Universidade Federal do Triângulo Mineiro

Marcela Rezende Lemes

Prospecção *in silico* de proteína para diagnóstico multiepítopo de Hanseníase

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Marcela Rezende Lemes

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Orientador: Prof. Dr. Marcos Vinícius da Silva
Co orientador: Prof. Dr. Siomar de Castro Soares

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Resumo

A hanseníase é uma doença causada por *Mycobacterium leprae* and *M. lepromatosis***,** as quais não são cultiváveis. Ela afeta primariamente países de baixa renda e pode levar até dez anos para manifestar sinais clínicos, que são a base de sua detecção. Para enfrentar esse problema, foi proposto uma proteína multiepítopo para o diagnóstico da hanseníase. Foram recuperadas 22 proteínas do banco de dados do NCBI e por meio de ferramentas de bioinformática foram preditos os melhores epítopos para células T e B contidos nessas proteínas. Após múltiplos filtros e análises de pontuações chegou-se a 29 epítopos, que foram, então, fundidos em um construto. As estruturas secundárias e terciárias desse constructo foram preditas e refinadas para incluir os resíduos de aminoácidos na melhor conformação possível. A proteína multiepítopo construída é estável, não apresenta homologia com o proteoma humano e não é nem alérgena ou tóxica. O constructo apresenta dois epítopos conformacionais de células B e tem potencial para induzir a produção de IFN-γ, IL-4 e IL-10. Com resultados tanto na resposta humoral quanto na celular, essa proteína terá condições de diagnosticar a hanseníase sem muita dificuldade por detectar ambos os polos da doença.

Palavras-chave: Bioinformática. Imunoinformática. Hanseníase. Multi-epítopo. Diagnóstico. Proteína quimérica.

Abstract

Leprosy is caused by *Mycobacterium leprae* and *M. lepromatosis*, both non-cultivable bacteria. It affects primarily low-income countries and may take up to 10 years to show any clinical signs, which is how physicians diagnose it. To tackle this issue, we propose here a multi-epitope protein for its diagnosis. We retrieved 22 proteins from NCBI and, using bioinformatics tools, predicted the best epitopes for B and T cells on these proteins. After multiple filtering and scores analysis we ended up with 29 epitopes, which were than merged into one construct. Its secondary and tertiary structure were also predicted and refined to comprise the amino acid residues in the best conformation possible. The multi-epitope protein constructed is stable, does not have any homology with human proteome nor is allergenic or toxic. It has two conformational B cell epitopes and has the potential to elicit IFN-γ, IL-4 and IL-10 secretion. With results in both the humoral and cellular response, this protein will be able to diagnose leprosy without much difficult due to the detection of both ends of its spectrum.

Keywords: Bioinformatics. Immunoinformatics. Leprosy. Multi-epitope. Diagnosis. Hansen’s disease. Chimeric protein.

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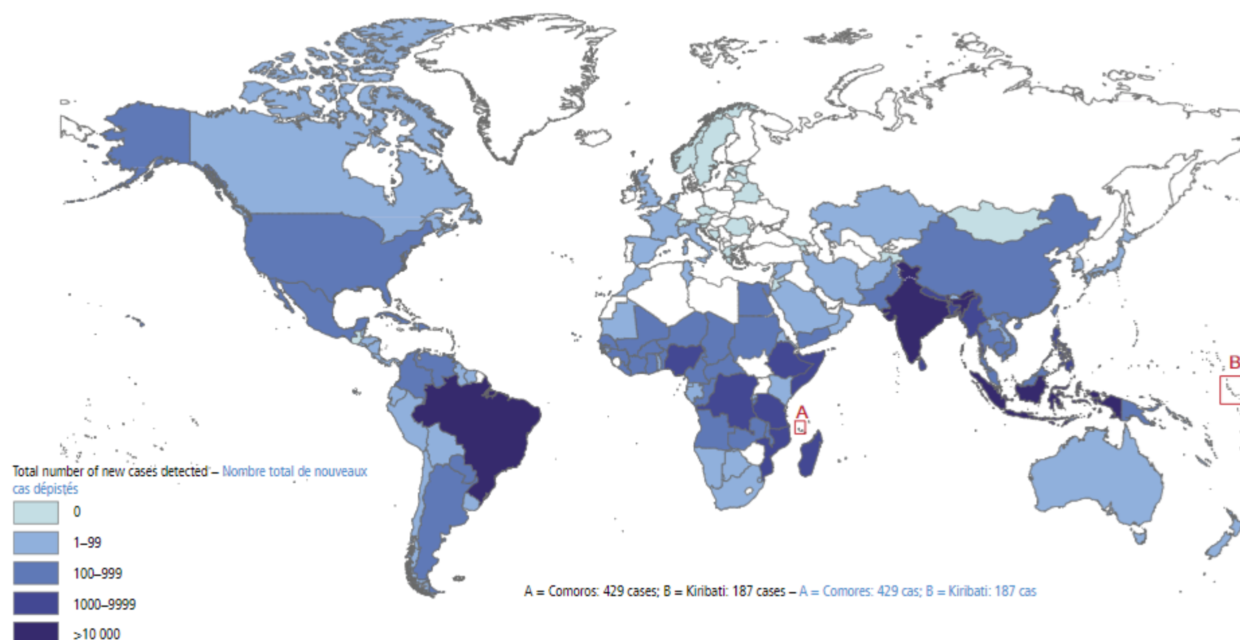
Introdução

Hanseníase

A hanseníase tem sido descrita desde a antiguidade (O'BRIEN; MALIK, 2017), entretanto seu agente etiológico só foi descoberto em 1873 por Gerhard Armauer Hansen (GRZYBOWSKI *et al.*, 2013). A bactéria de nome *Mycobacterium leprae*, assim como a *Mycobacterium lepromatosis*, descoberta em 2008 no México (HAN *et al.*, 2008), são bacilos álcool-ácido resistentes com uma diferença genética de apenas 9,1% (HAN *et al.*, 2009). Numa análise de 46 pacientes no Brasil, 10 (21,7%) foram detectados com *M. lepromatosis* (HAN *et al.*, 2014). Nenhuma das espécies são cultiváveis em meios de cultura, o que torna seu estudo mais desafiador (COLE *et al.*, 2001), necessitando do uso de animais para seu cultivo e isolamento e ambas conseguem infectar nervos periféricos (SCOLLARD, 2016).



Fonte: Adaptado de WHO (2018)

A doença de Hans, como também é conhecida a hanseníase, é uma doença dermatológica e neurológica periférica (DUTHIE *et al.*, 2018). Esses bacilos conseguem invadir as Células de Shwann (RAMBUKKANA, A., 1998), causando a desmielinização dos nervos periféricos por meio da desregulação

da comunicação entre nervo e célula (RAMBUKKANA, Anura, 2004), podendo gerar perda de sensação térmica, tátil e de dor (DUTHIE *et al.*, 2018), essas lesões nervosas são permanentes, não podendo ser revertidas com o uso de antibióticos (SHARMA *et al.*, 2020).

Considerada como uma das doenças infecciosas mais incapacitantes no mundo (DE OLIVEIRA, Ana Laura Grossi *et al.*, 2020), a hanseníase é também uma das mais estigmatizadas por alterar a aparência física do afetado, tendo sido inclusive considerada como uma punição de Deus em tempos remotos (MI; LIU; ZHANG, 2020). O período de incubação pode ser de até 11 anos (LOCKWOOD, D. N.J.; REID, 2001), sendo que a manifestação clínica consiste, na maioria das vezes, em lesões hipopigmentadas ou avermelhadas com perda de sensibilidade (OMS, 2016).

A resposta imunológica do hospedeiro, assim como seu *background* genético, impacta significativamente no aspecto clínico da doença (MI; LIU; ZHANG, 2020; NATH; SAINI; VALLURI, 2015), sendo esse muito diverso, compreendendo um espectro que varia desde a hanseníase tuberculóide, com altos níveis de resposta celular e baixa quantidade de bactérias até a hanseníase lepromatosa, também conhecido como hanseníase Virchowiana, com alta quantidade de bactérias e baixos níveis de resposta celular (SHARMA *et al.*, 2020).

Apesar do tempo de conhecimento da doença, seu diagnóstico ainda é um desafio, pois ainda se dá, em sua maioria, clinicamente, já que testes sorológicos ou até mesmo biopsias podem não ser definitivos ou não estarem disponíveis em locais endêmicos (MEDLEY *et al.*, 2018; OMS, 2016). Outro fator importante para o diagnóstico são os profissionais treinados para isso, os quais estão diminuindo cada vez mais (DUTHIE *et al.*, 2010, 2007).

Espectros Clínicos e Resposta Imunológica (RI)

Espectros Clínicos

Ridley e Jopling classificaram a hanseníase em cinco tipos de acordo com a clínica, histopatologia, quantidade de bactérias e nível de resposta

celular (RIDLEY; JOPLING, 1966): tuberculóide-tuberculóide (TT): algumas lesões com margens definidas sem presença do bacilo e com infiltrado inflamatório constituído por focos de células epitelióides cercadas por linfócitos; lepromatoso-lepromatoso (LL): múltiplas lesões com infiltrado inflamatório composto majoritariamente por macrófagos, poucos linfócitos e alguns plasmócitos. As demais classificações (borderline-tuberculóide – BT; borderline-borderline – BB; borderline-lepromatoso - BL) estão entre essas duas, podendo todas transitar para uma ou outra a depender da RI do hospedeiro. A hanseníase indeterminada não entra nessa classificação pois não há correlação entre clínica e histopatologia, pois ainda é o estágio inicial da doença, com a ausência de uma resposta celular clara (TALHARI; TALHARI; PENNA, 2015).

