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Cloning and Expression of H2-D(b) for Future Experimental Studies on its Role in Multiple Sclerosis

Nicole A. Otto
Worcester Polytechnic Institute

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Cloning and Expression of H2-D^b for Future Experimental Studies on its Role in Multiple Sclerosis

A Major Qualifying Project Report

Submitted to the Faculty of the

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by

Nicole Otto

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APPROVED:

Eric Huseby, PhD
Pathology
UMass Medical School
MAJOR ADVISOR

David Adams, PhD
Biology and Biotechnology
WPI Project Advisor

Destin Heilman, PhD
Chemistry and Biochemistry
WPI Project Advisor

ABSTRACT

Multiple sclerosis (MS) is an inflammatory disease characterized by damage to neuronal fatty myelin sheaths and neuronal dysfunction. Studies have shown that transgenic mice expressing a T-cell receptor (TCR) reactive to GFAP can succumb to spontaneous autoimmunity with pathologies comparable to MS. This study focused on the expression of a MHC-I type H2-D^b β -2m receptor domain in pBac & cloning H2-D^b into a pLM-1 expression vector. The β -2m domain expression vector was transformed into BL21 cells, and expression was confirmed via SDS-PAGE. The H2-D^b genes were then successfully cloned into the pLM-1 expression vector. Successful expression of β -2m receptor domain and of H2-D^b/GFAP ligand complex will be used for further experimentation to observe abnormalities within the H2-D^b epitope and its role in MS.

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BACKGROUND

Multiple Sclerosis (MS) is the most common neurological disease in young adults with a prevalence of over one million. It is an inflammatory disease, commonly described as autoimmune encephalomyelitis, that targets the central nervous system (CNS) characterized by damage to neuronal fatty myelin sheaths and neuronal dysfunction resulting in white and grey matter lesions (Huseby, 2012). Active lesions, which form focal demyelinating plaques, are predominantly composed of self-reactive T-cells, activated macrophages, and microglia that escape negative selection resulting in neurological degradation. This degradation disrupts the transmission of nerve signals from the spinal cord and brain to other parts of the body resulting in the primary symptoms of MS patients (hyperreflexia, ataxia, spasticity, and visual defects) which vary depending on the damage incurred (Huseby, 2012).

Symptoms variance plays a discrete role in the presentation of four main disease course pathologies. The disease course can be relapsing-remitting, primary-progressive, secondary-progressive, and progressive relapsing (Society, 2013). Relapsing remitting disease occurs in approximately 85% of MS patients who experience defined attacks of neurological function. These relapses are often followed by periods of remission during which no disease progression takes place (Society, 2013). The second course of disease progression is primary-progressive where MS patients show slow degeneration of neurological function with no distinct remissions. The rate of progression for this course of the disease varies over time and contains temporary plateaus with temporary minor improvements (Society, 2013). The third disease course is secondary-progressive. This disease course often follows an initial relapsing-remitting disease course. At this stage

many patients experience a steady disease progression with no occasional flare-ups or remissions (Society, 2013). The last disease course is progressive relapsing. This course affects approximately 5% of all MS patients and is characterized by a steadily increased disease rate from the beginning with clear neurological degrading attacks.

Autoimmune T-cell Responses

Multiple Sclerosis is hypothesized to be caused, in part, by autoimmune T-cell responses directed against antigens of the CNS (McFarland & Martin, 2007; Steinman, 2009). Following exposure to environmental triggers, myelin specific T-cells, specific for myelin basic protein (MBP), myelin associated glycoprotein (MAG), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), become activated and are able to escape negative selection (Sospedra & Martin, 2005; Goverman, 2009). This activation allows T cells to cross the blood brain barrier (BBB) and interact with myelin antigen triggers on antigen-presenting cells (APCs) of the CNS. Reactivation of T-cells in this manner recruits cells of the innate immune system, which then play a role in mediating axonal damage and demyelination resulting in CNS inflammation and plaque formation (Goverman, 2009). In order for CD4⁺ T-cells and CD8⁺ T-cells to initiate CNS inflammation they must first infiltrate the CNS from the periphery.

CD4⁺ mediated T-cell infiltration and inflammation begins with the priming by dendritic cells presenting myelin/ myelin cross-reactive epitopes (Figure 1). These myelin antigens can then be captured by antigen presenting cells of the CNS resulting in the migration across the cervical lymph nodes (Goverman, 2009). Local APCs can also phagocytize soluble myelin antigens which drain from the CNS to the lymph nodes. After T cell priming, CD4⁺ T cells cross the blood cerebrospinal fluid barrier via the choroid plexus or the meningeal venules. This then allows for these T cells to enter the

subarachnoid space (Goverman, 2009). Once in the subarachnoid space, MHC Class II expressing macrophages and dendritic cells containing myelin epitopes, re-activate T-cells (**Figure 1**).

