Tropical Biomedicine 43(1): 32-43 (2026)
https://doi.org/10.47665/tb.43.1.006

RESEARCH ARTICLE

In silico evaluation of human hookworm antigen, Glutathione-S-Transferase (GST-1) protein interaction with human dendritic cell responses towards eliciting immune response

Chong, S.X.[1,2], Lee, V.S.[3], Chong, W.L.[3], Sahimin, N.[1], Abu Bakar, S.[1], Lee, H.Y.[1*]

[1]Tropical Infectious Diseases Research and Education Centre (TIDREC), Higher Institution Centre of Excellence (HiCoE), Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[2]Institute of Advanced Studies (IAS), Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[3]Department of Chemistry, Centre of Excellence in Quantum Information Science and Technology (QIST), Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
*Corresponding author: leehaiyen@um.edu.my

ARTICLE HISTORY

Received: 1 July 2025
Revised: 7 January 2026
Accepted: 9 January 2026
Published: 31 March 2026

ABSTRACT

Hookworm disease is one of the tropical neglected diseases that significantly impacts human health to varying degrees. Hookworms produce various proteins to facilitate host invasion and immune evasion. Despite available treatments, reinfection is common, underscoring the need for effective vaccines. However, the complexity of the hookworm's life cycle poses a challenge in understanding the immune response in the vaccine candidates. Reverse vaccinology (RV) offers a powerful approach to understand the immune response by using various bioinformatics tools. This study begins by identifying hookworm antigens capable of inducing host immune responses, followed by docking analysis with different dendritic cell (DC) receptors to investigate the immunological response of antigenic peptides and further correlated to the immunogenicity findings in clinical trial. *Necator americanus* Glutathione-S-Transferase-1 (*Na*-GST-1), a known immunogenic protein from *Necator americanus*, was selected for docking due to its strong antigenic properties. Fifteen DC receptors were evaluated against *Na*-GST-1, of which seven receptors (TLR2, TLR3, TLR4, TLR7, DEC-205, CD206, and CD36) exhibited stronger predicted interactions, as indicated by stronger binding affinities with *Na*-GST-1 utilizing various immunoinformatic tools. These receptors are associated with the mediation of Th1/Th2 immune responses, suggesting a potential correlation between docking affinity and the predicted immunogenicity of *Na*-GST-1. Overall, this study provides valuable insights into DC receptor–antigen interactions and demonstrate a computational approach for assessing the potential of hookworm antigens to engage DC receptors, thereby supporting rational hookworm vaccine design. These findings support the application of early *in silico* strategies for advancing vaccine candidates against hookworm infection and strengthening control efforts for neglected tropical diseases.

Keywords: Neglected tropical diseases; vaccine; GST-1; parasite; hookworm.

INTRODUCTION

Hookworm disease, categorized as one of the tropical neglected diseases, has a significant role in the world's most vulnerable populations, with around 740 million people worldwide afflicted by hookworm infections (Diemert *et al*., 2018; Koopman *et al*., 2021). Chronic hookworm infection can cause abdominal pain, diarrhea, and blood loss in the gastrointestinal tract, which can then lead to anemia and protein loss, and it is particularly perilous to children and women who are of childbearing age (Diemert *et al*., 2018; Abuzeid *et al*., 2020). The principal causes of hookworm-related morbidity are *Necator americanus, Ancylostoma ceylanicum*, and *Ancylostoma duodenale* (Abuzeid *et al*., 2020). Hookworms produce an array of excretory/secretory products such as *Ancylostoma*-secreted protein (ASP-1 & 2), nematode anticoagulant peptide (NAP-1 & 5), glutathione S-transferase (GST-1, 2 & 3), peroxiredoxins (PRX-1), macrophage migratory inhibitory factor (MIF), excretory/secretory protein (ES-2), fatty acid- and retinol-binding protein (FAR-1), hookworm platelet inhibitor (HPI) to support their infection route in the host (Abuzeid *et al*., 2020). Hookworm infections that occur in rodents usually can be rapidly eliminated due to strong cellular and humoral immune responses (Sarkar *et al*., 2024). In humans, although a comparable immune phenotype is observed, swift elimination does not occur, and hence, adults often carry high-intensity hookworm infections well into old age (Sarkar *et al*., 2024). Hookworm infections are currently controlled mostly through mass drug administration (MDA) with anthelmintics like benzimidazole (Diemert *et al*., 2018; Koopman *et al*., 2021). The use of anthelmintics, however, only has the immediate ability to treat the illness; it is unable to stop re-infection, and resistance