Na clínica essa classificação é feita por quantidade de lesões: Paucibacilar (PB), com até cinco lesões ou Multibacilar (MB), com mais de cinco lesões (MINISTÉRIO DA SAÚDE, 2016). O diagnóstico da hanseníase no Brasil é essencialmente clínico e epidemiológico, com identificação de lesões e comprometimento neurológico e histórico de contato com outros pacientes. Quando disponível, faz-se a baciloscopia, que pode dar positivo, classificando o caso como MB, ou negativo, o que não descarta o diagnóstico clínico e nem classifica como PB (MINISTÉRIO DA SAÚDE, 2016).

Resposta Inata

Macrófagos e células dendríticas (CD) merecem destaque na hanseníase. Os macrófagos são as células mais estudadas por pesquisadores nessa doença, já que são os principais hospedeiros do bacilo (MI; LIU; ZHANG, 2020). Assim que adentra o organismo, o *Mycobacterium* é reconhecido por receptores de padrões moleculares associados à patógenos (PAMPs), os quais são expressos tanto em macrófagos quanto em células dendríticas (MODLIN, 2010), estando os receptores do tipo toll (TLRs) como TLR2 e TLR4 correlacionados ao desenvolvimento da resposta imune a *M. leprae* (BOCHUD; HAWN; ADEREM, 2003).

A polarização dos macrófagos em M1 (clássico) ou M2 (alternativo) ocorre por estímulos de citocinas. A ligação de IFN-γ e TNF-α a macrófagos M0 induzem sua polarização a M1 na hanseníase (DE SOUSA *et al.*, 2016; DE SOUSA; SOTTO; QUARESMA, 2017), levando essa célula a produzir citocinas pro inflamatórias e a enzima óxido nítrico sintase induzível (iNOS), a qual irá gerar óxido nítrico (NO) (LOCKWOOD, Diana N.J. *et al.*, 2011). Entretanto, estudos demonstraram que o *M leprae* consegue induzir a produção de NOS por meio do glicolipídio fenólico I (PGL-I) molécula exclusiva de *M. leprae* e *M. lepromatosis*, podendo gerar assim danos nas células de Shwann (MADIGAN *et al.*, 2017). O PGL-I é reconhecido pela proteína C3 do complemento, sendo que macrófagos possuem o receptor para ela, a bactéria utiliza disso invadir a célula, além de ser responsável por uma possível interferência na sinalização de IFN-γ, inibindo a polarização do macrófago em M1 (MADIGAN *et al.*, 2017).

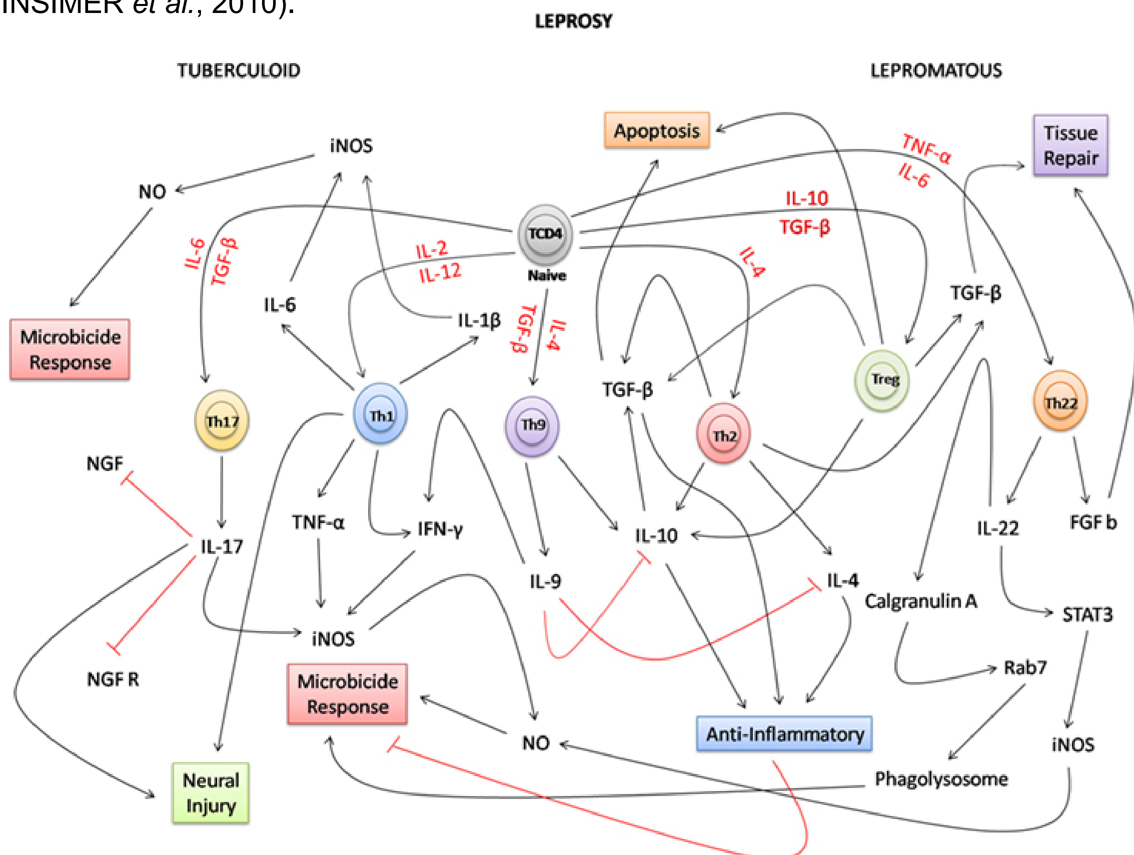
A polarização alternativa dos macrófagos (M2) foi relacionada com a forma lepromatosa da doença, sendo essa polarização induzida pelo meio contendo citocinas anti-inflamatórias como IL-4, IL-10 e IL-13 (DE SOUSA *et al.*, 2016). Esses macrófagos estão relacionados com reparo tecidual e estimulam a produção de arginase, a qual induz a produção de TGF-β e FGF-b, os quais já foram demonstrados como importantes em mecanismos apoptóticos, regulação de fibroblastos para reparo e ainda proliferação de células endoteliais e indução de angiogênese (BARBAY *et al.*, 2015; FULCO *et al.*, 2014; MONTOYA *et al.*, 2009; WIJNANDS *et al.*, 2015).

As Células de Langerhans (CL) são CDs residentes da epiderme e expressam CD1a e CD207, que são moléculas de apresentação de lipídeos (MI; LIU; ZHANG, 2020). As CL são as responsáveis pela aquisição dos antígenos e sua posterior apresentação (DANG *et al.*, 2019) e estudos apontam que elas estão mais presentes na área da epiderme lesionada de indivíduos com a forma tuberculoide da doença em comparação a indivíduos com a forma lepromatosa (GIMENEZ; GIGLI; TAUSK, 1989; HIRAI *et al.*, 2016; SIMÕES QUARESMA *et al.*, 2009). Sua presença mais acentuada em pacientes com a forma tuberculoide corrobora com a caracterização da resposta imunológica celular ser mais presente nessa forma da doença, ocorrendo o contrário na

forma lepromatosa, a qual possui uma resposta humoral mais acentuada (SHARMA *et al.*, 2020).

Resposta Imune Adaptativa

Classicamente, a resposta imune adaptativa na hanseníase é explicada pelo paradigma Th1/Th2 (MODLIN, 1994; SPELLBERG; EDWARDS, 2001), que ainda continua muito presente nos estudos recentes apesar da descoberta de outros padrões de resposta (ABDALLAH *et al.*, 2014; DE SOUSA; SOTTO; QUARESMA, 2017; MI; LIU; ZHANG, 2020; NATH; SAINI; VALLURI, 2015; SINSIMER *et al.*, 2010). Sua caracterização é dada por uma resposta Th1 no polo tuberculoide com IL-2, IFN-γ e IL-12 como principais citocinas e a expressão de CCR5 e CXCR3 em linfócitos, sendo ambos receptores de quimiocinas. O polo lepromatoso cursa com a presença de IL-4, IL-5 e IL-10 e expressão de CCR4, CCR8 e CCR3 numa menor quantidade (ABDALLAH *et al.*, 2014; DE SOUSA; SOTTO; QUARESMA, 2017; MI; LIU; ZHANG, 2020; NATH; SAINI; VALLURI, 2015; SINSIMER *et al.*, 2010).



Fonte: DE SOUSA; SOTTO; QUARESMA, 2017

E apesar das manifestações histopatológicas de cada um dos polos da hanseníase serem condizentes com a reposta imune acima citada, ela é mutável, de forma que o paciente pode ir de um polo ao outro da resposta, mudando completamente sua manifestação clínica (MI; LIU; ZHANG, 2020).