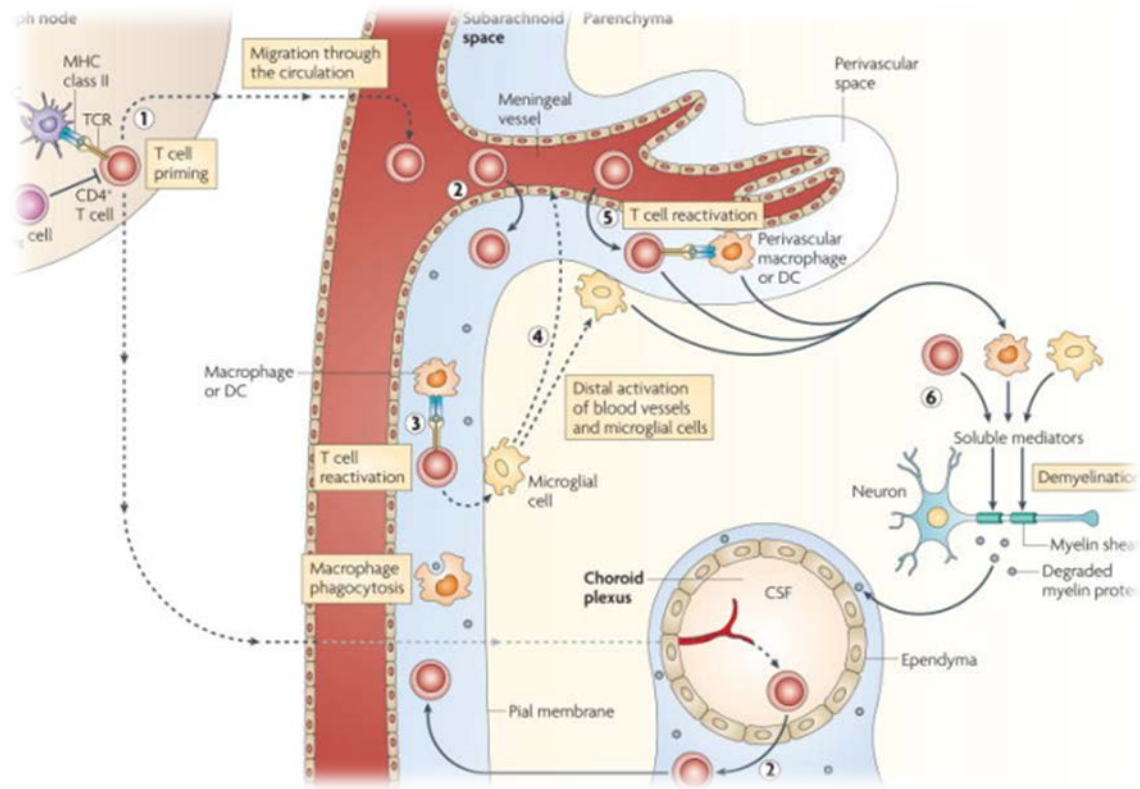


Figure 1: Activation of Myelin-Specific CD4⁺ T-Cells (Goverman, 2009)

These cells then activate microglial cells triggering the activation of other, distal microglial cells and blood vessels (Goverman, 2009). Activated CD4⁺ T-cells are then able to adhere to the blood-brain barrier and enter the perivascular space (Figure 1). They are then reactivated by perivascular dendritic cells and macrophages.

The re-activation of these self-reactive CD4⁺ T cells then results in the targeting of CNS proteins, such as MBP and PLP (Sospedra & Martin, 2005; Goverman, 2009). Disease causing myelin specific CD4⁺ T cells release Th1 and Th17 cytokines, which cause an increase in major histocompatibility complex (MHC) expression within the

CNS. MHC expression then leads to the activation of microglia and further recruitment of monocytes and other T cells to the site of inflammation. Cytokines released during this inflammatory response, include $\text{IFN}\gamma$, $\text{TNF}\alpha$, IL-6, IL-2, and IL-23, and are common soluble mediators (Tsuchida et al., 1994). These mediators result in the demyelination of neuronal cells and as such have been associated with the pathogenicity of the disease (Jurewicz et al., 1998; Medana et al., 2001).

Along with CD4^+ T cells, CD8^+ T-cells also play a role in the pathogenicity of the disease. CD8^+ T cells are also specific for CNS proteins such as MBP, MAG, and PLP (Huseby, 2012). These cells infiltrate the central nervous system in a similar way than CD4^+ cells except that CD8^+ T cells are activated by macrophages, microglial cells, and dendritic cells via cross-presentation. CD8^+ T-cell cross presentation occurs when endothelial cells can directly present the antigen to myelin epitopes (Goverman, 2009). Finally self-reactive T-cells enter the parenchyma. Soluble mediators expressed by CD8^+ T cells include the pro-inflammatory cytokines $\text{IFN}\gamma$ and $\text{TNF}\alpha$. The expression of these cytokines is in response to the presentation of CNS protein epitopes by MHC Class I molecules in activated macrophages and microglial cells (Huseby, 2012). These soluble mediators are capable of lysing oligodendrocytes expressing MHC class I and myelin epitopes resulting in demyelination of neuronal tissue and contributing to inflammatory response (Goverman, 2009).

Inflammation seen in autoimmune encephalomyelitis (EAE) mediated by these MHC Class II and MHC Class I-restricted CD4^+ and CD8^+ T cells, respectively; result in the accumulation and formation of lesions within the white and grey matter regions of the brain (Lassman, Wolfgang, & Lucchinetti, 2007). These lesions, characteristic of

multiple sclerosis, depict degraded neuronal tissue surrounded by activated T-cells. There are several mice models which depict a similar EAE response to MS patients.

