Conference Paper

Construction Hybrid immunoglobulin All Four Dengue serotype Using Mesenchymal Stem

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Received: 03 October 2017
Accepted: 10 October 2017
Published: 29 November 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the VMIC Conference Committee.

Abstract

The dengue virus is a member of vector-borne diseases that causes zoonotic disease and spreads rapidly in the world. No single treatment or vaccine yet is available that is recommended and there is no correlation with protectiveness against this disease. The heavy chain (VH) and light chain (VL) variables are molecules of immunoglobulin G (IgG) is the smallest part of the antibody. Although the part-time domain variable is short, it can be used as a long-term and rapid immune booster in the immune system. In this study we tried to clone an encoding gene that was able to influence the adaptive immune response to dengue 1-4 by using MSC as a gene carrier. The target scFv-IgG gene has been successfully integrated into the plasmid. Plasmids that we have linearly transfected into the MSC. From the cDNA synthesis results continued with PCR synthesis with primer FGHV and RGHA obtained bands in accordance with the target of 404 bp. The scFv gene encoding IgG can be integrated with MSC.

Keywords: immunetherapy; dengue; hybrid; scFv-IgG; mesenchymal.

1. Introduction

The dengue virus is a vector-borne diseases that causes zoonotic disease and spreads rapidly in the world [1, 2]. No single treatment or vaccine is available that is recommended and there is no correlation with protectiveness against the disease. Neutralization tests were not able to distinguish between specific serotypes and cross-immune antibodies in patients at the onset of infection, which led to overestimation of the long-term serotype antibody response.

Hybridoma and genetic engineering technologies can be used by utilizing and re-modifying individual antibodies obtained from immunized individuals [3].

The heavy chain (VH) and light chain (VL) variables are molecules of the immunoglobulin G (IgG) is the smallest part of the antibody. Although the part-time domain variable is short, it can be used as a long-term and rapid immune booster in the immune system.

Cell-based therapy has improved significantly over the last few decades and is poised to become a major pillar of modern medicine. Recent studies have shown that these cells have large immune modulation capacities [4]. Mesenchymal stem cells have immunomodulatory properties on some lineages such as T cells and B regulators. Mesenchymal stem cell (MSC) has also been shown to possess extensive immunoregulation abilities and be able to cope with adaptive and innate immune responses. MSC inhibits proliferation and maturation of immune cells and suppresses immune reactions both in vitro and in vivo [5].

Genetic stem cells can be modified to express intact antibody fragments or single chain antibody fragments (scFv) [6]. MSC has also has been investigated as a carrier medium for antibody delivery. In human MSC studies have been carried out nucleophysics to be able to express scFv on its cell surface against glioma III (EGFRvIII) [7]. This platform is able to function to express a series of antigen-specific paratops.

In this study we tried to clone a coding gene that was able to influence the adaptive immune response to dengue 1-4 by using MSC as a gene carrier. Cellular response to dengue virus initiated MHC I and MHC II bonds on CD4 + and CD8 + T cells. Activation of some of these cytokines has a greater concentration in DHF patients.

2. Material and methods

TArget Clone (Toyobo,Japan), DH5α cells (Toyobo Life Science, Japan), Lipofectamine 3000 (Carlsbad, CA), Opti-MEM^{TM} Medium (Gibco, USA), trizol® (Carlsbad, CA), First Strand cDNA Synthesis Kit (Invitrogen), PCR Reagent System (Carlsbad, CA), 1Kb plus DNA Markers (Carlsbad, CA), UltraPureTM Agarose (Carlsbad, CA), Zyppy^{TM} Plasmid Miniprep Kit (Zymo,USA), SYBR^{TM} Safe DNA Gel Stain (Carlsbad, CA), Tris Buffer EDTA 0,5x, BlueJuiceTM Gel Loading Buffer (Carlsbad, CA), QIAquick Gel Extraction Kit (Qiagen).

