Cloning and molecular analysis of Toxoplasma gondii Surface Antigen 2 (SAG2) gene cloned from Toxoplasma gondii DNA isolated from Javanese acute toxoplasmosis

W Monica1, A A Prasetyo1,2* and S Haryati1

1 A-IGIC (A-Infection, Genomic, Immunology & Cancer) Research Group, Sumber, Banjarsari, Surakarta, 57138, Indonesia
2 Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia

*afie.agp.la@gmail.com

Abstract. The Toxoplasma gondii Surface Antigens 2 (SAG2) is an immunogenic antigen covering the Toxoplasma gondii and one of the main proteins expressed on the surface of the parasite tachyzoites. However, data of the Toxoplasma gondii SAG2 from Indonesian isolate is limited. To study the Toxoplasma gondii SAG2 from Indonesian isolate the gene for SAG2 was amplified from genomic DNA of Toxoplasma gondii isolated from a Javanese acute toxoplasmosis blood samples patient. The clone was sequencing, and the results were subjected to bioinformatics analysis. Physicochemical analysis revealed the 190 aa of SAG2 had 19.47 kDa of weight. The isoelectric point and aliphatic index were 8.61 and 73.105, 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. These findings would contribute information about Toxoplasma gondii SAG2 from Indonesian isolate.

1. Introduction

Toxoplasma gondii is an apicomplexan parasite responsible for several important human pathologies, namely as Toxoplasmosis. Toxoplasmosis is worldwide distributed with medical importance especially in immunocompromised patients and congenitally infected newborns [1]. Toxoplasma gondii infection can cause Toxoplasmic encephalitis, a life-threatening disease in immunocompromised patients [2]. Toxoplasma gondii infection could be cause of several abnormalities from hydrocephalus, microcephaly, deafness, abortion and stillbirth in fetal to psychomotor retardation, intellectual disability, hearing loss, slower postnatal motor development during the first year of life; and chorioretinitis, cryptogenic epilepsy and autism spectrum disorders in newborns [3]. Chorioretinal Toxoplasma gondii infection, ocular toxoplasmosis, is the most common etiology of posterior uveitis in the world [4]. Toxoplasma gondii infection is related to decreased weight and autoimmune thyroid diseases in the mother [3]. Moreover, even though the Toxoplasma gondii chronic infection in immunocompetent individuals usually asymptomatic but many evidences associate Toxoplasma gondii chronic infection with diverse neurological and neuropsychiatric disorders such as cryptogenic epilepsy, Parkinson's disease, schizophrenia, anxiety, depression, self-
directed violence, bipolar disorder, and violent suicide attempts [3, 5, 6]. It is estimated over one-third of the human population are chronic-latently infected with *Toxoplasma gondii* [6].

*Toxoplasma gondii* has immune evasion strategies to parasite disseminate and reach immune privileged sites [7]. Infection of immunocompetent hosts leads to long-term parasite persistence mainly within neural and muscular tissues, as intraneuronal or intramuscular cysts, respectively [8, 9]. *Toxoplasma gondii* has the ability to downregulate inflammation that could be deleterious for the parasite to survive and host cell manipulation to reach the privileged sites. After stimulation of the host immune response, *Toxoplasma gondii* differentiates into a cyst form, which can reactivate. No drugs are available to clear the cyst form due to an incomplete understanding of both host immune and *Toxoplasma gondii* responses [10].

Protein antigens not only contain epitope structures to mediate immunological responses but can also contain structures that are unfavorable for protective immunity. Therefore, the study of antigenic epitopes from *Toxoplasma gondii* has not only enhanced the understanding of many aspects of immunology but also important in the development of new diagnostic and vaccines [11]. The cell surface of *Toxoplasma gondii* is covered by surface antigens, including the Surface Antigens 2 (SAG2), that will first interact with the target cell as part of its intracellular cycle [12]. *Toxoplasma gondii* SAG2 has been a target of intense research due to its immunodominant pattern, exhibiting potential as diagnostic, immunotherapy, and/or vaccine candidates for *Toxoplasma gondii* [13]. However, limited data is known about the *Toxoplasma gondii* SAG2 from Indonesian isolate. The present study aimed to clone and perform molecular analysis of *Toxoplasma gondii* SAG2 from Indonesian isolate.

