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# Delineation of B*m*SXP antibody V-gene usage from a lymphatic filariasis based immune scFv antibody library

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ABSTRACT

Phage display technology is an important tool for antibody generation or selection. This study describes the development of a scFv library and the subsequent analysis of identified monoclonal antibodies against B*m*SXP, a recombinant antigen for lymphatic filariasis. The immune library was generated from blood of lymphatic filariasis infected individuals. A TA based intermediary cloning approach was used to increase cloning efficiency for the library construction process. A diverse immune scFv library of $10^8$ was generated. Six unique monoclonal antibodies were identified from the 50 isolated clones against B*m*SXP. Analysis of the clones showed a bias for the IgHV3 and Vκ1 (45.5%) and IgHV2 and Vκ3 (27.3%) gene family. The most favored J segment for light chain is IgKJ1 (45.5%). The most favored D and J segment for heavy chain are IgHD6-13 (75%) and IgHJ3 (47.7%). The information may suggest a predisposition of certain V genes in antibody responses against lymphatic filariasis.



## 1. Introduction

B cells are generally responsible for antibody-mediated responses in the immune system. The B cell responses are defined by the generation of unique receptor molecules known as antibodies (Parra et al., 2013). The diverse nature of B cells to generate antibodies against a plethora of antigens is related to the immune repertoire available. The immune repertoire is generated by complex recombination and diversification mechanisms in vivo. The main mechanism involved is the V(D)J recombination variability (V), diversity (D) and joining (J) that occurs in the bone marrow. Further diversification of the antibody genes is carried out by somatic hypermutation and class switch recombination that occurs mainly in secondary lymphoid organs for B cells (Dudley et al., 2005; Lim et al., 2010).

Investigations on antibody repertoire have shown skewed preferences for certain diseases but generally carried out for several infected individuals to provide a global perspective. Therefore, the correlation of the molecular and genetic diversity inherent in antibody responses to a particular disease is difficult to study (Lim et al., 2014). However, diversity correlation for specific antigens provides a more directed correlation as opposed to population correlations whereby the antigen responsible for diversification is unknown.

There are two potential approaches to analyze antibody repertoires in response to a particular antigen. The first would require the cumbersome process of identifying antigen-specific cells in enriched populations and conducting single-cell PCR analysis. In this manner, native pairing information can be retrieved from antigen specific responses. Another approach requires the generation of combinatorial libraries of antibodies derived from donor cells. These libraries would then be screened against the target antigen to enrich target specific antibodies. Although more practical and adaptable for repertoire analysis, the combinatorial mixing of antibody genes during the construction process may result in the lost of native heavy (HC) and light chain (LC) pairing (Houimel, 2014). The screening process of such libraries is normally done using display technologies such as phage display. The physical interaction between the antibody genotype with phenotype allows for easy HC and LC pairing analysis of target specific enriched antibodies following the panning process.

There are three main types of antibody libraries available, mainly the naïve, synthetic and immune libraries, each differing by the source of the antibody genes (Ahmad et al., 2012). A Naïve library constitutes the antibody genes from B cells that have not been exposed to an immunological trigger. Naïve libraries are therefore highly diverse in terms of antibody repertoire making it ideal for the isolation of antibodies against a variety of antigens. Synthetic libraries are similar in terms of diversity as the

*Abbreviations:* PCR, polymerase chain reaction; Ig, immunoglobulin; scFv, single chain variable fragments; mAb, monoclonal antibody; 2xTY-GAK, teriffic broth supplemented with glucose, ampicilin and kanamycin; BSA, bovine serum albumin; RE, restriction enzyme; HC, native heavy chain; LC, light chain; aa, amino acid.