Tratamento

Os primeiros tratamentos quimioterápicos propostos para a hanseníase foram as sulfonamidas (FAGET; JOHANSEN, 1942), entretanto o primeiro sucesso veio apenas em 1943 com Promina, nos Estados Unidos, marcando a história do tratamento da doença (FAGET *et al.*, 1943). Em 1949 foram publicados estudos com ótimos resultados usando Dapsona (DDS) em doses menores devido à alta toxicidade (COCHRANE *et al.*, 1949; FAGET *et al.*, 1943). A resistência à sulfonas foi comprovada apenas em 1962, gerando medo pelo perigo do que isso representava no combate à hanseníase (SHEPARD; CHANG, 1962). Apesar de ter sua eficácia comprovada, a clofazimina não foi usada em grande escala, ficando apenas como um fármaco de segunda escolha para pacientes que não toleravam o uso de Dapsona. Entretanto hoje ela é uma das drogas da poliquimioterapia (PQT) recomendada pela Organização Mundial da Saúde (OMS) (WHO, 2018).

No Brasil o tratamento é o preconizado pela OMS, sendo dividido entre adultos e crianças, MB e PB e ocorre da seguinte forma: Rifampicina (RFM) e DDS durante 6 a 8 meses na hanseníase PB e RFM, DDS e Clofazimina (CFZ) entre 12 a 18 meses na hanseníase MB. A diferença entre adultos e crianças está na dose apenas, sendo todo o tratamento ambulatorial, com doses mensais e supervisionadas (MINISTÉRIO DA SAÚDE, 2016).

Diagnóstico

O diagnóstico da hanseníase é de suma importância tanto para sua perpetuação em uma região, pois o contato com pacientes multibacilares (MB) (com muitas bactérias) é um fator de risco para contração da bactéria (CORSTJENS *et al.*, 2019; DO CARMO GONÇALVES *et al.*, 2020), quanto para o aumento de chance de sequelas permanentes (MEDLEY *et al.*, 2018; VAN HOOIJ *et al.*, 2019). Muitas pessoas infectadas demoram a desenvolver sinais e sintomas clínicos, dificultando ainda mais esse diagnóstico (MEDLEY *et al.*, 2018; VAN HOOIJ *et al.*, 2019), já que ele é feito clinicamente em áreas endêmicas, o que é dificultado pela diminuição de profissionais com expertise em hanseníase (CORSTJENS *et al.*, 2019).

Figura 2 - Bacilo de Hansen (*M. leprae*) corado pelo método de Ziehl-Neelsen no esfregaço de linfa

Fonte: Moreira e col., 2006

Exames de baciloscopia também são empregados, entretanto em casos de hanseníase paucibacilar (PB) (com poucas bactérias), muitos são os casos em que essa baciloscopia é negativa, sendo que isso não exclui o diagnóstico de hanseníase feito pelo clínico (GROSSI DE OLIVEIRA *et al.*, 2020; MINISTÉRIO

DA SAÚDE, 2016). Uma forma muito precisa de diagnóstico é o uso da Reação em Cadeia da Polimerase (PCR), entretanto seu alto custo e necessidade de um ambiente controlado para uso, ou seja, em laboratório, dificulta sua utilização em locais mais isolados ou de baixa renda (MAYMONE *et al.*, 2020; MUNGROO; KHAN; SIDDIQUI, 2020; VAN HOOIJ *et al.*, 2017).

Uma revisão sistemática de 2019 fez um levantamento de estudos que buscaram diagnóstico sorológico por meio da pesquisa de anticorpos com diferentes antígenos (GROSSI DE OLIVEIRA *et al.*, 2020). Nesse artigo foram revisados 13 estudos, dos quais foi concluído que apesar de muitos desses antígenos apresentarem boas respostas na detecção de anticorpos em pacientes MB, entretanto os pacientes PB apresentam uma baixa resposta humoral, contrastando com sua resposta celular. Portanto, a chave para uma melhor ferramenta diagnóstica seria a detecção de ambas as respostas (humoral e celular) em apenas um teste (VAN HOOIJ *et al.*, 2017).

Ferramentas de bioinformática para aprimoramento diagnóstico

Com o aumento de informações provenientes da aplicação de tecnologias de alta performance na genômica e proteômica, foram desenvolvidas diversas ferramentas para se trabalhar esses dados, já que não é mais possível fazê-lo sem uma abordagem computacional.

Diversos algoritmos estão disponíveis para avaliação de proteínas em busca de epítopos de células B e T (DAVIES; FLOWER, 2007). Essas ferramentas utilizam métodos de treinamento para o aumento da assertividade dos preditores como redes neurais artificiais e métodos de matriz de pontuação juntamente com imensas bases de dados que são usadas para comparações (MARTINI *et al.*, 2020; VITA *et al.*, 2019). Outras ferramentas fazem a predição da estrutura terciária (KÄLLBERG *et al.*, 2012; KELLEY *et al.*, 2015; YANG; ZHANG, 2015), de parâmetros físico-químicos (GASTEIGER *et al.*, 2005) e melhora da estrutura (KO *et al.*, 2012) do produto construído (seja vacina ou proteínas para diagnóstico).

As ferramentas acima citadas são imensamente úteis na descoberta de peptídeos imunogênicos em proteínas, economizando tempo, dinheiro e até mesmo diminuindo o uso de animais por permitir múltiplas filtragens prévias antes de testes *in vitro* e *in vivo* (DAVIES; FLOWER, 2007). Doenças como hepatite B (DE SOUZA *et al.*, 2013), Chagas (DUTHIE *et al.*, 2016), criptococose (DE SERPA BRANDÃO *et al.*, 2018), leishmaniose (HEIDARI *et al.*, 2021; JAMEIE *et al.*, 2020), tuberculose (GUTIÉRREZ-ORTEGA *et al.*, 2021), entre outras, já possuem ótimos resultados com proteínas multiepítopo em diagnóstico.

Justificativa

O diagnóstico mais precoce da hanseníase é tido como um enorme desafio no cenário nacional pelo governo, sendo que no ano de 2015 o Brasil registrou mais de 26 mil novos casos, perdendo apenas para a Índia em todo o mundo, já em 2019 foram mais de 27 mil, mantendo o país em um parâmetro de alta endemicidade (SECRETARIA DE VIGILÂNCIA EM SAÚDE, 2020).

A utilização da bioinformática para fins diagnósticos permite uma abordagem *in silico*, sem o uso de animais, mais prática, rápida e barata, facilitando, dessa forma, a chegada da tecnologia até a população. Com essa ferramenta em uso os casos de hanseníase poderiam ser diagnosticados mais precocemente, diminuindo a possibilidade de sequelas permanentes e até mesmo as taxas de transmissão, demonstrando que o trabalho está de acordo com as demandas para cumprimento das metas do plano da OMS (OMS, 2016) para erradicação da doença.

Objetivos

Objetivo Geral

Encontrar peptídeos que sejam reconhecidos como epítopos para o diagnóstico da hanseníase.

Objetivos específicos

- Fazer a predição de epítopos de MHC I, MHC II e de células B
- Fazer a sobreposição dos epítopos humorais e celulares
- Construir uma proteína com os diversos fragmentos

Artigo

In silico Designing of a recombinant multi-epitope antigen for Leprosy Diagnosis

Marcela Rezende Lemes[A], Thaís Cristina Vilela Rodrigues[B], Sandeep Tiwari[B], Helioswilton Sales-Campos[C], Leonardo Eurípedes Andrade-Silva[D], Carlo Jose Freire Oliveira[A], Vasco Azevedo[B], Virmondes Rodrigues[A], Siomar C. Soares[A], Marcos Vinicius da Silva[A].

[A]Department of Immunology, Microbiology and Parasitology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro (UFTM), Uberaba 38025-180, MG, Brazil

[B]PG Program in Bioinformatics, Department of Genetics, Ecology and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte 31270-901, MG, Brazil

[C]Institute of Tropical Pathology and Public Health, Federal University of Goiás (UFG), Goiânia, Goiás, Brazil.

[D]Infectious Disease Department, Institute of Health Sciences, Federal University of Triângulo Mineiro (UFTM), Uberaba, MG, Brazil.

Corresponding author:

Sandeep Tiwari – sandip_sbti@yahoo.com

Postal address: Laboratory of Cellular and Molecular Genetics (LGCM)

PG Program in Bioinformatics,Department of Genetics, Ecology, and Evolution, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte 31270-901, MG, Brazil

Email list:

Marcela Rezende Lemes – marcela.lemes.r@hotmail.com

Thaís Cristina Rodrigues – thaiscristinavr@gmail.com

Sandeep Tiwari – sandip_sbti@yahoo.com

Helioswilton Sales-Campos – tonsales@hotmail.com

Leonardo Eurípedes Andrade-Silva – leonardoeuripedes@gmail.com

Carlo Jose Freire Oliveira – oliveiracjf@gmail.com

Vasco Azevedo – vascoariston@gmail.com

Virmondes Rodrigues – vrodrigues@mednet.com.br

Siomar C. Soares – siomars@gmail.com

Marcos Vinicius da Silva – marcosuftm@gmail.com

Abstract

Background: Leprosy is caused by *Mycobacterium leprae* and *M. lepromatosis*, both non-cultivable bacteria. It affects primarily low-income countries and may take up to 10 years to show any clinical signs, which is how physicians diagnose it. To tackle this issue, we propose here a multi-epitope protein for its diagnosis.

Method: We retrieved 22 proteins from NCBI and, using bioinformatics tools, predicted the best epitopes for B and T cells on these proteins. After multiple filtering and scores analysis we ended up with 29 epitopes, which were than merged into one construct. Its secondary and tertiary structure were also predicted and refined to comprise the amino acid residues in the best conformation possible.

Results: The multi-epitope protein constructed is stable, does not have any homology with human proteome nor is allergenic or toxic. It has two conformational B cell epitopes and has the potential to elicit IFN-γ, IL-4 and IL-10 secretion.

