVRMVQSLRTKNNPVLIGEPVGKTAVVEGLAQAIYHEVEPE |
|     | KGTEDEL - MQMLK |
|     | 1  |
|     | ALKDAQEK |
| CLPC | TDLGSLVAGSRYGRDFEEKLKKVLKEINTRDIIFLIFDELHLTVGAGAAEGER |
| TM  | AIDAASILPKLARGEIQGTTLDDEYRKYIEMDAALERRFQPVQGEPTVEHTIELK |
|     | LDKENALDR |
| CLPC | GLRDRYEAAHHRVSITDSAMVAATLADRYINDRFLPDKAILIDEAGARMRIRRTAPPD |
| TM  | LREFDKIAEAERKEASDAFAQFEKASLREKQVLQAERAEEQWRSGLDLVIAEVD |
|     | KATDAEADVSLNRAAEKAEQQA |
|     | 3  |
| CLPC | WMTSNLGTSDLKPVGLGFTQSQMUQNYMEKQKVMDNELKKHFPEFLNRDIIVFH |
| TM  | YVGYEEGQLTEKVRKPSVLFDEIKAHEIYNSLQVLEDGRLTDGQGRTVDFKNT |
|     | EERAELSEGK |
| CLPC | VLIPTSNLGTSKDFPVGLGFTQGSGENDYERMQKVMDNELKKHFPEFLNRDIIVFH |
| TM  | YVGYEEGQLTEKVRKPSVFVFDEIKAHEIYNSLQVLEDGRLTDGQGRTVDFKNT |
|     | EERAELSEGK |
| CLPC | QLSRDEIIKVMVLISRVANQLKVKTMLELTNKAKALLKRGDFVPLGARPLRTIQRE |
| TM  | VLIPTSNLGTSKDFPVGLGFTQGSGENDYERMQKVMDNELKKHFPEFLNRDIIVFH |
|     | EERAELSEGK |
| CLPC | IEDQLSEKLFEEVPGPVQVTVTVDNWDEGPEDVKTFTGIRKSPSTEPDLAKAGVHS |
| TM  | QLSRDEIIKVMVLISRVANQLKVKTMLELTNKAKALLKRGDFVPLGARPLRTIQRE |
|     | CTNDLKSLEA |
| CLPC | GGPEPVEQ |

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lead to autoimmunity, tissue injury and disease. We showed that significantly high levels of anti-myosin antibodies are present in all the groups of leprosy patients in comparison to HC. Highest level of anti-myosin antibodies was found in T1R (0.416 ± 0.18) which was followed by LL (0.339 ± 0.13), ENL (0.322 ± 0.12), BL (0.302 ± 0.10), BB (0.275 ± 0.08), and TT/ BT (0.264 ± 0.08) (Figure 1). We observed significantly high lymphoproliferation with myosin in leprosy patients across the spectrum except ENL in comparison to HC (Figure 2).

In the present study, porcine myosin was used to see the level of anti-myosin antibodies and lymphoproliferation in leprosy patients and experimental animals since, all the cytoskeletal proteins are conserved across the vertebrates and we observed high level of antibodies against this myosin in leprosy patients. We propose that molecular mimicry between putative epitopes of tropomyosin and M. leprae may potentially lead to loss of muscle functions in leprosy patients.

Leprosy is a chronic disease which affects both nerves and muscles. Leprosy is non-toxic disease and it was shown that most of the tissue and nerve damage occurs by host immune response to M. leprae antigens (30). Rambukkana et al. showed elegantly the immunological cross-reactivity between mycobacterial hsp 65 and human epidermal cytokeratin ½ (31). We recently reported existence of molecular mimicry between host cytokeratin-10 and HSP 65 (groEL2) of M. leprae (10) and between host myelin A1 and M. leprae 50S ribosomal L2 and lysyl tRNA synthetase proteins (11).

A central finding of this study is that MLSA induces antibodies against myosin in female BALB/c mice and this autoimmune reaction could be adoptively transferred to naïve mice. Hence, it supported our hypothesis that alteration in homeostatic mechanism may lead to autoimmune reaction and this autoimmunity is transferrable by autoreactive immune cells in naïve mice. Myosin reactive antibodies produced by immunization with MLSA could
be adoptively transferred to naïve mice even by T cell transfer could be explained by the proliferation of autoreactive B cells present in the secondary lymph nodes which are known to induce autoimmunity (32). It is also noted that MLSA induces antibodies against myosin in rabbit. It was earlier observed that mouse cytomegalovirus infection induces myocarditis in susceptible BALB/c mice by producing autoantibodies to cardiac myosin and it was concluded that there were common epitopes between both the proteins (33).