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naïve libraries but the repertoire is generated entirely by synthetic oligonucleotides. Immune libraries are generated from a specialized repertoire as the antibody genes are obtained from B-lymphocytes isolated from infected patients of a particular disease. Immune libraries in comparison to the earlier two libraries, contains the most abundant collection of antibodies towards a target of the particular disease. This is due to the polarization of the V-gene repertoire in the B cells for antigen-specific V-genes. The pairing of the most frequent variable heavy chain ($V_H$) and variable light chain ($V_L$) sequences has been shown to generate specific antibodies for a number of protein targets (Wang et al., 2013). Therefore immune antibody libraries best represent immune repertoire especially towards a specific disease.

Antibody repertoire analysis for neglected infectious diseases mainly parasitic infections is important. This is to understand the role these parasites play in shaping the repertoire distribution for antibody generation *in vivo*. Lymphatic filariasis (LF) is a neglected tropical disease transmitted by mosquitoes. It is caused by three species of tissue dwelling filarial nematodes that live in the human lymphatic system namely *Wuchereria bancrofti*, *Brugia timori* and *Brugia malayi* (Noordin, 2007). Most of the infections worldwide are caused by *W. bancrofti* but in Asia, the disease can also be caused by *B. malayi* and *B. timori*. A wide range of mosquitoes vectors such as *Anopheles*, *Aedes*, *Mansonia titillans*, and *Culex quinquefasciatus* can transmit the parasite depending on the geographical location. It is estimated that around 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis. Out of these, 66% live in the WHO South-East Asia Region and 33% in the African Region. People with the disease can suffer from disfigurement and permanent disabilities due to lymphedema, which is the swelling from fluid build-up, caused by improper functioning of the lymph system. Elephantiasis is a condition where the limbs or other parts of the body are grotesquely swollen or enlarged. People with the disease suffer from hidden internal damage to the kidneys and lymphatic system caused by the filariae (Tyrell, 2013; Wynd et al., 2007). In addition, the physiological and social stigma associated with the disease is significant and can adversely affect productivity and quality of life.

This study describes the generation of a LF immunized phage display antibody library from LF infected patients. Library panning was done against B*m*SXP, a diagnostic marker for LF to identify populations of antibodies specific to it (Khoo et al., 2012). This antigen is derived from the clone isolated from a *B. malayi* adult male worm cDNA library. The recombinant strain BmSXP/pROEXTM HT/TOP 10 was constructed with the open reading frame (ORF) SXP1 gene (462 bp) and cloned into the pROEXTMHTa expression vector. The cloned gene encodes for the BmSXP recombinant protein that has a size approximately 21.8 kDa. The enriched antibodies were analyzed for V–(D)–J usage as well as complementarity determining region (CDR) lengths. The isolation of similar antibody sequences was also evident during the selection process. The heavy chain V–(D)–J and light chain V–J segments that are preferred for B*m*SXP were characterized. Sequence analysis was done to investigate the somatic modification in terms of hypermutation and CDR compositions. It is noteworthy, that the results could shed light on B cell repertoire in parasitic infections and is also relevant for studies regarding the immune response towards LF and other parasitic infections.

## 2. Methods

### 2.1. Immunoglobulins variable regions amplification

Blood samples were collected from five lymphatic filariasis infected individuals. The human research ethics approval for the blood collection has been obtained from Universiti Sains Malaysia Human Ethics Committee. Total RNA was extracted using the QIAamp RNA Blood Mini Kit as recommended by the manufacturer (Qiagen) from human lymphocytes collected from infected donors. Full-length cDNAs were generated from 500 ng of total RNA per reaction using the Superscript II Reverse Transcriptase kit according to manufacturer's protocol (Invitrogen).