Conclusions: With results in both the humoral and cellular response, this protein will be able to diagnose leprosy without much difficult due to the detection of both ends of its spectrum.

Keywords: Bioinformatics; Immunoinformatics; leprosy; multi-epitope; diagnosis; Hansen's disease; chimeric protein

Introduction

Only in 2019 the WHO reported 202,226 new cases of Leprosy worldwide, with almost 80% of the cases in only 3 countries: India, Brazil and Indonesia (114,451; 27,863 and 17,439 respectively) (WHO, 2019). Leprosy is caused by *Mycobacterium leprae* and *M. lepromatosis*, which can invade Shwann cells (RAMBUKKANA, A., 1998) affecting both the dermis and peripheral nerves (DUTHIE *et al.*, 2018). The cell invasion causes nerve demyelization through nerve-cell communication deregulation (RAMBUKKANA, Anura, 2004). The damage to the myelin causes permanent loss of thermic, tactile and pain sensation (DUTHIE *et al.*, 2018). It may take up to 11 years until any clinical manifestation occurs, but even before that it is transmissible (MI; LIU; ZHANG, 2020; NATH; SAINI; VALLURI, 2015).

Immunological response in leprosy is highly dependent on host genetic background and it drives its clinic manifestations (MI; LIU; ZHANG, 2020; NATH; SAINI; VALLURI, 2015). Leprosy spectrum ranges from Tuberculoid Leprosy (TL) to Lepromatous Leprosy (LL). In TL the immune response is more cellular, with a Th1 profile with cytokines like IFN-γ, IL-2 and IL-12, on the other hand we have LL with a Th2/Th17 response, with more antibody titers, IL-10, IL-4 and IL-13 secretion and a higher bacillary load (DE SOUSA *et al.*, 2016; SHARMA *et al.*, 2020). Between the poles is borderline tuberculoid (BT), borderline-borderline (BB) and borderline lepromatous (BL) with mixed immunologic characteristics, ranging from the Th1 profile to the Th2/Th17 according to the poles (FROES; TRINDADE; SOTTO, 2020). The borderline presentations are where most of the patients fit and the nerve involvement is more severe, causing more disability (RIDLEY; JOPLING, 1966).

The leprosy diagnosis is mainly based on clinical and laboratorial evaluations. Due to the progressive cell damage the early diagnosis is very important. Actually, the best way to confirm leprosy is through bacilloscopic or PCR, since the bacteria is fastidious and do not grow in any culture media (SHARMA *et al.*, 2020), therefore, diagnosing leprosy in the lab is still a challenge. Most of the affected population lives in lower income country and do not have access to this type of technology (VAN HOOIJ *et al.*, 2017) and the serum or whole blood-based assays are not conclusive for all types of leprosy (VAN HOOIJ *et al.*, 2017). That leave this population with only the possibility of discovering the disease after clinical manifestation, increasing the transmission (DUTHIE *et al.*, 2010; SAMPAIO, Lucas H *et al.*, 2011), making it difficult to end the cycle.

Bioinformatics tools are of the outmost utility to assess immunogenic peptides within a protein, saving time, money and even diminishing the use of animals, since it provides multiple filters before *in vitro* and *in vivo* tests are performed (ACEVEDO *et al.*, 2020; DAVIES; FLOWER, 2007; DE SERPA BRANDÃO *et al.*, 2018; DE SOUZA *et al.*, 2013). Knowing that *M. leprae* and *M. lepromatosis* are not yet cultivable in any culture media (SHARMA *et al.*, 2020), bioinformatics is the best way to assess its proteins and their immunogenic potential. It also provides the possibility to create multi-epitope constructs, which can hold several antigenic epitopes, differing from natural proteins or whole-cell preparations (OLIVEIRA, Tatiane R. *et al.*, 2008). Multi-epitope proteins can be used in Enzyme-Linked Immunosorbent Assay (ELISA), Lateral Flow Tests, biosensors and also, in cellular assays. That possibility may also increase sensitivity and specificity, given that we can assess homology with other microorganisms and permits the

fusion of epitopes from different proteins from different sites of the organism. The multi-epitope constructs enables a higher immunogenic epitope density and diminishes the cross-reaction risk of whole bacterial antigen (YIN *et al.*, 2016). Diseases such as hepatitis B (DE SOUZA *et al.*, 2013), Chagas Disease (DUTHIE *et al.*, 2016), Cryptococcosis (DE SERPA BRANDÃO *et al.*, 2018), leishmaniosis (HEIDARI *et al.*, 2021; JAMEIE *et al.*, 2020), tuberculosis (GUTIÉRREZ-ORTEGA *et al.*, 2021), among others already have great results with multi-epitope proteins in their diagnosis. Here we propose a multi-epitope based antigen for leprosy diagnosis using bioinformatic tools.

Results

1.1 Prediction of B, CTL and HTL epitopes

All of the selected proteins had predicted antigenicity, as assessed by VaxiJen analysis, showing their capacity to be recognized as peptides of immunological relevance (Supplementary Table 1). The 21 proteins submitted at ABCpred generated a total of 729 epitopes and 2098 in LBtope. Using the *in-house* python script, we searched for overlapping epitopes that were predicted by both programs, in order to find common epitopes, and it returned 227 B cell shared epitopes (Supplementary Table 2). For cytotoxic T lymphocyte (MHC I) we used IEDB MHC-I Binding Predictions and NetCTL 1.2 server. 2273 epitopes were predicted by the former and 1146 by the latter, respectively, with 992 common epitopes predicted by the two software (Supplementary Table 3). IEDB MHC-II Binding Predictions and NetMHCII 2.3 were the tools used for MHC II epitope prediction, with 637 and 3734 epitopes, respectively. There were 586 common epitopes found (Supplementary Table 4). Figure 1A summarizes the filters.

1.2 Epitopes screening

In order to find epitopes with potential to induce both humoral and cellular immune responses, we applied the *in house* python script again, searching for overlaps between MHC II (637) and B (227) epitopes, with similarity of at least nine sequential residues. At this screening step, we reduced the total number of MHC II and B epitopes to 40 overlapping epitopes (Supplementary Table 5). Applying Class I Immunogenicity Tool, we predicted the 350 most immunogenic epitopes, with scores greater than 0.1 (Supplementary Table 5 and 6) using the previous common MHC I epitopes (992). Those 350 epitopes were, then, overlapped with the 40 ones resulted from humoral and cellular overlap, giving a total of 20 epitopes (Supplementary Table 5). Figure 1B summarizes the filters.

From the 20 selected epitopes, we compared the sequence and its percentile rank. MHC I epitopes with only one or two residues difference were excluded, using the percentile rank as guide, leaving the smaller ones (Table 1).

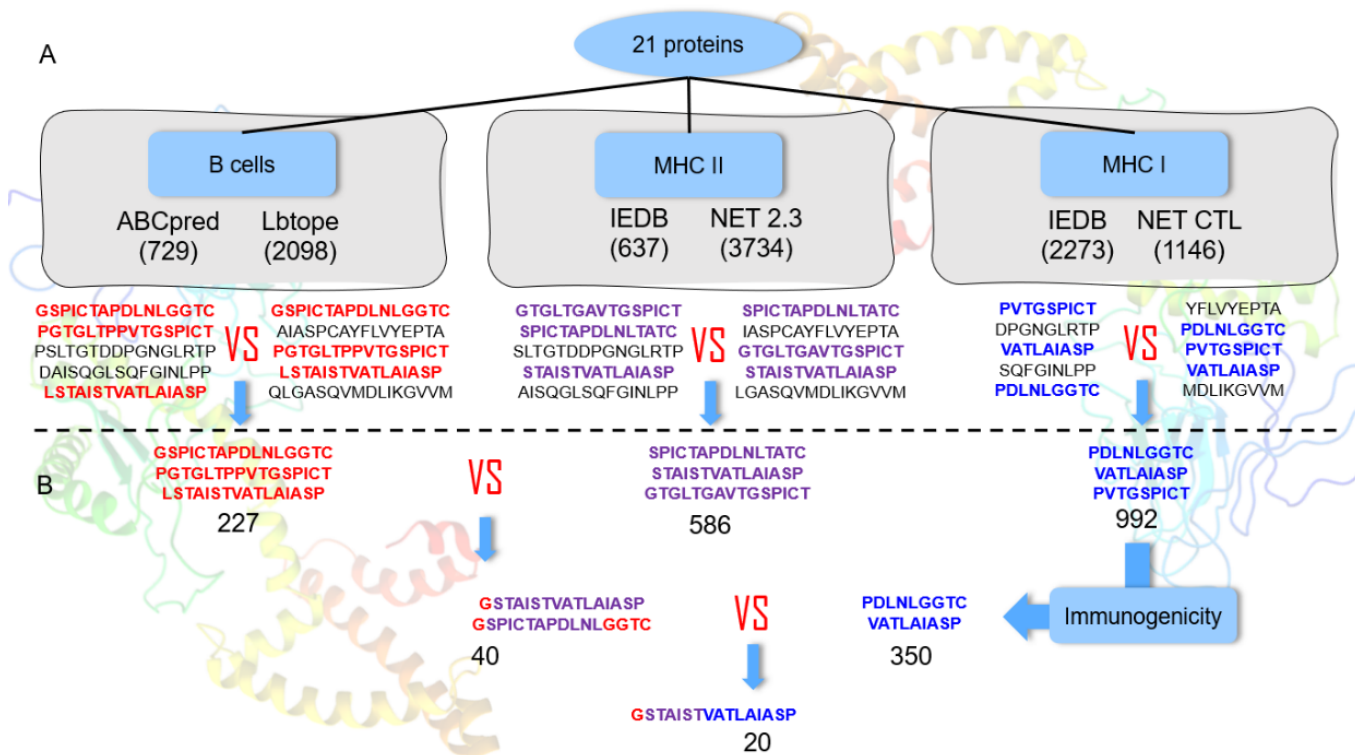


Figure 1 - Filters applied: A - Prediction of B, CTL and HTL epitopes. B - Epitopes screening