Mice Models for Multiple Sclerosis

Studies have generated two types of mice models that induce autoimmune encephalomyelitis. A commonly used mouse model resulting in EAE is focused upon the role of Th1 and Th17 phenotype CD4 T cells (Huseby, 2012). In this CD4-EAE model, TCR transgenic mice were crossed with RAG-1 gene-deficient mice. This cross yielded mice capable of expressing a transgenic T cells receptor (TCR) specific for MBP but no other lymphocytes (Lafaille et al., 1994). In studies conducted by Lafaille et al., all T/R-mice developed spontaneous EAE over a 12 month period. It was also found that the mice incurring spontaneous EAE associated with typical EAE lesions in the central and peripheral nervous system. Furthermore, this mouse model presented MBP-specific T cells in the brain but not in the peripheral lymphoid tissues. This study proved that CD4⁺ anti-MBP T cells are capable of mediating EAE within this mouse model and that spontaneous EAE may be triggered by in situ activation of CD4⁺ anti-MBP cells in the nervous system.

The second type of mouse model focuses primarily on the role of CD8⁺ T cells in autoimmunity. In a study conducted by Huseby et al., C2H MBP^{-/-} and MBP^{+/+} mice were infected with Ad/MBP. Results demonstrated that MBP-specific CD8 T cells are subject to immune tolerance. Furthermore, this study showed that the activation of MBP-specific CD8 T cells in mice resulted in severe demyelinating central nervous system autoimmunity (Huseby et al., 2001). Analysis of brain and spinal cord tissue, revealed lesions (mostly found in the brain) containing perivascular cuffing with common demyelinating and cytoplasmic swelling (Huseby, 2012). This study demonstrated that

viruses are capable of inducing CD8 T cell autoimmunity via the disruption of peripheral tolerance mechanisms (Huseby, 2012). In another study conducted by Fournier and colleagues, it was found that C57BL/6 mice over-expressing co-stimulatory CD86 molecules on peripheral T cells and resident CNS microglia, spontaneously succumbed to CD8 T cell-dependent CNS autoimmunity. This study collectively demonstrated that spontaneous demyelinating disease as a consequence of inflammatory response is initiated via the activation of CNS specific T cells (Brisebois et al., 2006). These mice models thereby, demonstrate evidence that supports the hypothesis that auto reactive CD8 T cells are capable of contributing to the pathogenicity of inflammatory diseases such as multiple sclerosis (Brisebois et al., 2006).

Preliminary Data: GFAP₂₆₄₋₂₇₂ specific T cell responses in BG1 Tg mice

Recent data has demonstrated the formation of plaques within grey and white matter regions indicating that proteins other than myelin may be targeted during the progression of MS in patients (Ontaneda, 2011). In order to look into the role of other CNS proteins in the pathogenicity of the disease, GFAP (glial fibrillary acidic protein), expressed primarily in astrocytes in grey and white matter regions of the brain and spinal cord, was examined in a mouse model expression GFAP-specific CD8 T cells. It was hypothesized that they immune cells would target grey and white matter regions of the CNS resulting in a disease progression similar to MS. To test this hypothesis, TCR transgenic mice expressing the V α 4 and V β 9 chains from GFAP-specific CD8 T cell clone BG1. It was found that CD 8 T-cells, reactive to GFAP, can induce central nervous system autoimmunity with pathologies similar to multiple sclerosis. BG1 TCR Tg mice were also found to develop paralytic disease with a disease progression resulting in mature lesions, demyelination of the cerebellum as well as classical EAE symptoms

(infiltration along most of the spinal cord) (Huseby, preliminary unpublished data). Furthermore, preliminary data has shown that BG1 TCR Tg mice, which express a TCR for GFAP, can succumb to spontaneous autoimmunity. In addition the T-cell repertoire of these mice is skewed towards the CD8 T-cell lineage, resulting in GFAP peptides bound to the MHC Class I H2-D^b molecule (**Figure 2A.**)

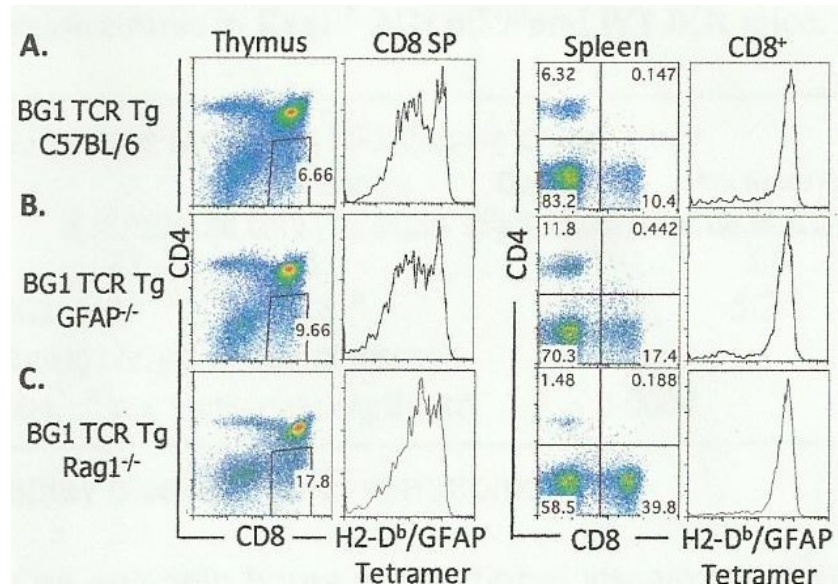


Figure 2: Representation of skewed CD8 T-cell lineage background stained with GFAP MHC Class I H2-D^b/GFAP₂₆₄₋₂₇₂. (Huseby Lab Preliminary (unpublished) Data)

MHC Class I molecules are composed of two polypeptide chains, α and β -chains. The α -chain is covalently bonded to the β -chain, and spans the membrane. The complete MHC Class I molecule is comprised of four domains, three formed from the MHC encoded α -chain and one encoded from the MHC encoded β 2-microglobulin (Murphy, 2012) The α 1 and α 2 domains of MHC the Class I molecule compose the peptide binding cleft (**Figure 3**).