2.1. Mesenchymal stem cellcCulture

Rat MSC obtained from Stem cell Research and Development Center, Airlangga University, Surabaya, Indonesia. Rat mesenchymal stem cell cultured in medium with Dulbecco's modified Eagle's medium (DMEM) and serum fetal bovine (FBS). Non-adherent cells are released carefully after 3 hours and replaced with fresh media. When the primary culture becomes almost confluent, culture treats 0.5 ml of 0.25% trypsin containing 0.02% ethylenediaminetetraacetic acid for 2 min at room temperature (25 °C).

2.2. Construction and identification of IgG heavy chain genes

Adapted mice treated with multivalent dengue vaccination 1-4 and control were given PBS at a dose of 0.3 ml each, Injection was performed on days 0, 7, 14, 21 and 28. The spleen was taken on day 32 after being treated with five vaccinations. Then extracted RNA using Trizol (Invitrogen) and amplified with primers set GHVF and GHVR.

RNA extraction was performed using TRIzol® Reagent Invitrogen (Allen Way, USA). Primer for PCR is designed using data from the National Center for Biotechnology Information (NCBI) site based on the sequence of the Mus musculus immunoglobulin heavy chain locus constant region of GenBank. Specific primers for IgG heavy chains are designed using Primary select software. GHVF Primary: 5'AGTGAAGCTTGAGGAGTCT 3', Primer GHVR: 5'GCTCCTGTTCCTGGTTTAC 3'. The length of the amplified segment is 400 bp.

The PCR product was detected by electrophoresis in 1% agarose gel. Target segments identified under Ultraviolet lights. Segment gene targets ligation and purification by using QIAquick Gel Extraction Kit.

2.3. Cloning scFv-IgG Gene

PCR Product coding scFv-IgG gene then sub Cloning using cassette vector pTA2, mixing on a 0.2 ml micro tube. 9 µl of the PCR product is fed into a 0.2 ml micro tube, then added a 0.1 µl 10x A- attachment mix then mixed by gently tapping the tube with the finger. The tube was incubated in a thermal cycler with a temperature of 60 °C 10 minutes. A 5 µl 2x reagent, 1 µl pTA2 (50ng / µl) vector, 3 µl of dA-attached PCR product and 1 µl T4 DNA Ligase ligation buffer were added. Incubated at room temperature (15-25 °C) was performed in a thermal cyler with a temperature of 20 °C for 30 minutes, then transformed. then mixed by gently tapping the tube with the finger. The tube was incubated in a thermal cycler with a temperature of 60 °C 10 minutes. A 5 µl 2x reagent,

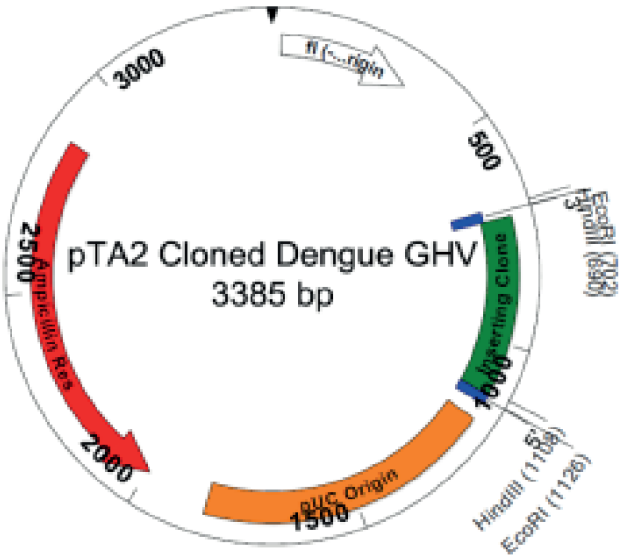

Figure 1: Map of plasmid vector.

1 µl pTA2 (50ng / µl) vector, 3 µl of dA-attached PCR product and 1 µl T4 DNA Ligase ligation buffer were added. Incubated at room temperature (15-25 °C) was performed in a thermal cyler with a temperature of 20 °C for 30 minutes, then transformed.