**Table 1**
Primers used for primary antibody variable region repertoire amplification.

| Primer name | Sequence 5′—3′ |
|---|---|
| To amplify VH antibody genes | |
| VH1 Fw | 5′-CAGGTCCAGCTKGTRCAGTCTGG-3′ |
| VH157 Fw | 5′-CAGGTGCAGCTGGTGSARTCTGG-3′ |
| VH2 Fw | 5′-CAGRTCACCTTGAAGGAGTCTG-3′ |
| VH3 Fw | 5′-GAGGTGCAGCTGKTGGAGWCY-3′ |
| VH4 Fw | 5′-CAGGTGCAGCTGCAGGAGTCSG-3′ |
| VH4 DP63 Fw | 5′-CAGGTGCAGCTACAGCAGTGGG-3′ |
| VH6 Fw | 5′-CAGGTACAGCTGCAGCAGTCA-3′ |
| Human ScFv IgG1-4 Rv | 5′-GACCGATGGGCCCTTGGTGGA-3′ |
| To amplify VK antibody genes | |
| VK 1 Fw | 5′-GACATCCRGDTGACCCAGTCTCC-3′ |
| VK 3 Fw | 5′-GAAATTGTRWTGACRCAGTCTCC-3′ |
| VK 5 Fw | 5′-GAAACGACACTCACGCAGTCTC-3′ |
| VK 246 Fw | 5′-GATATTGTGMTGACBCAGWCTCC-3′ |
| VK 2N1 Fw | 5′-AGATGCTGTGTGAMCCAGCCTC-3′ |
| ScFv Fab kappa CL Rv | 5′-ACACTCTCCCCTGTTGAAGCTCTT-3′ |
| To amplify VL antibody genes | |
| VL 1 Fw | 5′-CAGTCTGTSBTGACGCAGCCGCC-3′ |
| VL 1459 Fw | 5′-CAGCCTGTGCTGACTCARYC-3′ |
| VL 15910 Fw | 5′-CAGCCWGKGCTGACTCAGCCMCC-3′ |
| VL 2 Fw | 5′-CAGTCTGYYCTGAYTCAGCCT-3′ |
| VL 3 Fw | 5′-TCCTATGWGCTGACWCAGCCAA-3′ |
| VL 3DPL16 Fw | 5′-TCCTCTGAGCTGASTCAGGASCC-3′ |
| VL 338 Fw | 5′-TCCTATGAGCTGAYRCAGCYACC-3′ |
| VL 6 Fw | 5′-AATTTTATGCTGACTCAGCCCC-3′ |
| ScFv Fab Lambda CL1Rv | 5′-TGAACATTCTGTAGGGGCCACTG-3′ |
| ScFv Fab Lambda CL2 Rv | 5′-TGAACATTCCGTAGGGGCAACTG-3′ |

Primary antibody variable gene repertoires were amplified using each of the primers described in Table 1 (Lim et al., 2010). Briefly, a 30 cycles of PCR with the reaction consisting of 20 ng of cDNA, 2 μL of 10X Vent buffer with $MgCl_2$, 0.4 μL of 10 mM dNTP mix, 0.2 μL Vent Polymerase (New England Biolabs) and 10 μM of each forward and reverse primer was used. 15 μL of distilled water was added to a final volume of 20 μL. The amplification was performed using the following program: initial denaturation step (95 °C, 90 s), followed by 30 cycles made up of a denaturation step (95 °C, 90 s), annealing step (55 °C, 30 s) and an extension step (72 °C, 1 min). This was concluded by a final extension step at 72 °C for 5 min. An independent amplification reaction using each primer set was performed for each donor. All heavy and light chain PCR products resolved by electrophoresis on a 1% w/v agarose gel and purified using QIAquick gel extraction kit (Qiagen). The purified product was used for re-amplification to introduce the RE sites.

Second PCR amplification to introduce the RE sites were carried out under similar conditions as the first PCR reaction. However the amount of DNA used for the second amplification is 100ng of DNA in a 20 μL reaction. Primers used for the second amplification are shown in Table 2. The PCR cycling conditions used are also similar to that of the first PCR reaction. An independent amplification reaction using each primer set was performed for each donor. All the PCR products resolved by electrophoresis on a 1% w/v agarose gel and purified using QIAquick gel extraction kit (Qiagen). A final PCR amplification was carried out only with the variable region of the heavy chain. This is to introduce the glycine–serine linker sequence at the 3′-end. The PCR set up and program was the same as described before. All the PCR products resolved by electrophoresis