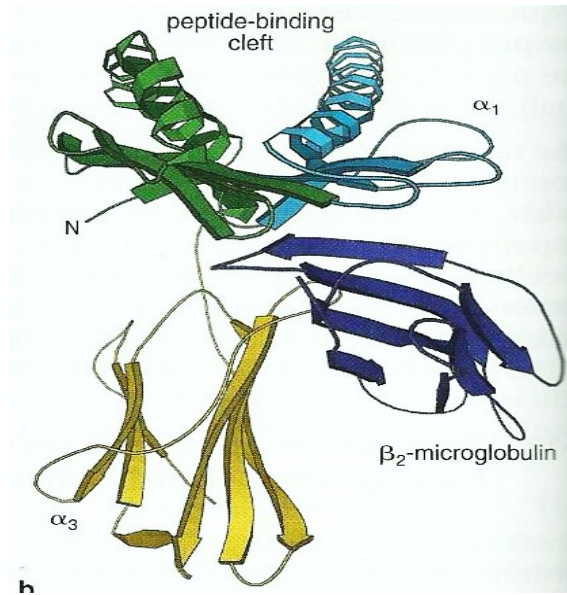


Figure 3: MHC Class I structure (Murphy, 2012)

Determining the structure of H2-D(b) + GFAP ligand complex will define the antigenic epitope, and could unravel the pathogenicity of the spontaneous autoimmunity seen in the BG1 mice models.

PROJECT PURPOSE

The purpose of this project was to express the β 2-microglobulin domain of the H2-D^b gene, and to clone and express its alpha domain for its future crystallization. The MHC Class I H2-D^b molecule was selected for crystallization as a direct result of the molecule's presentation of glial fibrillary acidic protein (GFAP). Studies have shown that transgenic mice expressing a T cell receptor (TCR) reactive to GFAP presented H2-D^b can succumb to spontaneous autoimmunity with pathologies comparable to MS. CD8⁺ T-cells, reactive to GFAP, can induce central nervous system autoimmunity with pathologies similar to multiple sclerosis.

METHODS

Expression of β 2m H2-D^b Domain

The β -2m receptor domain of MHC Class I H2-D^b in a pBac bacterial expression vector (**Figure 4**) was transformed into BL21 *E. coli* cells.

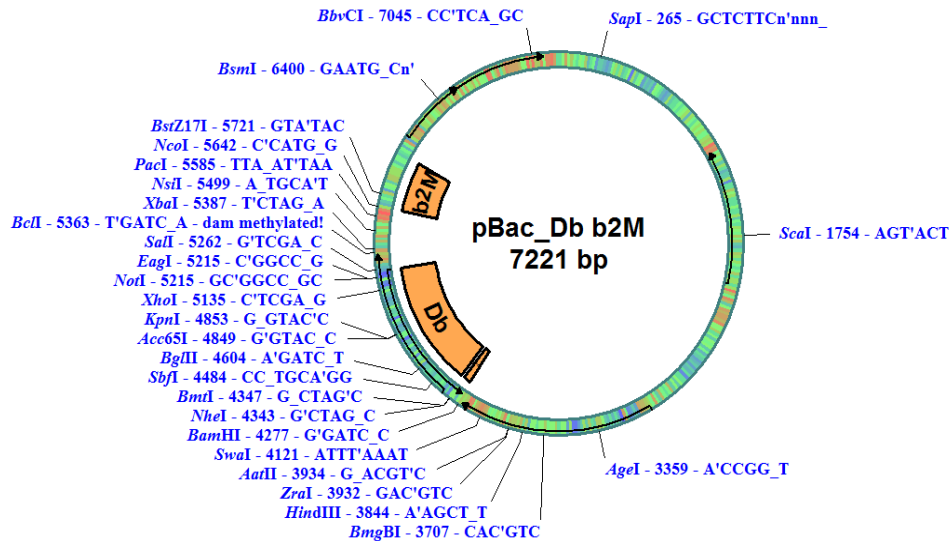


Figure 4: Map of the pBac Expression Vector Encoding the D β ^b2m Domain.

Transformed BL21 cells were then cultured at 37°C overnight in 2x TY medium containing 1000x Carbinicillin. 0.5mL of culture were removed from the sample and frozen via the addition 200 μ L of 80% glycerol. Isopropyl β -D-thiogalactopyranoside (IPTG) was added to 1 mL remaining culture and pre and post-induction cultures were incubated for 3 hours at 37°C shaking until a final O.D of 0.6 was achieved. Cultures were then centrifuged and bacterial pellets were boiled in 50 μ L 2X SDS sample buffer at 95 C for 5 minutes.