Protein	B/MHC II	PERCENTILE RANK	MHC I	PERCENTILE RANK
ML0091	TLAIASPCAYFLVYEP	8,3	SPCAYFLVY	0,05
ML2346	AVLWELGYRRFAYVDQ	5,5	LGYRRFAYV	0,95
			VLWELGYRR	0,35
			ELGYRRFAY	0,13
	GVTYHYIDVPARTFAS	3,7	YIDVPARTF	0,23
ML2380	HWGNWAKIFFNNKGVV	6,2	HWGNWAKIF	0,31
NP_301196.1	RWKWHDPYVHASLLAQ	2,6	RWKWHDPYV	0,53
NP_301958.1	GVLIFAAILVTGFLWP	2,7	VLIFAAILV	0,18
			VTGFLWPAW	0,88
			LVTGFLWPA	0,16
			FLWPAWLVT	0,12
			FAAILVTGF	0,52
			AILVTGFLW	0,51
NP_302056.1	MSTIFGQVTTKEKQCQ	1,4	IMSTIFGQV	0,16
NP_302185.1	VLVFDAHRGMVVGSPL	8,1	LVFDAHRGM	0,25
NP_302292.1	TNIGLVSCKRDVGAAV	2,4	MVVTNIGLV	0,25
NP_302342.1	TRFVAAHGAYLVWLEQ	1,1	FVAAHGAYL	0,1
NP_302503.1	TFTKPEILTRYLNLVS	2,1	KPEILTRYL	0,06

Table 1 - Final epitope selection

1.3 Multi-epitope sequence construction: Structural modelling, refinement and properties assessment

Different amino acid sequences were constructed in order to evaluate and select the one with the best structure quality. To give our construct flexibility to make its conformational changes, we joined these sequences with different peptide linkers since they assist in protein folding which is important for the conformational epitopes (FERDOUS *et al.*, 2019). For MHC I we used AAY linkers and for MHC II we used GPGPG, forming a 431 amino acid multi-epitope protein, however, we made changes in positions of epitopes. All sequencies were submitted to structural prediction in I-TASSER, Phyre2 and RaptorX. After, a Ramachandram plot was constructed for all the amino acid sequences.

The best structure quality obtained was the one modeled by RaptorX, with 86.2% of the residues in the most favored regions, 9.1% in the additional allowed regions, 2.1% in the generously allowed regions and 2.6% in the disallowed regions (Figure 2A and 2B). We performed the refinement with GalaxyWeb Server, getting the best result with model 5, with 86.6% of the residues in the most favored regions, 12.5% in the additional allowed regions, 0.6% in the generously allowed regions and 0.6% in the disallowed regions (Figure 2C and 2D).

1.5 Secondary structure prediction

According to PSIPRED and RaptorX, the tools used for secondary structure prediction, the 431-residue protein has 31% of Helicex 20% Beta-sheet and 47% Loop formation (Supplementary Figure 1).

1.6 Host homology and Physical-chemical properties

Host homology was performed through NCBI BlastP with *Homo sapiens* (taxid 9606) and no significant similarity was found.

The protein's molecular mass is 46779.35 (46.7 kDa), with a theoretical pI of 9.38, which means its behavior is in basic pH. The protein is considered stable since the instability index is 29.99. The aliphatic index is 91.79, also showing stability in changes of temperature. Positive GRAVY (great average of the hydropathy value) scores mean hydrophobicity, as our result is: 0.489.

The solubility analysis performed through Protein-Sol described the score 0.382, which is lower than the tool threshold, 0.45. The threshold corresponds to *E. coli* solubility and scores higher than 0.45 has a higher solubility average.

1.7 Antigenicity, cytokine-inducing potential, and conformational B cell epitopes

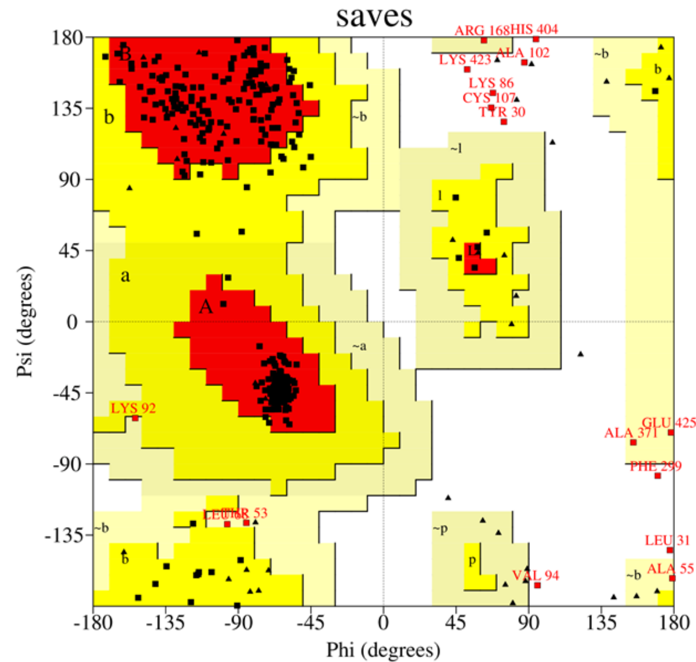
The application of multi-epitope protein in cell-based *in vitro* platforms depends on its ability to be antigenic and inducing cytokine production. The designed protein had a predicted antigenicity score 0.5596 through VaxiJen with means that is probably antigenic. The tool IFNepitope predicted two epitopes as probable IFN-γ inducer, IL-4Pred and ProInflam predicted five, IL-10Pred four epitopes. ElliPro predicted six linear and two conformational epitopes with scores greater than 0.7.

1

A PROCHECK

Ramachandran Plot

saves



Plot statistics

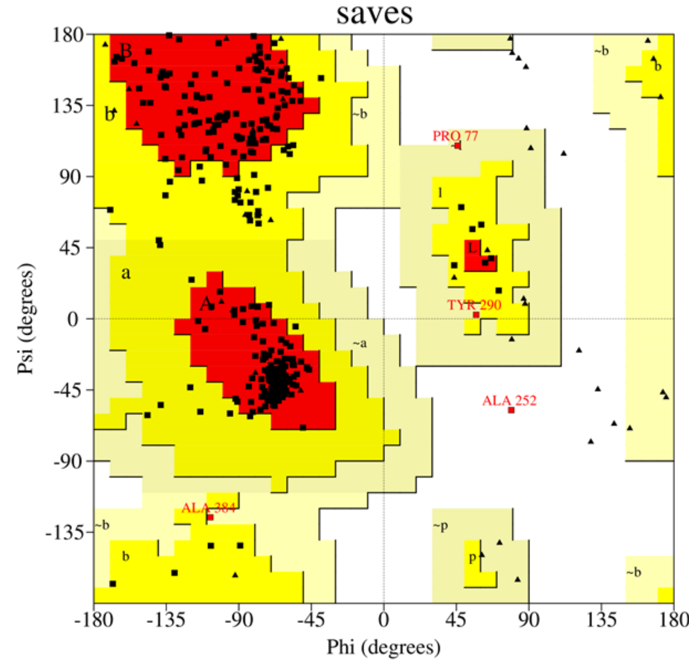
Residues in most favoured regions [A,B,L]	294	86.2%
Residues in additional allowed regions [a,b,l,p]	31	9.1%
Residues in generously allowed regions [~a,~b,~l,~p]	7	2.1%
Residues in disallowed regions	9	2.6%
	----	------
Number of non-glycine and non-proline residues	341	100.0%
Number of end-residues (excl. Gly and Pro)	4	
Number of glycine residues (shown as triangles)	53	
Number of proline residues	33	

Total number of residues	431	

C PROCHECK

Ramachandran Plot

saves



Plot statistics

Residues in most favoured regions [A,B,L]	297	86.6%
Residues in additional allowed regions [a,b,l,p]	43	12.5%
Residues in generously allowed regions [~a,~b,~l,~p]	2	0.6%
Residues in disallowed regions	1	0.3%
	----	------
Number of non-glycine and non-proline residues	343	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	53	
Number of proline residues	33	

Total number of residues	431	

B

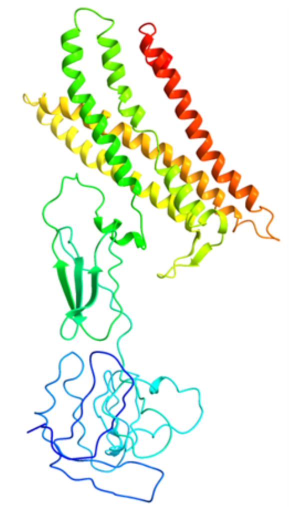


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Figure 1 - Ramachandram plot and tertiary structure

1.9 *In silico* cloning

The codon adaptation Jcat software analysis showed that the GC content of the constructed sequence is 56.07% and the CAI (Codon adaptation index) index is 1.0. Both within the parameters range, which is important to measure the cloning and expression potential. In order to construct the cloning vector, though SnapGene tool, restriction sites sequences of the enzymes BipI and BamHI were inserted in the expression vector pET28a(+) totalizing 6310 base pairs in the complete clone length (Figure 2).

