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Generation of a T cell receptor (TCR)-like single domain antibody (sDAb) against a *Mycobacterium Tuberculosis* (*Mtb*) heat shock protein (HSP) 16kDa antigen presented by Human Leukocyte Antigen (HLA)-A*02

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ABSTRACT

The discovery of heat shock protein 16 kDa antigen protein has deepen the understanding of latent tuberculosis since it was found to be primarily expressed by *Mycobacterium tuberculosis* during latent phase leading to the rapid optimization and development in terms of diagnosis and therapeutics. Recently, T cell receptor-like antibody has been explored extensively targeting various diseases due to its dual functionality (T cell receptor and antibody). In this study, a TCR-like domain antibody (A2/Ab) with the binding capacity to *Mtb* heat shock protein (HSP) 16 kDa antigen presented by major histocompatible complex (MHC) HLA-A*02 was successfully generated via biopanning against human domain antibody library. The generated antibody (A2/Ab) exhibited strong functionality and binding capacity against the target assuring the findings of this study to be beneficial for the development of latent tuberculosis diagnosis and immunotherapeutics in future.

1. Introduction

Tuberculosis (TB) is an infectious airborne disease caused by *Mycobacterium tuberculosis* (*Mtb*) and is known to be one of the oldest disease affecting mankind dating back to 4000 years ago (Zaman, 2010; Delogu et al., 2013). According to the Global Tuberculosis Report 2016 published by World Health Organization (WHO), an estimated 10.4 million new TB cases were reported worldwide which comprised of 56% male, 34% female and 10% children in 2015 (W.H. Organization, 2016). In general, TB can be divided into active and latent tuberculosis. Active tuberculosis is the stage in which the *Mtb* infected individual develops and exhibits symptoms depending on the infection site, as a result of the pathogen invading the immune defences (WHO, 2013). On the other hand, individuals with latent tuberculosis are infected by *Mtb* but the infection is asymptomatic, challenging to be diagnosed and may progress into active tuberculosis due to immunocompromisation (Lee, 2016) which makes latent tuberculosis the primary focus of this study.

Heat shock protein (HSP) 16.3 or 16 kDa antigen is one of two heat shock proteins acquired by *Mtb* and acts as molecular chaperons during protein complex assembly and disassembly (Verbon et al., 1992). The 16 kDa antigen is found to be primarily expressed by *Mtb* during the stationary phase in which the bacteria undergoes oxygen and nutrient deficiency, which highlights its crucial role in ensuring the survival of *Mtb* during latent infection (Siddiqui et al., 2011; Caccamo et al., 2002). In this study, peptides derived from the 16-kDa protein of *Mycobacterium tuberculosis* with the binding capacity to the major histocompatibility complex (MHC) class 1 HLA-A*02 were selected. The HLA-A*02 allele frequency is known to be globally common which makes it a suitable candidate for our study (Allele, 2017).

Antibody-based therapies are considered as a main approach to treat many infectious and cancer in recent years since the first breakthrough by Kohler and Milstein in 1975 through the production of monoclonal antibodies via hybridoma technology (Freysd'ottir, 2000). Since then, antibody-based therapies have been further explored with the rapid development in science and technology. Phage display technology is the alternative method for the generation of monoclonal

Abbreviations: ABTS, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; APC, Antigen presenting cells; APD, Antigen Phage Display; BSA, bovine serum albumin; DAb, human domain antibody; ELISA, enzyme-linked immunosorbent assays; Fv, Fragment variable; HLA, human leukocyte antigen; HSP, heat shock protein; IEDB, Immune Epitope Database and Analysis Resource; IPTG, Isopropyl-B-d-thiogalactopyranoside (IPTG); MHC, major histocompatibility complex; *Mtb*, *Mycobacterium tuberculosis*; PBS, phosphate buffer saline; PEG, polyethylene glycol; scFv, single chain fragment variable single; sDAb, single domain antibody; TB, tuberculosis; TCR, T cell receptor; Tregs, regulatory T-cells; UV, Ultraviolet; VH, variable heavy chain; WHO, World Health Organization; β2-M, Beta 2-microglobulin

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antibodies, bearing several significant advantages when compared to hybridoma technology (Dantas-Barbosa, 2012 #397). Discovered in 1985, phage display technology is based on the concept that phage genotype and phenotype are physically linked (Smith, 1985). The technology was illustrated by inserting a target DNA into the genome of a M13 filamentous bacteriophage which then generates phage particles displaying the protein encoded by the target DNA and depending on the cloning system used, phage DNA isolated from the selected clones, could be advance manipulated in vitro to generate soluble proteins and antibodies unlinked to the phage, but useful for diagnostic or therapeutic applications (Bazan et al., 2012). Besides that, generation of high variant peptide (Wu et al., 2016), antibody (Schofield et al., 2007) and ligand (Koivunen et al., 1999) libraries are possible through phage display.

T cell receptor (TCR)-like antibodies, also known as TCR-mimic antibody is a very interesting approach for therapeutics and diagnostics. It was first reported by Andersen et al and the idea has been exploited differently to treat many diseases including cancer, viral infection and auto immune disease since then (Andersen et al. 1996) (Poschke et al.) (Zhang et al. 2013)(Lai, 2017 #419). The highlight of TCR-like antibody is its capability of delivering the best of both humoral and cell-mediated immunity in a platform. T cell (T cell receptor) which belongs to cell-mediated immunity, distinguishes antigenic peptides presented on major histocompatible complex (MHC) of all nucleated cells (Wood, 2006). The MHC class I molecules in particular plays a crucial role in the internal surveillance of the immune system by detecting the synthesis of non-self or foreign proteins within the cell, resulting in the elimination of the infected or mutated cells by cytotoxic T cells (Janeway et al., 2001). B-cell receptors (humoral immunity) on the other hand recognizes three dimensional antigen structure (soluble or membrane-bound) and has a greater capability to eliminate diseases through antibodies (Ivan and Peter, 1971). Therefore, TCR-like antibody would be capable of internal surveillance (T cell) as well as efficiently eradicate a disease (antibody) with the help of NK cells or recruitment of complements. This will enable the detection of latent TB and potentially lead to an effective immunotherapy.