to the anthelmintics may develop (Diemert *et al*., 2018). Besides benzimidazole, emodepside has also been used for human soil-transmitted helminths (STH) infections, which is effective in treating onchocerciasis and in regions that have developed benzimidazole resistance (Sarkar *et al*., 2024). Although presently there is no report on the resistance to emodepside in humans, cases of multiple drug resistance against other drugs such as albendazole, moxidectin and benzimidazoles have been continuously discovered (Sarkar *et al*., 2024). For instance, the discovery of multi-drug resistant *A. caninum* against the combination of febantel-pyrantel moxidectin, albendazole, and moxidectin in the USA (Sarkar *et al*., 2024). Therefore, the need for new treatments for hookworm infections is in critical demand due to the rapid development of multi-drug resistance. There was one clinical study which studied the protective effect of UV-attenuated *N. americanus* larvae against the infection; however, the outcome of the study reflected that there was no significant reduction in fecal egg count and developed skin rash as an adverse effect (Hoogerwerf *et al*., 2023; Sarkar *et al*., 2024). There was another clinical study that evaluated the vaccine efficacy of repeated short-term exposures to *N. americanus* third-stage infective larvae (L3), followed by effective chemotherapeutics treatment with anthelmintics which resulted in an indistinct protection but a trend in reducing egg burdens in patients who had strong skin reaction to percutaneous infection (Baker *et al*., 2018). Hence, these clinical studies demonstrate that the human hookworm vaccine can be a promising approach against the disease.

Numerous hookworm ES products have been studied for their functions and aim to be used as potential vaccine candidates. For instance, ASP has been studied in several studies as a human hookworm vaccine (Bethony *et al*., 2005; Mendez *et al*., 2008; Xiao *et al*., 2008). However, in the study of Diemert *et al*. (2012), the human vaccine using ASP-2 as a target caused allergic reactions and urticaria after vaccination with recombinant *Na*-ASP-2, which caused the study to terminate. Besides, in the study of Diemert *et al*. (2017), the administration of recombinant *Na*-GST-1 to volunteers in Brazil and the United States had no significant vaccine-related events occur during the phase 1 trials. This indicated that *Na*-GST-1 will be a potential vaccine candidate that can be carried forward to the next round of children's clinical trials and eventually effectiveness studies (Diemert *et al*., 2017). Moreover, the co-administration of two hookworm vaccine candidates, *Necator americanus* Glutathione-S-Transferase-1 (*Na*-GST-1) and aspartic protease-1 (*Na*-APR-1) in healthy Gabonese adults showed that co-administration of these two vaccine candidates was safe and induced antibody, immunoglobulin G (IgG) to each of the antigens (Adegnika *et al*., 2021). More clinical trials using these hookworm antigens are being conducted because of the encouraging findings from earlier studies, for example, the co-administration of *Na*-APR-1 and *Na*-GST-1 in Gabonese children (Wong *et al*., 2023) and a phase 2 vaccination clinical trial in the USA using a controlled human hookworm infection model (Adegnika *et al*., 2021). In this study, various hookworm antigens were screened and based on the literature, the best candidate was chosen for site-specific docking with various DC receptors to investigate its role in inducing host immune response.