2.4. Transformation

The cultures of E. coli strain BL21 mix & go cells (zymo, USA) were grown on 0.5 L of LB medium and then grown on 50 ml of ZymoBroth ^{TM} medium in erlenmeyer. After completion it is then grown on LB to have ampicillin added 100 µl / ml then incubated in an incubator at 37°C for 48 hours according to the manufacturer's recommended protocol.

The plasmid is extracted from E.coli bacteria by using Zyppy ^{TM} Plasmid Miniprep Kit according to the manufacturer's recommended protocol (Zymo, USA). The plasmid restricted by HIND III. Plasmid pTA2 / GHV (1.2 µg) 5 µl, HIND III 1 µl, 10 × Buffer K1 µl, 10 × BSA 2 µl, sterile water added up to 20 µl, cutting was done on dry bath at 37°C for 4 Hour, then detected by electrophoresis with 1% agarose then the result continued sequencing.

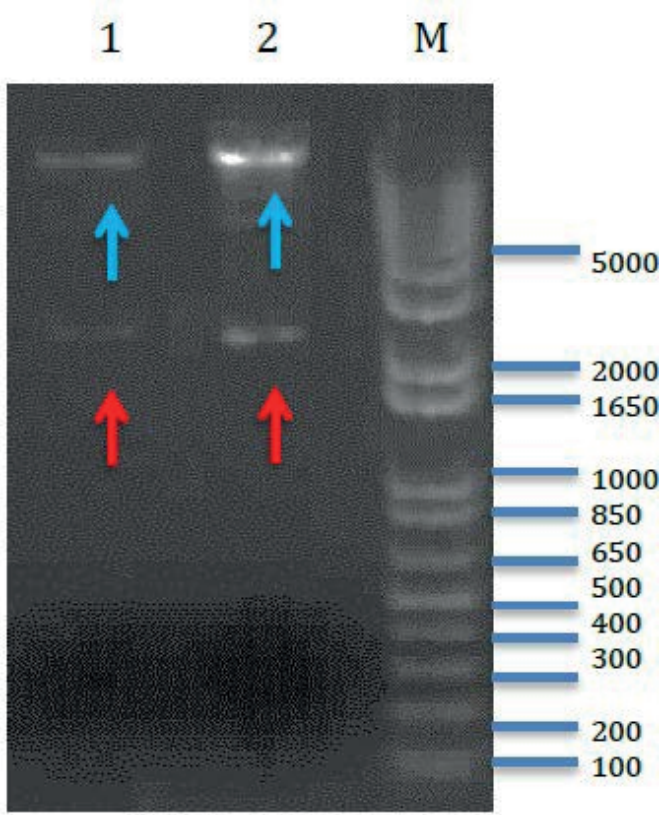

Figure 2: Positive clone analysis. Recombinant plasmid digestion product with 1% agarose electrophoresis shows, red arrow is for target gene fragment, blue arrow for plasmid vector. 1. GHV Control; 2. GHV dengue; M. Marker 1 kb Plus.

2.5. Transfection MSC

Adapted mice treated with multivalent dengue vaccination 1-4 and control were given PBS at a dose of 0. In this study used conventional lipofectamine method to transfection on rat MSC. Briefly, Lipofectamine 3000 (Invitrogen, Carlsbad, CA) is mixed with a DNA plasmid on Opti-MEM (Invitrogen, Carlsbad, CA) media according to the manufacturer's recommendation. MSC mouse cells were incubated on Opti-MEM medium with DNA- lipofectamine complex for 6 hours, then media replaced with complete media that FBS added.

3. Result

To verify the genes we cloned can be integrated with the plasmid vector and have been transformed to competent E. coli, the plasmids were digest with HINDIII, the result of restriction was electrophoresis. From the electrophoresis obtained two bands, targeting cloning and plasmid vectors (fig.2).