In this study, TCR-like monoclonal domain antibody against the heat shock 16-kDa antigen protein of *Mycobacterium tuberculosis* with the binding capacity to the major histocompatibility complex (MHC) class 1 HLA-A*2 was favourably generated using a human domain antibody library. The findings from this study are expected to contribute detection and potentially lead to an effective immunotherapy of latent TB.

2. Materials & methods

2.1. Bioinformatics analysis of targets to be used in generating the peptide-MHC molecule

The photoliable peptide and 16 kDa Mtb antigenic peptide (target peptide) with the binding capacity to the major histocompatibility complex (MHC) class 1 HLA-A*02 were selected from the Immune Epitope Database and Analysis Resource (IEDB) database (Vita et al., 2015). The suitability of the peptides was evaluated based on the SYFPEITHI score and literature review before peptide synthesis by First Base Malaysia Sdn Bhd.

2.2. Generation of 16 kDa antigen target peptide-MHC complex via Ultraviolet (UV)-induced peptide exchange

The HLA*A2 (heavy chain) and beta 2-microglobulin (β2-M) (light chain) vectors were kindly provided by Prof. Dr. Ton Schumacher and the generation of 16 kDa antigen target peptide-MHC complex via UV-induced peptide exchange was performed according to their published protocol (Rodenko et al., 2006). The success of the peptide exchange was measured via enzyme-linked immunosorbent assays (ELISA). The assay was used for the detection of β2-M which illustrated MHC degradation and the success of the peptide exchange. Briefly, HLA*A2 (heavy chain) and beta 2-microglobulin (β2-M) (light chain) vectors obtained were transformed into Bl21 (DE3) pLysS cell via electroporation before plating the cells on 2 YT agar plates supplemented with 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol. A single colony was picked and grown in 10 mL 2 YT media overnight at 37 °C for protein expression the next day. To induce protein expression, 100 μL of 1 M IPTG was added to each 2 L Erlenmeyer flask containing 500 ml of bacterial culture. The pellet of both culture were obtained by centrifugation at 4 °C, 4000 *g* for 30 min before the inclusion bodies were isolated. The heavy chain, β2-M and the photoliable peptide were refolded based on the established protocol adapted in order to generate the photoliable peptide-MHC complex (Rodenko, 2006 #164).

The generated photoliable peptide-MHC complex was used in the UV peptide exchange reaction in which three different conditions (C1, C2, C3) were tested. The success of the peptide exchange was analyzed via enzyme-linked immunosorbent assay (ELISA). In short, each well was coated with 2 μg/mL streptavidin in PBS and incubated at 37 °C for two hours. The wells were washed with 1 X PBST wash buffer three times, discarding the wash buffer after each wash. 300 μl of 2% BSA were added to each well before incubating at room temperature for an hour in order to block the wells. After tipping out the blocking buffer and washing the wells with 1 X PBST, MHC monomer solutions of different conditions were added to each well. The plate was incubated for 1 h at 37 °C. Following this, the wells were washed three times with 300 μl of wash buffer per well before adding 100 μl of horseradish peroxidase (HRP) conjugated anti-β2 M solution and incubation at 37 °C for an hour. The wells were washed three times with 300 μl of wash buffer and dried carefully by tipping out the excess liquid on a tissue. 100 μl of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) coloring solution was added to each well followed by incubation at room temperature for 10–15 minutes with careful monitoring of color development. The absorbance was measured at 414 nm in a plate reader

2.3. Preparation and purification of phage antibody repertoire

The human domain antibody library (DAb) used in the study was purchased from Source Bioscience. Preparation and purification of phage antibody repertoire via 20% polyethylene glycol (PEG)-NaCl precipitation was adapted based on the published protocol (Lee et al., 2007). The purified antibody phage was subjected to titration to determine the library size and stored at 4 °C until further use.

2.4. Biopanning of 16 kDa antigen peptide-MHC complex against human domain antibody library (DAb)

Biopanning was performed using the purified domain antibody library (DAb) against 16 kDa antigen peptide-MHC complex. The conventional biopanning process was carried out with several modifications (Rahumatullah et al., 2015). The first modification was the usage of 2% bovine serum albumin (BSA) as blocking buffer. Next, 10 μL of 10^{11} KM13 helper phage was used instead of M13K07 helper phage. Briefly, the target 16 kDa antigen target peptide-MHC complex (10 μg/ml) was immobilized on 96 Costar microtitre wells (Corning) with 1xPBS kept overnight at 4 °C. The next day, a new empty well was blocked with 300 μl of 2% BSA for an hour, 700 rpm. After 1 h, the target antigen coated well was washed three times with 1xPBST to remove unbound antigen before the well was blocked with 300 μl of 2% BSA for an hour at 700 rpm. At the same time, the empty well was washed with 1xPBST three times and approximately 1×10^{12} Dab library was incubated into the well for 1 h at 700 rpm. After 1 h, the pre-incubated phage library was transferred to the antigen-coated well and incubated for 2 h at room temperature, 700 rpm. Following that, the well was washed three times with 1xPBST. In order to elute the bound