2. Materials and methods

Our research group (A-IGIC/ A-Infection, Genomics, Immunology & Cancer) performed a blood-borne molecular epidemiology study in Central Java, Indonesia, including that for *Toxoplasma gondii* [14-19]. Briefly, blood samples aliquots were examined with a DRG *Toxoplasma gondii* IgM Elisa Kit (DRG International, Springfield, NJ) to detect IgM anti-toxoplasma and a DRG *Toxoplasma gondii* IgG Elisa Kit (DRG International) to detect IgG anti-toxoplasma antibodies, according to the manufacturer’s instructions. All samples were tested at least in duplicate. To present study, an anti-*Toxoplasma gondii* IgM positive but anti-*Toxoplasma gondii* IgG negative blood sample derived from a Javanese patient was used. *Toxoplasma gondii* DNA was extracted from blood samples aliquots by using Genomic DNA Mini Kit (Geneaid, New Taipei City, Taiwan). Molecular assays were performed by a nested PCR using the MyTaq HS Red Mix (Bioline, London, UK) according to the manufacturer’s instructions, to confirm the presence of the *Toxoplasma gondii* genomic DNA. The *Toxoplasma gondii* SAG2 gene (complete coding sequence) was then cloned from the *Toxoplasma gondii* genomic DNA using MyFi Mix (Bioline, London, UK) and KOD -Plus- Mutagenesis (Toyobo, Osaka, Japan). The PCR product was purified using Zymoclean Gel DNA Recovery (Zymo Research, Irvine, CA) then molecular sequenced three times for confirmation.

The BLAST (Basic Local Alignment Search Tool) analysis was performed to confirm the *Toxoplasma gondii* SAG2 sequencing results. Multiple alignments of reference sequences were reconstructed using ClustalW as implemented in CLC Main Workbench 8.0.1 software. The *Toxoplasma gondii* SAG2 predicted protein analysis was performed using CLC Main Workbench 8.0.1 software. The parameters for *Toxoplasma gondii* SAG2 hydrophobicity plot was set by Kyte-Doolittle, Eisenberg, Engelman, Hopp-Woods, Janin, Rose, Cornette hydrophobicity scale, respectively. The non-redundant protein sequences (nr) database with standard database genetic code was used for the blastp *Toxoplasma gondii* SAG2 protein sequence analysis. Both Wellmg and Kolaskar-Tongaonkar antigenic scale was used to create the antigenicity plot of *Toxoplasma gondii* SAG2, with a BLOSUM62 matrix with gap cost existence set as 11 and extension as 1 were used. The T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource was used to predict the *Toxoplasma gondii* SAG2 immunogenicity prediction and major histocompatibility complex-binding.
3. Results and discussion

3.1. Sequencing results
*Toxoplasma gondii* genomic DNA was successfully isolated from an anti- *Toxoplasma gondii* IgM positive but anti- *Toxoplasma gondii* IgG negative blood sample derived from a Javanese patient. The *Toxoplasma gondii SAG2* gene (573 bp) was successfully cloned from *Toxoplasma gondii* genomic DNA. The Basic Local Alignment Search Tool analysis was performed to confirm the *Toxoplasma gondii SAG2* gene sequences.

| Table 1. The *Toxoplasma gondii* SAG2 protein from Indonesian isolate molecular characteristics. |
|---------------------------------------------|
| **Protein statistics** | **Characteristics** |
| Sequence information | |
| Length | 190aa |
| Weight | 19.47 kDa |
| Isoelectric point | 8.61 |
| Aliphatic index | 73.105 |
| N-terminal Methionine Half-life | |
| Half-life mammals | 30 hours |
| Half-life yeast | >20 hours |
| Half-life *E. coli* | >10 hours |
| Atomic composition | |
| hydrogen (H) | 0.503 (n = 1,372) |
| carbon (C) | 0.310 (n = 846) |
| nitrogen (N) | 0.081 (n = 222) |
| oxygen (O) | 0.102 (n = 279) |
| sulfur (S) | 0.004 (n = 11) |
| Count of residues | |
| Hydrophobic (A,F,G,I,L,M,P,V,W) | 0.484 (n = 92) |
| Hydrophilic (C,N,Q,S,T,Y) | 0.353 (n = 67) |
| Count of charged residues | |
| Negatively Charged (D & E) | 0.074 (n = 14) |
| Positively Charged (R & K) | 0.084 (n = 16) |