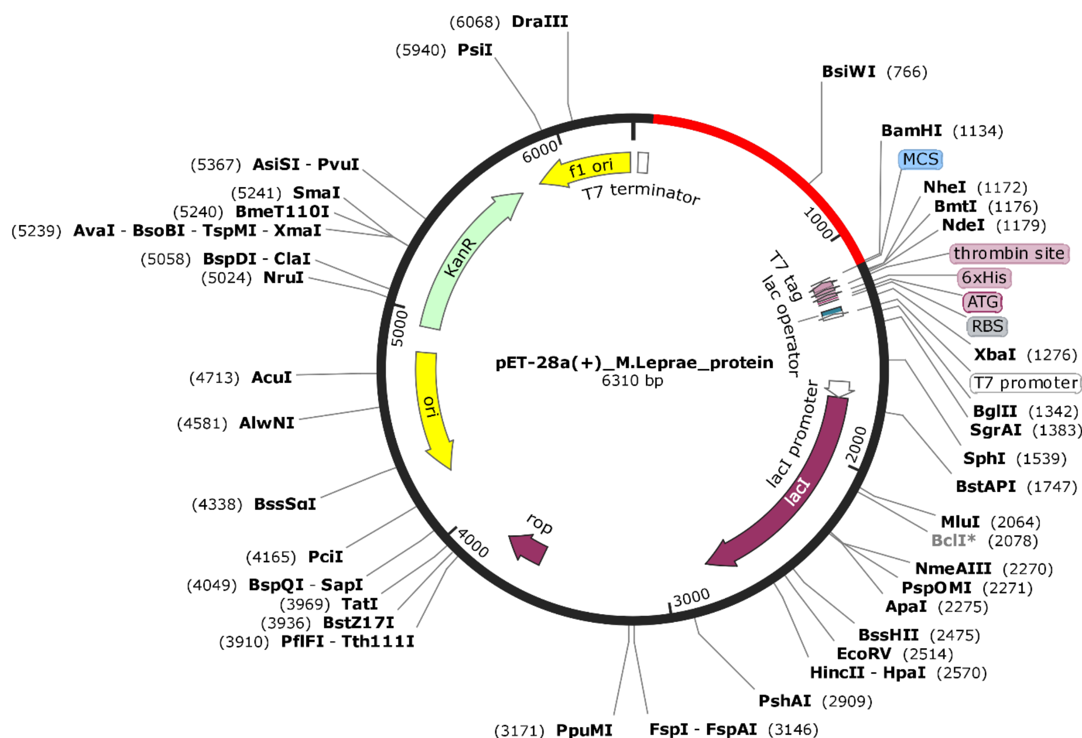

Figure 2 – In silico *cloning through SnapGene tool*

Discussion

For being fastidious bacteria, *M. leprae* and *M. lepromatosis* were never cultivated in axenic media (SHARMA *et al.*, 2020), which heightens the difficulty to work with them. That said, bioinformatics enables us to run innumerous tests without the bacteria, only with its genome.

ML0091, ML2380 and ML2346 were first described by Cole *et al.* in 2001 (COLE *et al.*, 2001). The first ones are similar to *M. tuberculosis* Rv3810 and Rv0455c, respectively, but, as it was described, no homology was found in our construct. On the other hand, ML2346 have no known homology (COLE *et al.*, 2001; SAMPAIO, Lucas H *et al.*, 2011). As to its function, ML0091 is a 28 KDa antigen precursor, ML2380 is a possible secreted protein and ML2346 is a hypothetical protein (COLE *et al.*, 2001).

Duthie *et al.* tested ML0091 and ML2346 against patients' sera from Goiânia, Brazil, having positive response in 71% and 29% of the cases, respectively (DUTHIE *et al.*, 2007). The protein ML2328 was also used in the construction of LepVax (a subunit vaccine against *M. leprae*) by Duthie *et al.* (DUTHIE *et al.*, 2018).

The other proteins used were predicted by our group (JAISWA *et al.*, 2021) in a reverse vaccinology approach, NP_301958.1, NP_302056.1, NP_302292.1, NP_302503.1 are secreted proteins and NP_302185.1, NP_301196.1 and NP_302342.1 are putative surface-exposed proteins. All these proteins are components of the core genome from 4 strains of *M. leprae* and 2 strains of *M. lepromatosis*.

Given that diagnosis of Leprosy is essential for treatment initiation and the earlier it begins, the better the response (GASTEIGER *et al.*, 2005), many are the attempts to create a diagnostic approach that can detect leprosy in all its spectrum (DHANDA *et al.*, 2013) and before any clinical sign (DHANDA *et al.*, 2013; GASTEIGER *et al.*, 2005; NAGPAL *et al.*, 2017), since infected individuals can spread the disease even before that (RIDLEY; JOPLING, 1966; VAN HOOIJ *et al.*, 2017). Even though PCR is effective (DHANDA *et al.*, 2013), leprosy is endemic in areas of difficult access or poverty, which makes its laboratory diagnosis hard (GUPTA *et al.*, 2016). Here, we propose a protein that has potential to detect both humoral and cellular responses, which is established as efficient in diagnosing leprosy (CHEN, Xiaoying; ZARO; SHEN, 2013; FERDOUS *et al.*, 2019; FROES; TRINDADE; SOTTO, 2020; PONOMARENKO *et al.*, 2008) even in a not so controlled environment and with lower cost than PCR.

Immunoinformatics is a *in silico* approach that helps in predicting epitopes which have greater chance to be immunogenic, nevertheless, it may not be accurate if we take into consideration that proteins undergo unique biological complex processes driven by genetics to be presented as an epitope by a cell. (DAVIES; FLOWER, 2007; MIRZAPOUR *et al.*, 2020; SAMPAIO, Lucas H *et al.*, 2011). Nonetheless, several multi-epitope constructs are already described as being effective, for example, LID-1, a fusion construct of ML0405 and ML2331 that is capable of diagnosing MB leprosy 6 to 8 months before the onset of clinical symptoms (DUTHIE *et al.*, 2007). LepReact, a delayed-type hypersensitivity skin test, made from LID-1, was able to detect antigen-specific immune responses from *M. leprae* in guinea pigs and armadillos (DUTHIE *et al.*, 2020). As to other diseases, Chagas disease detection can be improved by the use of TcF43 and

TcF26, proteins derived from fusion of selected *T. cruzi* TR proteins (DUTHIE *et al.*, 2016); Yin et al. validated a high accuracy IELISA assay using a recombinant protein for diagnosis of human brucellosis (YIN *et al.*, 2016); Ebrahimi et al. developed a multi-epitope protein with potential epitopes for the diagnosis of human toxocariasis (EBRAHIMI *et al.*, 2020).

Most of these tests are directed to humoral immunity, which is one of our goals, since it is less expensive and have good accuracy in LL and MB leprosy, where antibodies are more present (DE SOUSA *et al.*, 2016; FROES; TRINDADE; SOTTO, 2020; SHARMA *et al.*, 2020), however, cellular assays may enable the detection of TT and PB leprosy. Sampaio *et al.* described IFN-γ secretion upon antigen-specific stimulation as an indicator of progression to the tuberculoid pole and IL-4 or IL-5 as an indicator of progression to the lepromatous pole (SAMPAIO, L. H. *et al.*, 2012).

T-cell interferon-gamma release assays (IGRA) were developed as an alternative for delayed-typed hypersensitivity test for latent tuberculosis diagnosis, reducing false-positive results (LALVANI; PAREEK, 2010; MORI *et al.*, 2004). Since we found two IFN-γ, five IL-4, four IL-10 inducing epitopes, and five epitopes that induce pro-inflammatory responses within our protein, these properties point to a multi-epitope protein that can be used in a cytokine-production assay, similar to the aforementioned IGRA, being able to detect different cellular immune profiles associated to different clinical manifestations of leprosy. IL-10 together with IL-4 is known to be associated with LL pole and MB leprosy while IFN-γ associated with other proinflammatory cytokines

characterize the TT pole and PB leprosy (FONSECA *et al.*, 2017; SADHU; MITRA, 2018; SAMPAIO, L. H. *et al.*, 2012; WEISS *et al.*, 2016)..

With results in both the humoral and cellular response, this protein will be able to diagnose leprosy without much difficult due to the detection of both ends of its spectrum. Other studies using immunoinformatics to construct multi-epitope proteins for diagnosis purpose had good results *in silico*, but it lacked sensitivity and specificity in *ex vivo* (ALIBAKHSHI *et al.*, 2020) or had strong specificity and weak sensibility (YIN *et al.*, 2021).

This protein has a huge immunological potential, is stable and can be lyophilized to be used in ELISA plates or even in biosensors, which are user-friendly diagnosis tools, facilitating translation into human sample tests.

