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EDITORIAL

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Biochemical Aspects of *Mycobacterium leprae* Binding Proteins: A Review of Their Role in Pathogenesis

Leprosy is one of the oldest human neurological diseases resulting from infection with an obligate, intracellular pathogen *Mycobacterium leprae*. Neuropathy in leprosy is usually a subacute, demyelinating and remitting event involving the cutaneous and trunk nerves. The invasion of Schwann cells and axons by *M. leprae* leads to demyelination and axonal degeneration.[1]

The specificity of bacteria-host cell interaction and the pattern of tissue distribution of host cell receptors determines the tissues which are ultimately infected by a pathogen.[2] A recent advance in understanding the pathogenesis of leprosy—in particular, the affinity of *M. leprae* to the Schwann cell—is the discovery of host Schwann cell proteins that bind to *M. leprae*. Receptor-mediated mechanisms have been suggested to be involved in Schwann cell–*M. leprae* interaction.[3] Rambukkanna, *et al.* (1998), showed that laminin (LN)-α dystroglycan (α DG) bridge present in the basal lamina of Schwann cells could mediate the entry of *M. leprae*.[4] Subsequently, a complementary 21-kDa protein present on the *M. leprae* surface was shown to bind to laminin, explaining the neural affinity of *M. leprae*.[5] However, other proteins such as fibronectin (FN),[6, 7] β integrin[6] and a 25-kDa glycopro-

Abbreviations used are as follows: α DG = alpha dystroglycan, CIG = cold insoluble globulin, DAPC = dystrophin-associated protein complex, EHS-tumor LN = Engelbreth-Holm-Swarm-tumor laminin, FN = fibronectin, LAM = lipoarabinomannan, LCMV = lymphocytic choriomeningitis virus, LNα2G = laminin merosin heavy chain, PO = myelin protein.

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tein[8] have also been shown to bind to *M. leprae.*

Although *M. leprae* is known to bind to different proteins, their relative role in pathogenesis is not yet clearly defined. Here we have reviewed some of the biochemical aspects of *M. leprae* binding proteins, and have followed with a discussion of the tissue proteins that were found to bind to *M. leprae.*

FIBRONECTIN (FN)-β INTEGRIN

Byrd, *et al.* (1993),[6] carried out experiments on nasal septal cell interactions with *M. leprae.* The methodology involved studies on *M. leprae* binding to nasal epithelial cells in the presence and the absence of various modulators, such as mannoside, β galactoside, fibronectin, antibodies to fibronectin, CD11a, CD29, CD54 and also to a peptide gly-arg-gly-arg-ser (The Table). The result was evaluated either by an ELISA or visual observation of *M. leprae* binding. The study showed that *M. leprae* binds to nasal epithelial cells after binding to fibronectin, using β-integrins as receptors. [The corresponding component on *M. leprae,* which binds to fibronectin (Fibronectin binding protein FAP) has been identified and characterized.[7, 9] It is a protein of 29.5-kDa and has sequence homologies with other mycobacteria—*M. vaccae, M. avium* and *M. tuberculosis.*] Fibronectin significantly enhanced both attachment and ingestion of *M. leprae* by T24 epithelial and JS1 Schwannoma cell lines.

Interestingly, further experiments showed that even in the absence of soluble fibronectin *M. leprae* was found to bind to the nasal epithelial cells, suggesting an independent β-integrin–*M. leprae* interaction. β-Integrin is a known receptor for fibronectin.[6] Therefore, fibronectin–integrin mediated binding mechanisms could play a role in the pathogenesis of cells expressing these proteins.

Fibronectin is also known as cold insoluble globulin (CIG) with a molecular weight of 262.05-kDa. It binds to cell surfaces and to various compounds including collagen, fibrin, heparin, DNA and actin. FNs are involved in cell adhesion, cell motility, opsonization, wound healing and maintenance of cell shape.[10, 11, 12] FN occurs mostly as heterodimers, multimers of alternatively spliced variants, connected by 2 disulfide bonds near the carboxyl ends. Plasma FN (soluble dimeric form) is secreted by hepatocytes and cellular FN (dimeric or cross linked multimeric forms) is synthesized by fibroblasts, epithelial and other cell types.[12, 13] FN is deposited as fibrils in the extracellular matrix. Phosphorylation, glycosylation and sulfation sites are present in this protein.[13]

β-Integrin (also known as fibronectin receptor, Beta sub unit CD29 or integrin VLA4 beta sub unit) has a molecular weight of 88.465-kDa. It is a type-I membrane protein and is a receptor for fibronectin, laminin and vitronectin.[14] It is widely expressed in skin, liver, skeletal muscle, cardiac muscle, placenta, umbilical vein, endothelial cells, neuroblastoma cells and astrocytoma cells.[15, 16] It is a transmem-

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Mastek, M., Clark-Curtiss, J. E., Ratliff, J. L. and Brown, E. J. A *Mycobacterium leprae* gene encoding fibronectin binding is used for efficient invasion of epithelial cells and Schwann cells. Infect. Immun. **63** (1995) 2652–2657.