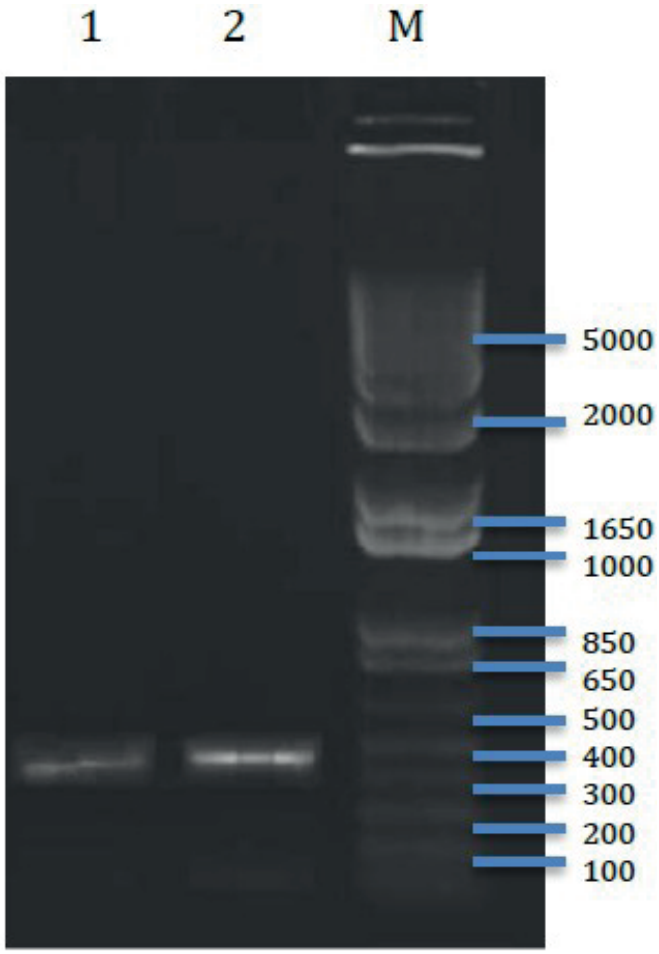

Figure 3: PCR DNA After Transfusion PCR DNA gene encoding scFv-IgG GHV Transfected. 1: Control Group; 2: Dengue Treatment Group; M: 1kb Plus MarkerDiskusi.

The target scFv-IgG gene has been successfully integrated into the plasmid. Plasmids that we have linearly transfected into the MSC. After cells confluent reach 90% we do RNA extraction from MSC cells to find out whether cells can enter the MSC cell nucleus. RNA was extracted using Trizol (Invitrogen, Carlsbad, CA). RNA obtained reverse transcription with First Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). From the cDNA synthesis results continued with PCR synthesis with primary FGHV and RGHA obtained bands according to the target of 404 bp (fig.3).

MSC that has been transfected with the gene encoding scFv is expected to express scFv-IgG on its cell surface, so can produce IgG. MSC is a unique multipotent stem cell, currently widely exploited as a vector for gene therapy, including for cancer and autoimmune diseases [8–10, 10–13].

MSC is easy to transfection with certain protein- coding plasmids either temporary or long-term and stable. The transduced MSC can produce more IL-10 to produce collagen to suppress arthritis in mouse models [15]. MSC immunoregulation also depends on

external signals. With the presence of inflammatory cytokines or stimulants. Previous MSC therapies have been suppressive and may become immunostimulants [16,17, 18].

4. Conclusion

The scFv gene encoding IgG can be integrated with MSC. The modified MSC to express scFv-IgG on its cell surface is expected to respond quickly to antigen specific to scFv-IgG. The idea for the use of MSC to express scFv-IgG against denv-1, 2, 3 and 4. Specifically, the potential of MSC as an immune therapy medium is remarkable, therefore a deep evaluation is needed for its effectiveness.

Although some studies have proven that MSC is very effective for expressing and secreting immune therapy. Analysis of the impact of specific immunity to target organs, including humoral and cellular responses of memory cells.

Acknowledgments

We would like to thank to the Stem Cell development and research of Airlangga University, This work was supported by grants DIPA Ditlitabmas Year 2015 No. 519 / UN3 / 2015.

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