Materials and methods

1.1 Data selection

The proteins ML0091, ML0405, ML1636, ML2055, ML2331, ML2346 and ML1556 were previously proved to detect leprosy in some level (DUTHIE *et al.*, 2007). ML2028, ML2055, ML2380 and ML2531 were tested as immunizer in mice, and it reduced the bacterial burden (DUTHIE *et al.*, 2018). NP_301196.1, NP_301663.1, NP_301805.1, NP_301958.1, NP_302056.1, NP_302185.1, NP_302232.1, NP_302292.1, NP_302342.1, NP_302490.1, NP_302503.1 were obtained from our group previous results (JAISWA *et al.*, 2021). The sequences of these proteins were retrieved from NCBI (Nacional Center for Biotechnology Information) in FASTA format. Antigenicity of these selected proteins were

evaluated by VaxiJen analysis (DOYTCHINOVA; FLOWER, 2007; HOME - PROTEIN - NCBI, [*s. d.*]).

1.2 Prediction of epitopes that binds to MHC I alleles

The epitopes able to bind to MHC I alleles and activate cytotoxic T lymphocytes (CTL) were predicted by two different platforms to improve its confidence. The Immune Epitope Database and Analysis Resource (IEDB) contains thousands of high and low-affinity epitopes used on training to enhance the accuracy of the predictor (MARTINI *et al.*, 2020; VITA *et al.*, 2019). Aiming to develop a diagnostic tool to be used in all the endemic areas, we selected all 27 alleles with high frequency in the global population. The lengths of our peptides were 9 amino acids residues (GREENBAUM *et al.*, 2011). Default parameters were chosen for the prediction since they combine Artificial Neural Network (ANN), Scoring Matrix Method (SMM), and combinatorial library. Epitopes with percentile rank smaller than 1% were selected for our study, due to its enhanced probability to be immunogenic. NetCTL 1.2 server is able to assess binding affinity and antigenic processing and transportation integrated to the epitope prediction, using both ANN and SMM to make the predictions (LARSEN, Mette Voldby *et al.*, 2005; LARSEN, Mette v *et al.*, 2007). The same alleles used in IEDB were used here.

1.3 Prediction of epitopes that binds to MHC II alleles

For epitopes that activate helper T-lymphocyte (HTL) (MHC II biding epitopes), we also used two different predictors, IEDB tool (MARTINI *et al.*, 2020) and

NetMHCII 2.3 server (JENSEN *et al.*, 2018). The MHC II cleft size is able to accommodate epitopes from 13 to 25 amino acids, thus we chose to use 15 residues length as a standard since NetMHCII 2.3 server also uses this length, allowing the comparison between both programs. In IEDB we selected only epitopes with percentile rank lower than 3%. For the IC50, which is used to determine the epitopes' affinity with the MHC, we chose an IC50 < 1000 nM (JENSEN *et al.*, 2018). ANN is also used by NetMHCII 2.3 server with various epitope databases to increase data training and predict the epitopes (NIELSEN; LUND, 2009).

1.4 Prediction of B cell epitopes

To predict linear B cell epitopes, we used ABCpred (CHEN, J. *et al.*, 2007; GALANIS *et al.*, 2019) which uses ANN for predictions and LBtope server (SINGH; ANSARI; RAGHAVA, 2013) which uses the SVM (Support Vector Machine) based models for the prediction of linear B cell epitopes. We chose the epitope's length as 16 due to its better accuracy properties (CHEN, J. *et al.*, 2007; EL-MANZALAWY; DOBBS; HONAVAR, 2008; SAHA; RAGHAVA, 2006).

1.5 Filtering and Immunogenicity assessment of MHC I epitopes

All the epitopes predicted were filtered through a python *in-house* script which compared the results from both programs for each kind of epitope (Figure 1). After the recognition of epitopes predicted by the two programs, the same script was used to find overlapping epitopes between B cells and MHC II with at least nine sequential amino acids residues. The last time the script was used it assessed the overlap between the epitopes predicted as immunogenic by Immunogenicity Tool and the lasting filtered epitopes. Class I Immunogenicity

Tool (CALIS *et al.*, 2013) uses amino acids properties and its position within the peptide to predict immunogenic properties. Only peptides with a score greater than 0.1 were chosen.

1.6 Sequence construction

The epitopes that passed through all those filters were than merged into different constructs with the sequence AAY for MHC I epitopes and GPGPG for MHC II were used as spacer sequences which help in protein folding (CHAUHAN *et al.*, 2019).

1.7 Evaluation of Host homology and Physical-chemical properties

To evaluate similarity with human proteins and therefore reduce autoimmunity possibilities, a BLAST13 was carried out. The whole multi-epitope vaccine sequence and its epitopes individually were submitted against UniProtKB Human database.

Molecular mass, theoretical pI, extinction coefficient, aliphatic index, grand average of hydropathicity (GRAVY), estimated half-life for three model organisms (*Escherichia coli*, yeast, and mammal cells), and the instability index were analyzed through the final construct sequence using ProtParam (GASTEIGER *et al.*, 2005). Solubility index was also assessed by Protein-Sol (HEBDITCH *et al.*, 2017), which evaluates several properties based on *E.coli* expression data.

1.8 Secondary structure prediction

The secondary structure of the final epitope construct was predicted by RaptorX template-based protein structure modeling server (KÄLLBERG *et al.*, 2012) and

PSIPRED. PSIPRED predict the secondary structure and generate the pictures by applying complex ANN and Position-Specific Scoring Matrix (PSSM) (YANG; ZHANG, 2015).

1.9 Structural modelling, refinement, and properties assessment

To predict the tertiary structure (3D), we used three different programs and then best 3D structure was chosen based on its structural quality. For the evaluation of structural quality PROCHECK was used through SAVES v6.0 (BOWIE; LÜTHY; EISENBERG, 1991; LÜTHY; BOWIE; EISENBERG, 1992) to generate the Ramachandran plot. Phyre2 intensive method comprises the multiple alignments of the sequence of interest with homologous sequences using threading and *ab initio* followed by the secondary structure's prediction with the PSIPRED. Then, a hidden Markov model is determined with the information from these two steps combined. The models with the best scores are used, from a search in an HMM database of known protein structures, to determine the modeling and error correction (KELLEY *et al.*, 2015). Multiple-template threading (MTT) and scoring methods are used in RaptorX to predict the 3D structures and to indicate the quality of models predicted (KÄLLBERG *et al.*, 2012). Finally, I-TASSER uses an interactive method based on templates according to fragment assembly simulations with further refinement to construct the models (YANG; ZHANG, 2015).

To enhance local and global quality of modelled 3D structure, we used GalaxyWeb Server which applies methods for refinement of amino acid side chains using light and aggressive relaxation (KO *et al.*, 2012).

1.10 Antigenicity, IFN-γ, IL-4 and Il-10 inducing potential

The final construct sequence was analyzed for crucial aspects related to the induction of immune responses, toxicity, allergenicity and solubility. We used VaxiJen to assess the antigenic capacity through the automatic cross covariance method. Thus, analyzing the physical-chemical properties and predicting the ability to induce immune responses without the need to do alignments (DOYTCHINOVA; FLOWER, 2007).

The search for epitopes able to induce IFN-γ production was performed with the IFNepitope predictor, using MHC II epitopes. This predictor uses a SVM hybrid method based on motifs to perform the prediction (DHANDA; VIR; RAGHAVA, 2013). IL-4 and IL-10 were also assessed by different predictors (IL-4Pred and IL-10Pred), by the same method (DHANDA *et al.*, 2013; NAGPAL *et al.*, 2017). The tool ProInflam was used as well to predict the pro inflammatory potential of the peptides included in the protein (GUPTA *et al.*, 2016).

1.11 Conformational B cell epitopes prediction

The ElliPro web-based tool was used to predict conformational B cell epitopes from the refined structure (PONOMARENKO *et al.*, 2008). These epitopes are generally conformational, which means they are away in linear distance but close in spatial proximity (FERDOUS *et al.*, 2019).

1.12 *In silico* cloning

To verify the capacity of cloning and expression of the multi-epitope protein in an appropriate expression vector, we performed *in silico* cloning. Using JCat we

adapted the codon of our peptide according to the *E. coli* expression system's codon usage through reverse translation. With the cDNA sequence, the codon optimization for *E. coli* k12 was performed, and it returned the codon adaptation index (CAI), which must have a score higher than 0.8 and the GC content rate should be between 30-70%. Furthermore, to clone the final constructs optimized gene sequence in *E. coli* we used pET28a(+) vector, with Blpi, and BamHI restriction sites. Finally, the optimized sequence was inserted into the pET28a(+) vector using the SnapGene tool to ensure protein expression.

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Conflict of interest

The authors declare no conflicts of interest.