Based on The Basic Local Alignment Search Tool analysis the clone was confirmed as Toxoplasma gondii SAG2 gene and translated as Toxoplasma gondii SAG2 with 190 amino acids (Table 1). The *Toxoplasma gondii SAG2* gene had 99% homology (572/573 nucleotide base pairs) with strain RH (GenBank Accession Number AM055943) [20].

3.2. Protein analysis results
The *Toxoplasma gondii SAG2* predicted protein analysis was performed using CLC Main Workbench 8.0.1 software. The extinction coefficient of *Toxoplasma gondii SAG2* at 280 nm for non-reduced cysteines was 6,820 (absorption at 280nm 0.1%= 0.35) while for reduced cysteines was 6,400 (absorption at 280nm 0.1%= 0.329). Ten alpha helix secondary structures were found in *Toxoplasma gondii SAG2* (at position 6-17, 30-36, 39-42, 69-71, 84-86, 101-104, 109-111, 138-149, 167-170, and 175-184). Seven beta strand secondary structures were found in *Toxoplasma gondii SAG2* (at position 21-23, 49-54, 58-59, 72, 115-119, 128-133, and 156-159).
3.3. Immunogenicity prediction results

The immunogenicity and major histocompatibility complex-binding prediction for the *Toxoplasma gondii* SAG2 were performed using the T Cell Epitope Prediction Tools from Immune Epitope Database (IEDB) Analysis Resource. In total, seven epitopes were found had a high affinity (percentile rank < 0.1) for Major histocompatibility complex II-binding (Table 2), consistent with the antigenicity plot.

Table 2. *Toxoplasma gondii* SAG2 protein MHC-II binding prediction results.

| Allele                | Position |
|-----------------------|----------|
| HLA-DRB1*09:01        | 2-16     |
| HLA-DRB1*09:01        | 3-17     |
| HLA-DRB1*01:01        | 4-18     |
| HLA-DRB1*08:02        | 154-168  |
| HLA-DRB1*08:02        | 155-169  |
| HLA-DRB1*01:01        | 3-17     |
| HLA-DRB1*01:01        | 5-19     |

*Toxoplasma gondii* SAG2 has potential in activating humoral responses and has been used in characterizing the acute phase and in the serological diagnosis of *Toxoplasma gondii* infection [12]. *Toxoplasma gondii* SAG2 could interact with HZF (hematopoietic zinc finger) protein, a protein important for dendritic cells localization [21, 22]. *Toxoplasma gondii* proteins also can be processed and presented by Major histocompatibility complex-I for CD8+ T-cell recognition [23]. However, the alternative binding conformation of naturally occurring peptides from *Toxoplasma gondii* was reported, suggest they are presented via alternative means, potentially including cross-presentation via the MHC class II pathway [24]. In the present study, we found the amino acids of *Toxoplasma gondii* SAG2 at position 2 until 19 and 154-169 had a high affinity for Major histocompatibility complex II-binding with the high antigenic property, therefore, may be potential as a target to improving diagnostic, therapeutic, or vaccine efficacy against *Toxoplasma gondii*.

4. Conclusions

The present study will contribute information about our *Toxoplasma gondii* SAG2 especially the Indonesian isolate and benefits for further works willing to develop a diagnostic kit, immunotherapeutic, or vaccine strategies against *Toxoplasma gondii*.

Acknowledgement

This work was supported in part by grants from the Indonesian Ministry of Research, Technology and Higher Education 2018 (No. 089/ SP2H/ LT/ DRPM/ 2018) and PNBP UNS 2018 (No. 543/ UN27.21/ PP/ 2018).