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brane receptor protein present in the extracellular matrix and cytoskeleton.[17] This protein is phosphorylated and glycosylated.[18]

LAMININ 2-α DYSTROGLYCAN/ β-INTEGRIN

The laminin-2 isoform is present at the Schwann cell-axon units, in the peripheral nerves. It is composed of α2 heavy chains together with β1 and γ1 light chains. However, the α2 chain has a tissue restricted distribution, predominately expressed in the basal lamina of Schwann cells and muscles, while the β1 and γ1 chains have a wide range of distribution.[19–23] Laminin-2 or laminin merosin heavy chain (LNα2G) has a molecular weight of 342.766-kDa. This glycoprotein binds to cells via a high affinity receptor such as α-dystroglycan (αDG) or β-integrin. It is present in the placenta, striated muscle, peripheral nerve, cardiac muscle, pancreas, lung, spleen, kidney, adrenal gland, skin, testis, meninges, choroid plexus and brain, but is not present in liver, thymus or bone.[24, 25]

α-Dystroglycan or dystrophin-associated glycoprotein (molecular weight 97.5-kDa) is a type-I extracellular membrane protein. This protein has phosphorylation and glycosylation sites. It is present in a variety of adult and fetal tissue. It forms a dystrophin-associated protein complex (DAPC), which may link the cytoskeleton to the extracellular matrix. αDG not only binds to *M. leprae* but also to several types of arena viruses [lymphocytic choriomeningitis virus (LCMV), Lassa fever, Oliveros and Mobala viruses[26]].

Dystroglycan is a laminin receptor encoded by a single gene and cleaved by post-translational processing into two proteins, the peripheral membrane αDG and transmembrane βDG.[27] While αDG interacts with laminin-2 in the basal lamina, βDG appears to bind to dystrophin-containing cytoskeletal proteins in muscles and peripheral nerve.[28] The loss or a defect of laminin 2-αDG interaction causes certain types of muscular dystrophy and peripheral neuropathy.[4, 29] Experiments were carried out on immobilized αDG binding to *M. leprae* in the presence of different modulators. This binding was assessed by ELISA. The other experiments were on Schwann cell cultures with and without modulators such as human FN, type IV collagen, murine Engelbreth-Holm-Swarm-tumor laminin (EHS-tumor LN), etc. (The Table).[4, 30] The results showed that αDG participates in LNα2G mediated *M. leprae* interaction with Schwann cells. *M. leprae* binding to LNα2G was increased by >95% with increased concentration of LNα2G. However,

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THE TABLE. *A review of M. leprae-binding proteins of host cells and methodologies.*

Reference	Source of *M. leprae*	Cells	Type of binding assay	Time of assay	Modulators of the binding protein	Conclusion
1. Byrd, *et al.;* 1993, Clin. Immunol., Immunopatho. **69**, 266–271.	Armadillo derived 10^7/ml	Human nasal septal cell line	—Uptake & counting, No. of bacteria for 300 cells —ELISA binding assay	2 hr	Fibronectin 20-ln–200 µg/ml α mannoside 1–4% β galactoside 1–4% Ab[a] to Fn[b] 20–200 µg/ml Ab to CD11 10–40 µg/ml Ab to CD54 10–40 µg/ml Ab to CD29 10–40 µg gly-arg-gly-arg-ser peptide 50 µg/ml	*M. leprae* binds to nasal epithelial cells after binding to fibronectin and uses β-Integrins as receptors.
2. Shorey, *et al.;* 1995, Infect. Immun. **63**, 2652–2657.	Armadillo derived irradiated 10^5/ml	—JS1 Schwannoma cells —T24 epithelial cells	—Uptake & counting, No. of bacteria for 100 cells —ELISA binding assay	3 hr	FN 50 µg/ml Ab to FN 50 µg/ml Ab to FN adherence protein (FAP) 100 µg/ml	*M. leprae* appears to use fibronectin as a conventional opsonin.
3. Rambukkanna, *et al.;* 1997, Cell, **88**, 811–822.	Armadillo derived 10^8/ml	—Primary rat Schwann cells —Dorsal root ganglion culture (DRG) —Human mammary cell line HBL-100 —Human erythroleukemic cell lines K562, Cos-7	—ELISA binding assay —Uptake and counting, No. of bacteria for 100 cells	1 hr	*Matrix proteins:* LN-2[c] 20–200 µg/ml; Type 4 collagen 200 µg; Murine EHS[d]-tumor LN(LN-1); Human FN; Heparin sulfate proteoglycan *Affinity purified Ab's:* pAb[e] to Human Placental LN, LN-2/4 and EHS-LN (LN-1); mAb[f] to γ-1/β1 LN chain. β-2/S LN chain; pAb to 300-kDa fragment of α2LN; pAb to fragment LNα4/5 mAb to type IV collagen; mAb to α2/M LM	Molecular basis of neurotropism of *M. leprae* is attributable to the specific binding of *M. leprae* to laminin-2 on the Schwann cells axon units.