Presence of autoantibodies are common in leprosy patients (34). A key question is whether these autoantibodies are produced because of mimicking epitopes between host protein/s and M. leprae protein/s. Significantly elevated level of antibodies against myosin is observed in leprosy patients across the spectrum in comparison to HC indicates that anti-myosin antibodies are produced because of the presence of some cross-reactive regions between both the proteins. Significantly high lymphoproliferation with myosin antigen is also noted in leprosy patients in comparison to HC. It is possible that high CMI level with host antigen might also be because of similarity of myosin protein with M. leprae protein/s. This study indicates that the cross-reactivity is at the 35 kDa of porcine myosin with leprosy patients’ sera and at 97 kDa of MLSA with anti-myosin rabbit sera. We propose that this cross-reactivity between myosin and MLSA may be because of presence of mimicking B cell epitopes in both the proteins. Further, these proteins are identified as tropomyosin of host and probable ATP-dependent clp protease ATP-binding subunit of M. leprae. We used porcine myosin for 2-D gel electrophoresis and western blotting but the reactive spot of porcine myosin with pooled leprosy patients’ sera identified by MALDI-TOF analysis was tropomyosin. We expected to find myosin as the reactive spot but it turned out to be tropomyosin, and this reactivity might be because of the presence of tropomyosin in the porcine myosin that reacted with pooled leprosy patients’ sera. Earlier reports from our group showing the presence of seven mimicking B cell epitopes of cytokeratin-10 and HSP 65 (10) and four mimicking B cell epitopes of myelin A1 and 50S ribosomal L2 and lysyl tRNA synthetase (11) were cross-reactive indicated their role in skin and nerve damage. Further, in the present study it is noted that four putative B cell epitopes are mimicking between tropomyosin and probable ATP-dependent clp protease ATP-binding subunit of M. leprae. These putative mimicking B cell epitopes might be responsible for “leprous myositis” leading to muscle damage in leprosy patients which has been reported earlier (17, 18). We have already reported in experimental mice that hyperimmunization with M. leprae antigen leads to lowering of Treg cells along with production of high levels of antibodies against M. leprae in addition to the production of high levels of autoantibodies against host proteins (10). Thus, these findings support our hypothesis that M. leprae infection can induce imbalance in homeostatic mechanism in immune system of the host and is responsible for the auto-reaction in leprosy patients.

For the first time, we identified the cross-reactive proteins between tropomyosin of host and probable ATP-dependent clp protease ATP-binding subunit of M. leprae. Further, it is noted that four B cell epitopes are putative mimicking B cell epitopes of both the proteins. We observed elevated level of antibodies against myosin and high level of CMI with myosin in leprosy patients in comparison to HC. The cross-reactive protein is at 97 kDa of M. leprae and at 35 kDa of myosin.

We also observed that this auto-reaction can be induced in experimental animals (rabbit and mice) after hyperimmunization with MLSA. This auto-reaction is transferrable to naïve mice with the help of immune cells. Hence, we conclude from our study that M. leprae infection can induce imbalance in the homeostatic mechanism of the host and can induce auto-reaction in leprosy patients. This induction in auto-reaction in leprosy patients is due to the presence of molecular mimicry between tropomyosin and probable ATP-dependent clp protease ATP-binding subunit of M. leprae which might be responsible for “leprous myositis” and muscular weakness.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of “Indian Council of Medical Research guidelines, National JALMA Institute for Leprosy & OMD Human Ethics Committee” with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the “National JALMA Institute for Leprosy & OMD Human Ethics Committee”. This study was carried out in accordance with the recommendations of “guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),” “National JALMA Institute for Leprosy & OMD Animal Ethics Committee”. The protocol was approved by the “National JALMA Institute for Leprosy & OMD Animal Ethics Committee.”

AUTHORS CONTRIBUTION

US conceived and designed the study; IS, KM, PS, VP, and AY performed the experiments and analyzed and interpreted the data; IS drafted the manuscript; KM, KK, DB, UG, and US critically reviewed the manuscript.

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