Molecular analysis of *Toxoplasma gondii* Surface Antigen 1 (SAG1) gene cloned from *Toxoplasma gondii* DNA isolated from Javanese acute toxoplasmosis

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Abstract. *Toxoplasma gondii* Surface Antigen 1 (SAG1) is often used as a diagnostic tool due to its immunodominant-specific as antigen. However, data of the *Toxoplasma gondii* SAG1 protein from Indonesian isolate is limited. To study the protein, genomic DNA was isolated from a Javanese acute toxoplasmosis blood samples patient. A complete coding sequence of *Toxoplasma gondii* SAG1 was cloned and inserted into an *Escherichia coli* expression plasmid and sequenced. The sequencing results were subjected to bioinformatics analysis. The *Toxoplasma gondii* SAG1 complete coding sequences were successfully cloned. Physicochemical analysis revealed the 336 aa of SAG1 had 34.7 kDa of weight. The isoelectric point and aliphatic index were 8.4 and 78.4, respectively. The N-terminal methionine half-life in *Escherichia coli* was more than 10 hours. The antigenicity, secondary structure, and identification of the HLA binding motifs also had been discussed. The results of this study would contribute information about *Toxoplasma gondii* SAG1 and benefits for further works willing to develop diagnostic and therapeutic strategies against the parasite.

1. Introduction

*Toxoplasma gondii* is an obligate intracellular protozoan parasite with a broad host range including humans [1]. The parasite is of great importance to public health, highlighted by the high diversity of hosts [2]. The parasite could cause toxoplasmosis, a disease that can create serious health problems in humans [3]. Not only humans which are immune compromised, the immune competent humans are also susceptible to toxoplasmosis [4]. Because of the clinical importance of toxoplasmosis, it's timely and accurate diagnosis has a major impact on disease-fighting strategies [3].
**Toxoplasma gondii** surface antigen 1 (SAG1) is a well-characterized **Toxoplasma gondii** antigen [1]. SAG1 is an immunodominant-specific antigen, therefore is often used as a diagnostic tool [3]. However, data of the SAG1 protein from Indonesian isolate is limited.

2. **Materials and Methods**

2.1. **Toxoplasma gondii Sample Selection**

Since 2009, our research group (A-IGIC/A-Infection, Genomics, Immunology, & Cancer Research Group) has been conducting a molecular epidemiology study of human blood-borne pathogens in Central Java, Indonesia [5-24]. To select the **Toxoplasma gondii** sample for cloning, all blood samples were subjected for immunological and molecular assays to find out the HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), HCV (Hepatitis C Virus), HDV (Hepatitis D Virus), TTV (Torque Teno Virus), GBV-C (GB Virus C), HTLV-1/2 (Human T-cell Lymphotrophic Virus-1/2), and **Toxoplasma gondii** infection status as described previously [5-24]. All samples were tested at least in duplicate.

2.2. **Cloning and Sequencing**

A complete coding sequence of **Toxoplasma gondii** SAG1 was cloned from a Javanese acute toxoplasmosis blood samples by nested PCR. The PCR product was inserted into *Escherichia coli* expression plasmid. For cloning, each primer was attached with a start or stop codon sequences. The recombinant plasmid was then transformed into competent cells, propagated, purified, and sequenced. The sequencing step was performed in both orientations for confirmation, three times for each orientation.

2.3. **BLAST Analysis**

A BLAST analysis was performed to confirm the sequencing results. The sequences were submitted to the BLAST program to check their similarity to related strains deposited in GenBank/EMBL/DDBJ. Multiple alignments of reference sequences were reconstructed using ClustalW as implemented in CLC Main Workbench 7.9.1 software. The frequency of nucleotide substitution at each base was estimated using the Kimura 2-parameter method. Synonymous and non-synonymous amino acid substitution events were computed using the Nei-Gojobori method with the Jukes-Cantor correction implemented in CLC Main Workbench 7.9.1 software.