References

[1] P Latré de Laté, M Pineda, M Harnett, W Harnett, S Besteiro and G Langsley 2017 *Biomed. J.* **40** 23-30
[2] M P Connolly, E Goodwin, C Schey, J Zummo 2017 *Pathog. Glob. Health.* **111**(1) 31-44
[3] S Fallahi, A Rostami, M Nourollahpour, Shiadeh, H Behniafar and S Paktinat 2018 *J. Gynecol. Obstet. Hum. Reprod.* **47** 133-140
[4] C Ozgonul and C G Besirli 2017 *Ophthalmic Res.* **57** 1-12
[5] C Del Grande, L Galli, E Schiavi, L Dell'Osso and F Bruschi 2017 *Pathogens.* **6** E3 (2017)
[6] M Foroutan and F Ghaffarifar 2018 *Clin. Exp. Vaccine Res.* **7** 24-36
[7] T R Brasil, C G Freire-de-Lima, A Morrot and A C Vetö Arnholdt 2017 *Front. Immunol.* **8** 1080
[8] C G K Lüder and T Rahman 2017 Microb. Cell. 4 203-211
[9] E A Wolhert, I J Blader and E H Wilson 2017 Trends Parasitol. 33 519-531
[10] K J Pittman and L J Knoll 2015 Microbiol. Mol. Biol. Rev. 79 387-401
[11] Y Wang, G Wang, J Cai and H Yin 2016 Parasitol. Res. 115 459-468
[12] J A Leal-Sena, J L Dos Santos, T A R Dos Santos, E M de Andrade, T A de Oliveira Mendes, J O Santana, T W P Mineo, J R Mineo, J P da Cunha-Júnior and C P Pirovani 2018 Appl. Microbiol. Biotechnol. 102 2235-2249
[13] M A Bezerra, L M Pereira, A Bononi, C A Biella, L Baroni, L Pollo-Oliveira and A P Yatsuda 2017 Parasitol. Int. 66 173-180
[14] A A Prasetyo and K U N Zaini 2015 Southeast Asian J. Trop. Med. Public Health. 46 662-668
[15] A A Prasetyo, M N Desyardi, J Tanamas, Suradi, Reviono, Harsini, S Kageyama, H Chikumi and E. Shimizu 2015 Intervirology. 58 57-68
[16] A A Prasetyo and R Sariyatun 2015 Asian J. Microbiol. Biotechnol. Environ. Sci. 17 349-355
[17] A A Prasetyo and R Sariyatun 2015 J. Teknol. 77 67-70
[18] A A Prasetyo, R Dharmawan, I Raharjo and Hudiyono 2016 J. Global Infect. Dis. 8 75-81
[19] A.A. Prasetyo 2017 AIP Conference Proceedings 1788 030100
[20] A Khan, U Böhme, K A Kelly, E Adlem, K Brooks, M Simmonds, K Mungall, M A Quail, C Arrowsmith, T Chillingworth, C Churcher, D Harris, M Collins, N Fosker, A Fraser, Z Hance, K Jagels, S Moule, L Murphy, S O’Neil, M A Rajandream, D Saunders, K Seeger, S Whitehead, T Mayr, X Xuan, J Watanabe, Y Suzuki, H Wakaguri, S Sugano, C Sugimoto, I Paulsen, A J Mackey, D S Roos, N Hall, M Berriman, B Barrell, L D Sibley and J W Ajioka 2006 Genome Res. 16 1119-1125
[21] M Y Lai, Y L Lau 2017 Parasit Vectors. 10 456
[22] M Y Lai and Y L Lau 2018 Acta Parasitol. 63 106-113
[23] C Buaillon, N A Guererro, I Cebrian, S Blanié, J Lopez, E Bassot, V Vasseur, J Santi-Rocca, N Blanchard 2017 Eur. J. Immunol. 47 1160-1170 (2017)
[24] S G Remesh, M Andreatta, G Ying, T Kaever, M Nielsen, C McMurtrey, W Hildebrand, B Peters and D M Zajonc 2017 J. Biol. Chem. 292 5262-5270