SDS-Gel Assay for β 2m H2-D^b Domain

10 μ L of the 50 μ L samples boiled in 2X SDS sample buffer, were loaded into a 12% SDS-PAGE gel and run at 200V at 0.3 A for 55 minutes at RT. Gel was then stained for 20 minutes using Coomassie Blue. Gel was then subjected to a fast de-stain of 15 minutes using a 50% methanol, 10% acetic acid. An additional fast de-stain was then performed. This was then followed by an overnight slow de-stain composed of 5% methanol and 7% acetic acid.

Cloning of H2-D^b Genes into pLM-1 Expression Vector

To construct the expression vector for H2-D^b, the gene fragment coding for amino acids 888-1745 was amplified by polymerase chain reaction (PCR) using the oligonucleotides GGGGGGGAATTCATGGGCCCACACTCGATGCG (forward primer labeled 5315) & CCCCCAAGCTTTTACCATCTCAGGGTGAGGGGCTCAGG (reverse primer labeled 5299). The PCR reaction mixture consisted of 4 μ L of 10X PCR buffer; 4 μ L of dNTP, 4 μ L of each 10X primer, 1 μ L of Taq DNA polymerase, and 1 μ L of p3630 plasmid DNA containing H2-D^b gene sequence. The PCR cycle consisted of an initial denaturing phase at 94°C for 1:00 minutes followed by 30 amplification cycles (94°C for 30 seconds, 60°C for 15 seconds, 72°C for 1:50 minute. The reactions were held at 4°C until retrieved from the PCR machine.

4 μ L of the resulting fragment and 5 μ L of the pLM-1 expression vector were then digested using a 20 L solution containing 1 μ L EcoRI-HF, 1 μ L HindIII, and 2 μ L of 10x Buffer 2, and 11 and 12 μ L of ddH₂O respectively. The two samples were then incubated in a 37°C water bath for one hour. Samples were then run in 2% agarose gel and gene cleaned using 10% acetic acid.

Ligation was then performed using a ligation mixture of 6 μ L of H2-Db fragment, 1 μ L of pLM-1 vector, 1.2 μ L of 10x T4 Ligase buffer, 1 μ L of T4 Ligase, and 3 μ L of ddH₂O. A negative control was also done using a ligation mixture of 6 μ L of pLM-1 vector, 1.2 μ L of 10 T4 Ligase buffer, 1 μ L of T4 Ligase, and 4 μ L of ddH₂O. Ligation was performed at 16 °C for 18 hours.

6 μ L of ligation and control sample were placed into two 14 μ L aliquots of *E. coli* competent cells and transformed. 100 μ L of 2x TY medium containing 1000x Carbinicillin was then added to each aliquot and were allowed to grow overnight on Carbinicillin plates. 24 colonies were then selected from the experimental ligation plate and 40 μ L of 2x TY medium containing 1000x Carbinicillin were added to a 96 well plate with one colony per well. These were then incubated for 3.5 hours in 37°C. PCR Screen of *E. coli* containing pLM-1 Expression Vector Encoding H2-D^b was then performed using solution containing 1 μ L of bacterial DNA, 1 μ L of reverse primer, 1 μ L of forward primer, 1.5 μ L of dNTP, 1.5 μ L of 10X PCR Buffer, 0.05 μ L of Taq DNA polymerase, and 9 μ L of ddH₂O. The PCR cycle consisted of an initial denaturing phase at 94°C for 1:00 minutes followed by 30 amplification cycles (94°C for 30 seconds, 60°C for 15 seconds, 72°C for 1:50 minute

5 colonies, labeled clones 10, 12-15, were then selected and were grown overnight in 3mL of 2x TY medium containing 1000x Carbinicillin. Mini-prep was then performed and a spectrophotometer was used to measure DNA concentration in each clone. A 1:25 dilution was performed on all samples.

Expression of H2-D^b

Previously screened clones were then transformed into BL21 *E. coli* cells and were then incubated on Carbinicillin plates overnight at 37°C. Colonies from

each clone (10, 12, 13, 14 and 15) were then PCR screened. The PCR cycle consisted of an initial denaturing phase at 94°C for 1:00 minutes followed by 30 amplification cycles (94°C for 30 seconds, 60°C for 15 seconds, 72°C for 1:50 minute. Samples were then run on a 2% agarose gel with pLM-1 vector without H2-D^b used as a negative control.

Transformed BL21 cells were then cultured at 37°C overnight in 2x TY medium containing 1000x Carbinicillin. 0.5mL of culture were removed from the sample and frozen via the addition 200 µL of 80% glycerol. Isopropyl β-D-thiogalactopyranoside (IPTG) was added to 1 mL remaining culture and pre and post-induction cultures were incubated for 3 hours at 37°C shaking until a final O.D of 0.6 was achieved. Cultures were then centrifuged and bacterial pellets were boiled in 50 µL 2X SDS sample buffer at 95 C for 5 minutes.

SDS-Gel Assay for H2-D^b

10 µL of the 50 µL samples boiled in 2X SDS sample buffer, were loaded into a 12% SDS-PAGE gel and run at 200V at 0.3 A for 55 minutes at RT. Gel was then stained for 20 minutes using Coomassie Blue. Gel was then subjected to a fast de-stain of 15 minutes using a 50% methanol, 10% acetic acid. An additional fast de-stain was then performed. This was then followed by an overnight slow de-stain composed of 5% methanol and 7% acetic acid.