[a] AB = antibody
[b] FN = fibronectin
[c] LN-2 = laminin
[d] EHS = Engelbreth-Holm-Swarm
[e] pAb = affinity purified antibody
[f] mAb = monoclonal antibody

THE TABLE. *Continued.*

Reference	Source of *M. leprae*	Cells	Type of binding assay	Time of assay	Modulators of the binding protein	Conclusion
4. Rambukkanna, *et al.;* 1998, Science, **282**, 2076–2079.	Armadillo derived 10^8/ml	—Rat Schwann cells —Immortalized human Schwann cells	—ELISA binding assay —Uptake and counting, No. of bacteria for 100 cells	2 hr	*Matrix proteins:* αDG 50 μg Human placental LN Murine EHS tumor LN Human FN Heparin sulphate proteoglycan Human Merosin (rLNα2G, LN-2/4) Human Type IV collagen *Abs: 100 μg/culture* mAb to β1 integrin mAb to β4 integrin pAb to COOH terminus of β4 mAb to α6 of integrin mAb11h6ch	α-Dystroglycan was shown to serve as a Schwann cell receptor for *M. leprae* binding. This binding occurred only in the presence of laminin-2.
5. Suneetha, *et al.;* 1998, Neurochem. Res. **23**, 909–913.	—Human leproma derived —Mouse footpad derived 10^7/ml	Human peripheral nerve, proteins partially purified 25-kDa glycoprotein from human peripheral nerve	—Nitrocellulose blot assay —Binding assay in a microfuge tube	2 hr	Pronase treatment, lipase, per iodate treatment; Peripheral nerve phosphorylated proteins.	*M. leprae* binds to a 25-kDa phosphorylated glycoprotein from the human peripheral nerve.

similar results were also obtained with a mixture of LN-2 and LN-4. Glycosylation and Ca^+ ions seem to regulate αDG's interaction with *M. leprae* through the LNα2G domain.[4] Schwann cell experiments in the presence of antibodies to αDG did not completely inhibit binding. Additionally, purified αDG was unable to compete 100% for LNα2G-mediated binding suggesting other Schwann cell laminin receptors. Hence, the experimental data on laminin and αDG interaction do not exclude other mechanisms of *M. leprae* binding to Schwann cells.

Among the several forms of integrins the α6β4 integrins, particularly the β4 subunit, appear to be involved in *M. leprae* binding.[31] Further experimentation by Rambukkanna, *et al.* (1998),[4] showed that the HBL-100 and Cos-7 cells which strongly express α6β4 integrins—the laminin receptors—exhibited significant adherence of *M. leprae,* whereas erythroleukemic k562 cells which lack known LN receptors including α6β4 integrins showed negligible binding. Hence, LNα2G-associated *M. leprae*-Schwann cell binding is also mediated by α6β4 integrin receptors on Schwann cells.

25-kDa HUMAN PERIPHERAL NERVE GLYCOPROTEIN

Protein phosphorylation is a post-translational modification of proteins important in signal transduction and is prominent in nerve tissue.[32, 33] Recent studies have shown the involvement of phosphorylation events in binding of bacteria to host cells.[34–36] Earlier studies have shown that purified *M. leprae* could inhibit the phosphorylation of 28-kDa to 30-kDa protein of the rat peripheral nerve.[37] One of the mechanisms proposed for this observation is the interaction between *M. leprae* and phosphorylated proteins. Binding experiments revealed that *M. leprae* bound to a major 28-kDa to 30-kDa protein and a few minor proteins of 45-kDa to 50-kDa range. However, *M. bovis* and *E. coli* also bound to these phosphorylated proteins but to a significantly lesser extent.[38] Similar observations were found with phosphorylated human peripheral nerve binding experiments (The Table). Biochemical characterizations of the 25-kDa protein showed that it is a major phosphorylated protein of the human peripheral nerve, which was found to bind to *M. leprae.*[8] It is a complex carbohydrate containing protein. When Triton X-100 was excluded in the homogenization buffer, the yield of this protein was negligible suggesting that it may be a membrane bound protein.