2.4. **Predicted Protein Analysis**

Protein analysis was performed using CLC Main Workbench 7.9.1 software. The parameters for hydrophobicity plot was set by Kyte-Doolittle, Eisenberg, Engelman, Hopp-Woods, Janin, Rose, and Cornette hydrophobicity scale, respectively. To create the antigenicity plot, both Welling and Kolaskar-Tongaonkar antigenic scale was used. The Immune Epitope Database (IEDB) (http://tools.immuneepitope.org/mhcii/) was used to predict the peptide binding of SAG1 sequences to HLA molecules.

3. **Results and Discussion**

Blood samples collected by our research group were always screened for HIV, HBV, HCV, HDV, TTV, GBV-C, HTLV-1/2, and **Toxoplasma gondii** infection status by immunological and molecular assays. Blood sample positive for IgM anti-**Toxoplasma gondii** and **Toxoplasma gondii** DNA (acute toxoplasmosis), but negative for anti-HIV, HIV RNA, HBsAg, HBV DNA, anti-HDV, HDV RNA, TTV DNA, GBV-C RNA, anti-HTLV-1/2, HTLV-1 RNA, HTLV-2 RNA, and IgG anti-**Toxoplasma gondii** was then used for cloning the **Toxoplasma gondii** interest gene, including the **Toxoplasma gondii** SAG1. A complete coding sequence of **Toxoplasma gondii** SAG1 was successfully cloned, and the BLAST and nucleotide analysis were performed to confirm the sequences. Based on the analysis of 1011 nucleotides bases, the cloned was confirmed as **Toxoplasma gondii** SAG1.
We then perform the predicted protein analysis. The predicted protein had 336 amino acid sequence length with 34.731 kDa in weight. The isoelectric point was 8.4 and the aliphatic index was 78.423. The predicted protein had methionine N-terminal half-life in *Escherichia coli* more than 10 hours, however, shorter than in mammals (30 hours) and in yeast (>20 hours), respectively. The extinction coefficient at 280 nm for non-reduced cysteines was 21,750 (absorption at 280nm 0.1%= 0.626) while for reduced cysteines was 20,910 (absorption at 280nm 0.1%= 0.602). The count of positively and negatively charged residues was 0.074 and 0.068, respectively, in frequency.

*Toxoplasma gondii* SAG1 is an appropriate antigen with high specificity and sensitivity for the detection of *Toxoplasma gondii* [25]. The predicted SAG1 protein had antigenic positions (figure 1). Beta alpha helix secondary structures were frequent in our predicted SAG1. Only three alpha helix secondary structures were found, at aa (amino acids) 21-26, 37-39, and 243-249, respectively. The induction of CD8+ T cell responses is important in the formulation of successful immune reaction against *Toxoplasma gondii* [26]. The CD8+ T cell response is initiated by the MHC-I presentation resulting in the death of infected cells [27]. Further analysis of the possible antigenic regions presented in figure 1, nine epitopes were found had high probability of eliciting an immune response (score >0.1), i.e. IPEAEDSWWT (0.3516), LTPTEHNHTL (0.27859), KAVRRATVAG (0.24846), SNGATLTIN (0.20798), FAMVTLIGS (0.17702), ILTVPIEKF (0.14864), PVTTQTFVVG (0.14582), SSVVNNVARC (0.14446), and SENPWQGNAS (0.13894), respectively.

![Antigenicity plot](image)

**Figure 1.** Antigenicity Plot of *Toxoplasma Gondii* SAG1 Predicted Protein

### 4. Conclusions
Finally, we concluded that our clone was *Toxoplasma gondii* SAG1. Our predictive protein should be a mature protein, tended to be positively charged, had both α-helix and β-sheet secondary structures, and 9 possible antigenic regions high probability of eliciting an immune response. Taken all data together,
it will increase our understanding of our *Toxoplasma gondii* SAG1 and benefits for further works willing to develop diagnostic and therapeutic strategies against the parasite.

5. References

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