RESULTS

Studies have shown that CD8⁺ T-cells, reactive to GFAP, can induce central nervous system autoimmunity with pathologies similar to multiple sclerosis. MHC Class I H2-D^b molecule was selected for crystallization as a direct result of the molecule's presentation of glial fibrillary acidic protein (GFAP). The purpose of this project was to express the β 2-microglobulin domain of the H2-D^b as well as to clone and express H2-D^b α domain for its future crystallization.

SDS-PAGE Gel Assay depicts successful Expression of the β 2-microglobulin H2-D^b Domain

First, the β 2-microglobulin domain of MHC Class I H2-D^b in a pBac bacterial expression vector was transformed into BL21 *E. coli* cells. Transformed BL21 cells were then cultured at 37°C overnight in 2x TY + 1000x Carbinicillin, and induced at 0.1M IPTG.

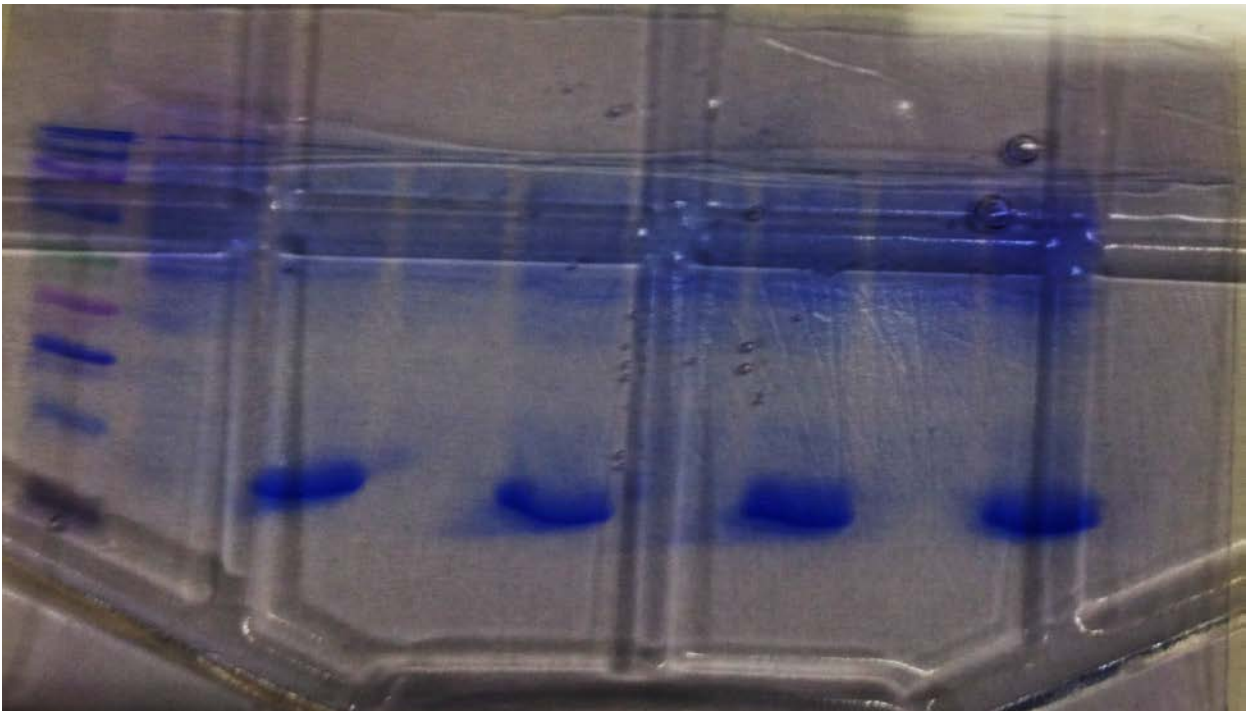


Figure 5: SDS-PAGE Gel Assay for the Expression of the β 2-Microglobulin Domain of MHC Class I.

The SDS-PAGE Gel Assay (**Figure 5**) was performed to confirm the expression of the β 2-microglobulin domain of the MHC Class I H2-D^b molecule. Dark bands in lanes 2, 4, 6, and 8 at approximately 800 bp depict post-induction clones (1-4) of the β 2-microglobulin demonstrating successful expression. Lanes 1, 3, 5, and 7 depict the pre-induced clones.

Cloning of H2-D^b Genes into pLM-1 Expression Vector

To construct the expression vector for H2-D^b α domain (Figure 6) , the gene fragment coding for amino acids 888-1745 was amplified by polymerase chain reaction (PCR) using the oligonucleotides GGGGGGAATTCATGGGCCACACTCGATGCG (forward primer) & CCCCCAAGCTTTTACCATCTCAGGGTGAGGGGCTCAGG (reverse primer). Both fragment and vector were cut using HindII and EcoRI-HF. The resulting expression vector contained the H2-D^b gene fragment ligated at 888 bp and 1745 bp. This expression vector also contained genetic sequence for ampicillin resistance (**Figure 6**).