Protein phosphorylation and autoradiography studies of the human peripheral nerve proteins showed a major band of a range of 23-kDa to 28-kDa. This could be due to a mobility shift caused by heterogeneous phosphorylated species.[39] Binding of this 25-kDa glycoprotein to the lectins, artocarpin and concanavalin A to a major extent, and to winged bean agglutinin and jacalin to a minor extent, indicated that the lectin binding epitopes have branched N-linked oligosaccharides with mannose and galactose residues similar to that reported for the P0 protein of human peripheral nerve myelin.[8, 40] Earlier, other groups had

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extensively studied the phosphorylation and glycosylation of the myelin protein (P0) from human and rat peripheral nerve, using SDS-PAGE and autoradiography.[41, 42] Hence, the biochemical characteristics of the 25-kDa protein namely its molecular weight, carbohydrate content and phosphorylatable nature are similar to those reported for the P0 protein of peripheral nerve myelin.[43–46] Further work recently carried out in our laboratory has immunologically identified the 25-kDa protein as P0 of the peripheral nerve myelin (unpublished observation). *M. leprae* binding to P0 (the 25-kDa glycoprotein) could have a major implication as it would throw light on the *M. leprae*-target interaction and consequent pathological manifestations such as demyelination and axonal degeneration. P0 is a highly abundant, phosphorylated and glycosylated membrane protein of the human peripheral nerve that has two major domains: an extracellular immunoglobin-like domain and an intracellular basic region.[47] It is known to be involved in myelin compaction.[48, 49]

OTHER STUDIES AND LIMITATIONS

Other experiments have been carried out in different cells on *M. leprae* binding and internalization. A major difficulty in interpreting all of these reports is that the conditions for the *in vitro* binding studies were different with respect to the source of Schwann cells or the source, storage and pre-treatment of *M. leprae*. Glial cell lines display no specificity in the uptake of *M. leprae*, whereas Lewis TC98 Schwann cells which mimic mouse Schwann cells show a preferential uptake of *M. leprae*, and not heat-killed *M. leprae* or *M. lepraemurium*.[50] Observations on human nerve-teased fiber preparation showed that Schwann cells engulfed *M. leprae*, *M. tuberculosis* and carbon particles without any discrimination.[51] In another study, *M. leprae* uptake was blocked by anti-mycobacterial antibodies directed against phenolic glycolipid-1 in disassociated Schwann cells suggesting the involvement of phenolic glycolipid.[52] LAM (lipoarabinomannan) and PGL-I (phenolic glycolipid-I) of *M.*

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leprae has been shown to bind to macrophages and human peripheral nerve.[53, 54, 55] Binding and internalization of *M. leprae* by human endothelial cells has recently been demonstrated, but the mycobacterial and host cell molecules involved have yet to be identified.[56]

Considering these varied observations on tissue culture and human nerve experiments, binding and internalization may not necessarily be directly related, and each may or may not have significance in pathogenesis and neural predilection. That is, in simple biochemical terms, all binding may not lead to internalization and all internalization may not mean neural predilection.

CONCLUSION

α-Dystroglycan, laminin-2, β-integrin, fibronectin and the 25-kDa glycoprotein (P0) are all membrane glycoproteins of phosphorylatable nature present in different tissues, and they all have an affinity to bind to *Mycobacterium leprae*. The only protein, with nerve tissue specificity, is P0 and whether it adds on to the present understanding of *M. leprae*'s neural predilection needs further experimentation. The molecular mechanisms of the relative binding affinities of *M. leprae*-binding proteins, their specificity of binding to *M. leprae*, their interaction with other membrane glycoprotein complex components, influence of phosphorylation, glycosylation, other modulators such as calcium, the complementary components on the surface of *M. leprae* (FAP/21-kDa surface protein/PGL/any other membrane components), and also the host/bacterial signalling mechanisms which could stimulate *M. leprae* to multiply in Schwann cells are areas which need further research.

Since we now have the whole *M. leprae* gene sequence, computational studies on homology modelling of *M. leprae*-binding proteins and their ligands on *M. leprae* may provide new directions in understanding the pathogenesis of nerve injury in leprosy.

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