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Supplementary Materials

Protein	Name	Overall Prediction for the Protective Antigen
ML0091	28 KDa antigen precursor	0.5993
ML0405	conserved hypothetical protein	0.5507
ML1556	initiation factor IF-2	0.7560
ML1633	possible secreted hydrolase	0.4079
ML2028	antigen 85A, mycolyltransferase	0.5759
ML2055	probable cell surface protein	0.4885
ML2331	possible secreted protein	0.7658
ML2346	L-lactate dehydrogenase	0.4681
ML2380	possible secreted protein	0.3004
ML2531	possible cell surface protein	0.5242
NP_301196.1	Antigen 85-C precursor 85C] Antigen 85 complex C] Ag85C] Mycolyl transferase 85C] EC 2.3.1.-]	0.4657
NP_301663.1	Putative membrane protein MmpS3	0.6988
NP_301805.1	Putative lipoprotein IprE Precursor	0.3909
NP_301958.1	Possible membrane protein	0.3909
NP_302056.1	Hypothetical protein	0.4972
NP_302185.1	Hypothetical protein	0.4572
NP_302232.1	Invasion protein – Putative exported p60 protein homologue	0.5827
NP_302292.1	Hypothetical protein	0.5468
NP_302342.1	PPE Family protein	0.6320
NP_302490.1	Protease	0.6261
NP_302503.1	Multimodular transpeptidasetransglycosylase EC 2.4.1.129]	0.5410

Supplementary Table 1 - VaxiJen Results

B cell epitopes			
Protein	**ABCpred**	**Lbtope**	**Overlapping**
ML0091	22	70	6
ML0405	39	106	10
ML1556	98	253	25
ML1633	51	178	17
ML2028	32	82	16
ML2055	30	88	11
ML2331	23	54	4
ML2346	30	94	11
ML2380	14	46	6
ML2531	10	17	2
NP_301196.1	31	66	9
NP_301663.1	28	93	9
NP_301805.1	20	52	6
NP_301958.1	31	110	15
NP_302056.1	36	95	8
NP_302185.1	37	140	13
NP_302232.1	25	51	6
NP_302292.1	25	91	9
NP_302342.1	44	103	13
NP_302490.1	22	29	2
NP_302503.1	81	280	29
Total	**729**	**2098**	**227**

Supplementary Table 2 - B cell epitopes

MHC I			
Protein	**IEDB**	**NET CTL**	**Overlapping**
ML0091	48	33	29
ML0405	114	63	56
ML1556	235	140	122
ML1633	136	78	69
ML2028	83	60	49
ML2055	69	42	36
ML2331	56	37	30
ML2346	95	57	48
ML2380	51	31	29
ML2531	20	13	10
NP_301196.1	293	48	48
NP_301663.1	285	52	52
NP_301805.1	49	28	22
NP_301958.1	57	33	29
NP_302056.1	83	53	47
NP_302185.1	94	56	46
NP_302232.1	55	33	27
NP_302292.1	60	37	29
NP_302342.1	141	97	84
NP_302490.1	52	37	29
NP_302503.1	197	118	101
Total	2273	1146	992

Supplementary Table 3 - MHC I epitopes

MHC II			
Protein	**IEDB**	**NET 2.3**	**Overlapping**
ML0091	23	65	21
ML0405	74	155	56
ML1556	67	303	65
ML1633	28	222	28
ML2028	64	154	57
ML2055	7	273	7
ML2331	31	92	31
ML2346	61	145	50
ML2380	33	58	25
ML2531	6	40	4
NP_301196.1	5	211	5
NP_301663.1	14	127	14
NP_301805.1	12	108	12
NP_301958.1	2	63	2
NP_302056.1	10	207	10
NP_302185.1	29	240	28
NP_302232.1	3	145	3
NP_302292.1	13	120	13
NP_302342.1	90	352	90
NP_302490.1	18	143	18
NP_302503.1	47	511	47
Total	637	3734	586

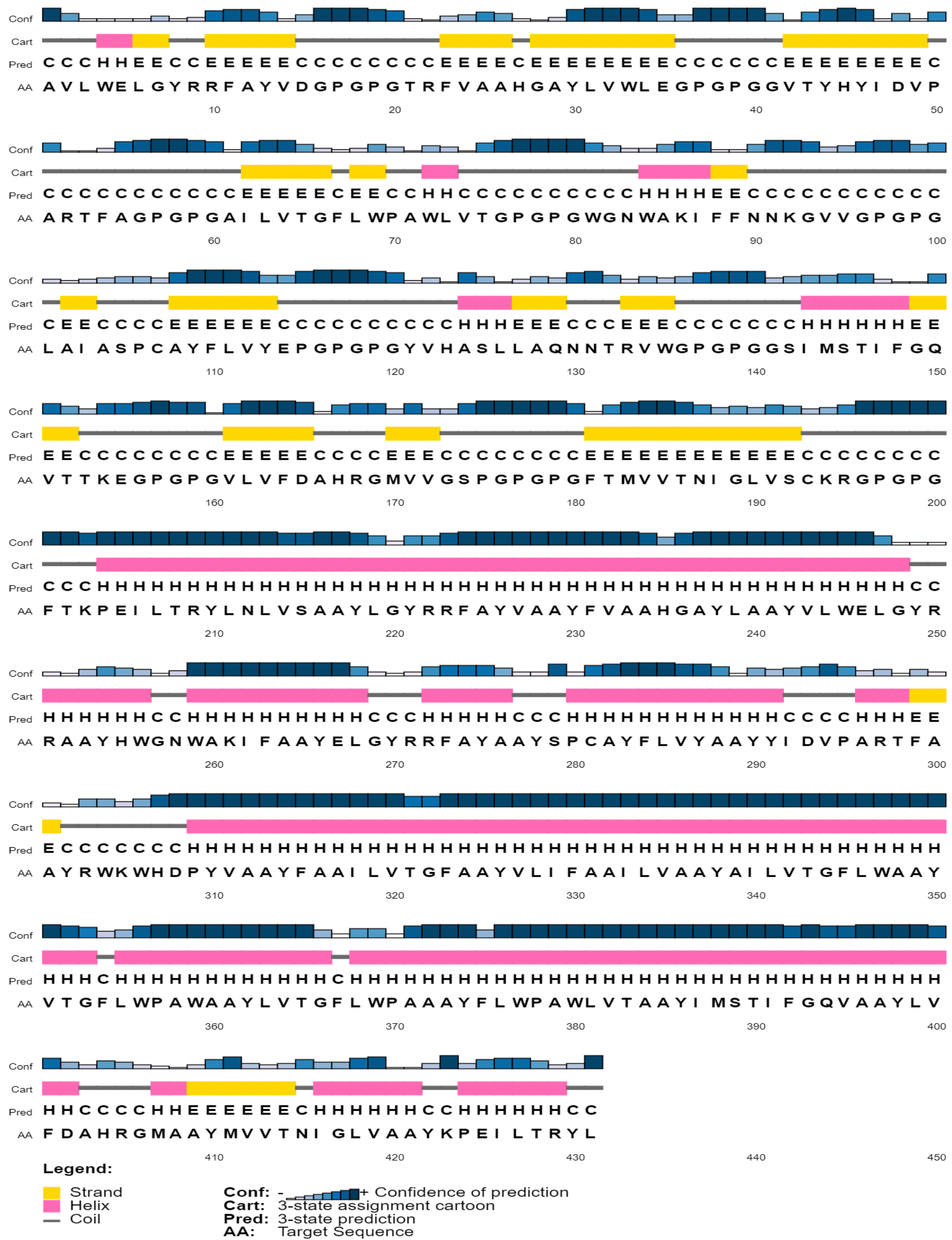
Supplementary Table 4 - MHC II epitopes

	MHC II FILTERING	MHC I FILTERING	
Protein	**B cell epitopes overlapping**	**Immunogenicity**	**Overlapping of MHC II with overlapping in B**
ML0091	1	7	1
ML0405	4	27	0
ML1556	1	49	0
ML1633	2	21	0
ML2028	5	15	0
ML2055	1	9	0
ML2331	2	9	0
ML2346	8	21	5
ML2380	3	15	1
ML2531	0	1	0
NP_301196.1	1	21	1
NP_301663.1	1	0	0
NP_301805.1	0	11	0
NP_301958.1	1	11	6
NP_302056.1	1	15	1
NP_302185.1	1	16	1
NP_302232.1	0	11	0
NP_302292.1	1	11	1
NP_302342.1	5	39	1
NP_302490.1	0	10	0
NP_302503.1	2	31	2
Total	40	350	20

Supplementary Table 5 – Number of epitopes in MHC II and I filtering

Protein	Immunogenicity	
	Epítopo	**Score**
ML0091	SPCAYFLVY	0,14323
ML2346	LGYRRFAYV	0,24234
	VLWELGYRR	0,22087
	ELGYRRFAY	0,22806
	YIDVPARTF	0,14113
ML2380	HWGNWAKIF	0,15278
NP_301196.1	RWKWHDPYV	0,19375
NP_301958.1	VLIFAAILV	0,34177
	VTGFLWPAW	0,34001
	LVTGFLWPA	0,33072
	FLWPAWLVT	0,32211
	FAAILVTGF	0,22724
	AILVTGFLW	0,19996
NP_302056.1	IMSTIFGQV	0,18608
NP_302185.1	LVFDAHRGM	0,19235
NP_302292.1	MVVTNIGLV	0,19356
NP_302342.1	FVAAHGAYL	0,14633
NP_302503.1	KPEILTRYL	0,23368

Supplementary Table 6 – Immunogenicity Scores



Supplementary Figure 1 - PSIPRED secondary structure prediction

Comentários ou considerações finais

- Encontramos peptídeos que são reconhecidos como epítopos pelas células ou anticorpos na resposta imunológica gerada na hanseníase.
- Separamos epítopos que são reconhecidos por MHC I, MHC II e células B.
- Filtramos os epítopos por meio da sobreposição dos epítopos humorais e celulares.
- Construímos uma proteína com os diversos fragmentos, chegando à um constructo estável, com 431 aminoácidos e com ótimos parâmetros físico-químicos.

Conclusão

Apresentamos aqui uma proteína multiepítopo que foi desenhada para o diagnóstico da hanseníase tanto pela resposta humoral quanto pela resposta celular, buscando uma forma diagnóstica única para todo o espectro da doença. A possibilidade da produção da proteína na forma liofilizada permite sua utilização tanto em placas de ELISA para sensibilização da mesma quanto seu uso em biossensores, esse último permitindo até mesmo diagnósticos em locais remotos e sem infraestrutura laboratorial.

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