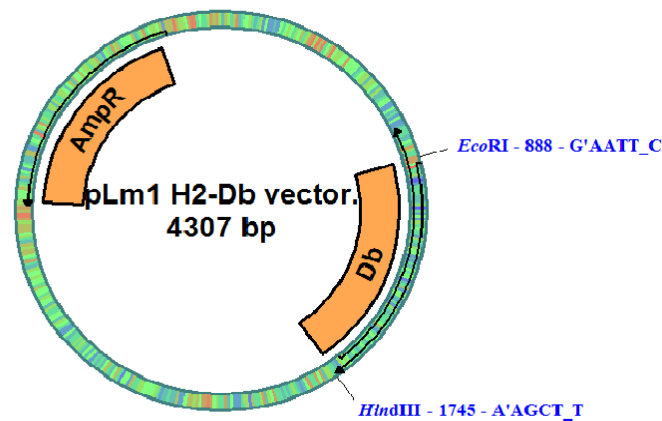


Figure 6: Map of the pLM-1 Expression Vector Encoding H2-D^b

A PCR screening of 24 clones containing the pLM-1 vector with the H2-D^b insert was then conducted (**Figure 7**). Bands at approximately 800 bp, (in lanes 1, 3, 4, 9, 10 12, 13, 14, 15, 17-19, 20-21) depict the successful cloning of H2-D^b into its expression vector (Figure 7).

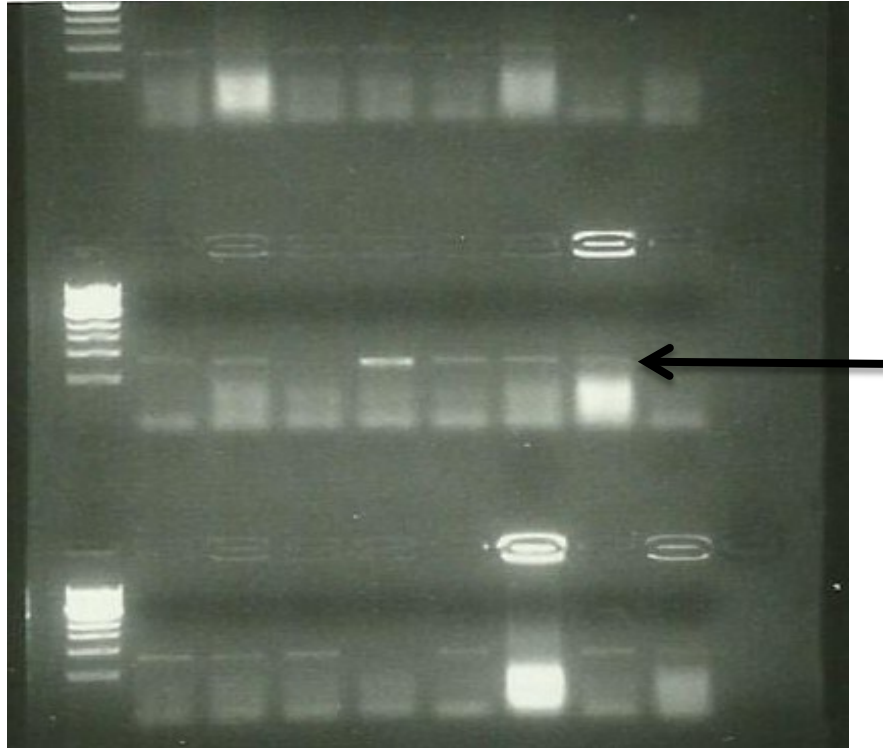


Figure 7: PCR Screen of E. coli containing pLM-1 Expression Vector Encoding H2-D^b.

5 colonies, labeled clones 10, 12-15, were then selected from data of PCR screen (Figure 7) and were grown overnight in 3mL of 2x TY medium containing 1000x Carbinicillin. Mini-prep was then performed and a spectrophotometer was used to measure DNA concentration in each clone (**Table 1**).

Table 1: DNA concentration of clones 10, 12, 13, 14, 15 (ng/ μ L)

Clone Number	DNA Concentration (ng/ μ L)
10	334.2
12	243.7
13	362.7
14	364.5
15	280.4

The results demonstrated high DNA concentrations for clones 10, 13, and 14 of over 300 ng/ μ L. Clones 12 and 15 showed lower concentration at 243.7 ng/ μ L and 280.4 ng/ μ L respectively.

Expression of H2-D^b

DNA from clones 10, 12, 13, 14 and 15 were transformed into BL.21 cells, plasmid DNA was isolated and PCR was done for H2-D^b (**Figure 8**). Lanes 2 through 5 represent clonal DNA taken from clones 10, 12, 13, 14, and 15, respectively, with each containing a strong band at 800 bp, indicative of H2-D^b. Lane 6 represents negative control clonal DNA from pLM-1 plasmid vector without H2-D^b.

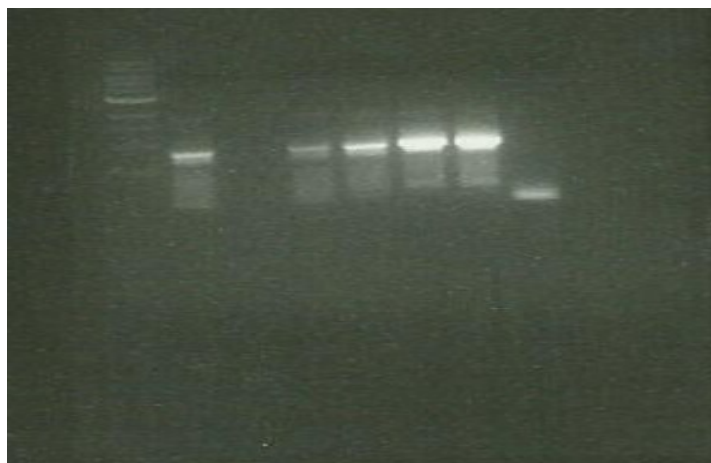


Figure 8: PCR Screen of Potential Positives Transformed into BL.21 Cells.

MHC Class I H2-D^b in a pBac bacterial expression vector was transformed into BL21 *E. coli* cells. Transformed BL21 cells were then cultured at 37°C overnight in 2x TY + 1000x Carbinicillin, and induced at 0.1M IPTG. The SDS-PAGE Gel Assay (**Figure 9**) was performed to confirm the expression of the MHC Class I H2-D^b α domain. Absence of bands at approximately 800bp, show that expression of the α domain was unsuccessful. Sequencing of clones 10, 12, 13, and 14 resulted in no priming capabilities.

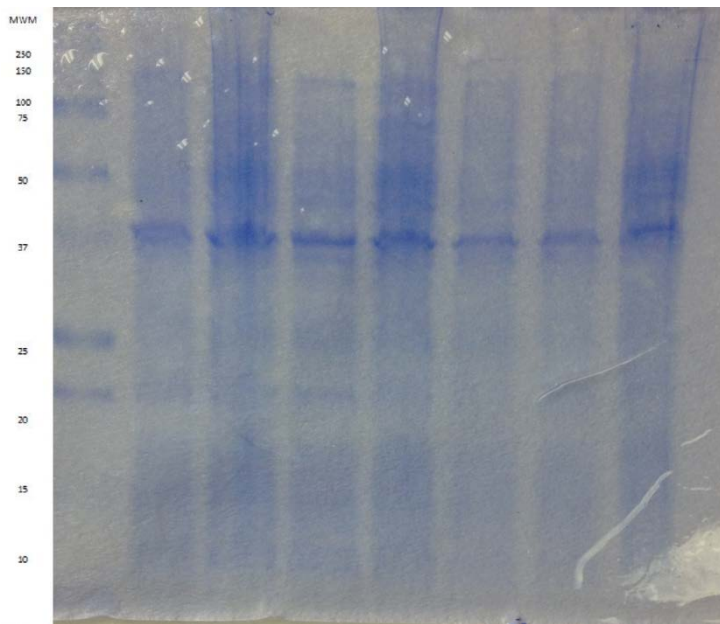


Figure 9: SDS-PAGE Gel Assay for the Expression of the α H2-D^b Domain of MHC I molecule.

DISCUSSION

Studies have shown that transgenic mice expressing a T cell receptor (TCR) reactive to glial fibrillary acidic protein presented by the MHC Class I H2-D^b molecule can succumb to spontaneous autoimmunity with pathologies comparable to Multiple Sclerosis (Huseby, preliminary unpublished data). MHC Class I molecules are composed of two polypeptide chains covalently bonded, α and β -chain (Murphy, 2012). The purpose of this project was to express the β 2-microglobulin domain of the H2-D^b as well as to clone and express H2-D^b α domain for its future crystallization

Data collected from PCR screens (Figure 8 and 7) demonstrate a successful cloning of H2-D^b into the pLM-1 expression vector. These agarose gels depict clones containing fragments of size equal to that of H2-D^b molecule (~800bp). Large concentrations of bacterial DNA taken from these clones (Table 1) further supported the success of cloned fragment into the PLM-1 expression vector. Sequencing for these clones, however, revealed a lack of priming. This could have resulted from non-functional or contaminated primers as well as mutations within the H2-D^b containing pLM-1 vector.

Prior to obtaining sequencing results, clones (Table 1) were induced and tested for expression. The induction and expression of these clones proved to be unsuccessful (Figure 9). This could indicate that the H2-D^b fragment did not clone successfully into the pLM-1 vector or that detrimental mutations within the sequence occurred during cloning preventing protein expression. In addition, mutations within the pLM-1 expression vector could have prevented the successful ligation of an accurate gene

sequence. Other possible causes for lack of expression could have resulted from the use of non-functional enzymes or contaminated IPTG.

Experimental data concerning the β 2-microglobulin domain of the MHC Class I demonstrated the successful induction and expression (Figure 5). This will then allow for the future refolding of the whole refolding of the H2-D^b MHC Class I molecule once the α domains are successfully expressed. Following the re-folding, crystallization of this MHC molecule bound to the GFAP ligand, seen in BG1 Tg mice, will reveal its structure. Structural analysis of MHC Class I H2-D^b + GFAP will depict any abnormalities within the H2-D^b epitope and shed light the molecule's role in the spontaneous autoimmune disease seen in BG1 Tg mice (Huseby, unpublished preliminary data).

The ability to define the antigenic epitope of the MHC molecule bound to the GFAP peptide will determine its pathogenic role and will open ways for production of this molecule in ex vivo analysis, in vivo imaging reagents, and in the efficient visualization of disease progression within the BG1 Tg mice models and eventually in humans. Defining the antigenic epitope of the MHC H2-Db molecule will allow for the introduction of retrogenic methods in order to determine the pathogenicity of T cells. The determination and defining of molecular targets of these auto reactive T cells will then allow for a modulation of this autoimmune response through the use of antigen-specific therapies. These therapies could then, eventually, prevent the development of this autoimmune disease in mice, and eventually in MS patients.

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