Dendritic cells (DCs) are a heterogeneous population found in both lymphoid and non-lymphoid tissues, including the blood, which possess diverse origins, anatomical locations, and migratory patterns (Gutiérrez-Kobeh *et al*., 2018). Their broad functional repertoire makes them essential players in the immune response (Gutiérrez-Kobeh *et al*., 2018). DCs function by capturing antigens in peripheral tissue and processing and presenting the antigens in lymphoid organs via MHC I and MHC II pathways (Gutiérrez-Kobeh *et al*., 2018). In addition to antigen presentation, DCs regulate immune responses by producing cytokines, influencing Th1/Th2 differentiation, modulating cytotoxic T lymphocytes, and contributing to immune tolerance (Gutiérrez-Kobeh *et al*., 2018). DCs act as surveillance cells, distinguishing self from non-self by detecting pathogen-associated molecular patterns (PAMPs) from different infectious agents (Gutiérrez-Kobeh *et al*., 2018). They achieve this through specialized receptors such as scavenger receptors (SRs), toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I like receptor (RLRs), and NOD-like receptors (NLRs) (Wang *et al*., 2015; Gutiérrez-Kobeh *et al*., 2018). TLRs (i.e. TLR2, TLR3, TLR4, TLR7, TLR9) are pattern recognition receptors (PRRs) which responsible for PAMP recognition and immune activation (Gutiérrez-Kobeh *et al*., 2018). CLRs (i.e. DEC-205, DC-SIGN, CD206, BDCA-2, DCIR, Clec9A, Clec10A, Langerin) are involved in antigen uptake, pathogen recognition and immune regulation (Tong *et al*., 2023). SRs function in lipid uptake, bacterial recognition and immune modulation (Taban *et al*., 2022). Other DC receptors, such as RLRs and NLRs, primarily function as intracellular receptors that recognise danger signals within the cytosol (Gutiérrez-Kobeh *et al*., 2018). Our study aims to assess the application of *in silico* models to enhance the evaluation of vaccine antigens and optimize design and development strategies within the reverse vaccinology framework. Therefore, this study will focus on the immune response triggered upon antigen recognition, with a particular emphasis on DC receptors that primarily function as antigen presentation, uptake, and immune activation or modulation (i.e. SRs, TLRs, CLRs) (Gutiérrez-Kobeh *et al*., 2018; Tong *et al*., 2023). Consequently, RLRs and NLRs will not be assessed in this study. However, future studies may include additional DC receptors to investigate a more comprehensive immune response during infections.

MATERIALS AND METHODS

The overall workflow of the study is illustrated in Figure 1.

Protein preparation

Preparation of protein models via Protein Data Bank (PDB)

The protein sequence of DC receptors, TLR2 (GenBank: NP_001305725), TLR3 (GenBank: KAI4027901), TLR4 (GenBank: KAI4008213), TLR7 (GenBank: KAI3998883), TLR9 (GenBank: KAI4030015), DEC-205 (GenBank: AAC17636), DC-SIGN (GenBank: Q9NNX6), CD206 (GenBank: NP_002429), CD14 (GenBank: P08571), CD36 (GenBank: P16671), BDCA-2 (GenBank: AAL37036), DCIR (GenBank: Q9UMR7), CLEC9A (GenBank: ABZ04557), CLEC10A (GenBank: NP_878910), and Langerin (GenBank: CAB62403) were obtained from the National Center of Biotechnology Information (NCBI). The protein sequences were compared with the Protein Data Bank (PDB) using Blastp to search for homologous receptor proteins. The best protein model was selected based on query coverage and percent identity.

Determination of the active site of dendritic cell receptors

The active site of each DC receptor was determined via the PrankWeb server (https://prankweb.cz) (Krivák & Hoksza, 2018; Jendele *et al*., 2019; Jakubec *et al*., 2022). PrankWeb is a machine learning-based ligand binding site prediction tool that predicts the active site based on the ligandability of the local chemical neighbourhoods of a protein (Krivák & Hoksza, 2018).

Preparation of parasite antigens from hookworm

The protein sequence of the hookworm antigen was obtained from the NCBI. The protein sequences were compared with the PDB using Blastp to search for homologous proteins. The best protein model was selected based on query coverage and percent identity. The hookworm antigens used for docking were ASP-1 (GenBank: AAD13340), ASP-2 (GenBank: AY288089), NAP-1 (GenBank: AAK81733), GST-1 (GenBank: FJ711440), PRX-1 (GenBank: JX124321), MIF (GenBank: ABO31935), ES-2 (GenBank: AAS13463), FAR-1 (GenBank: 4UET), NAP-5 (GenBank: 2P3F), HPI (GenBank: AF399709), GST-2 (GenBank: FJ711441) and GST-3 (GenBank: FJ711442).

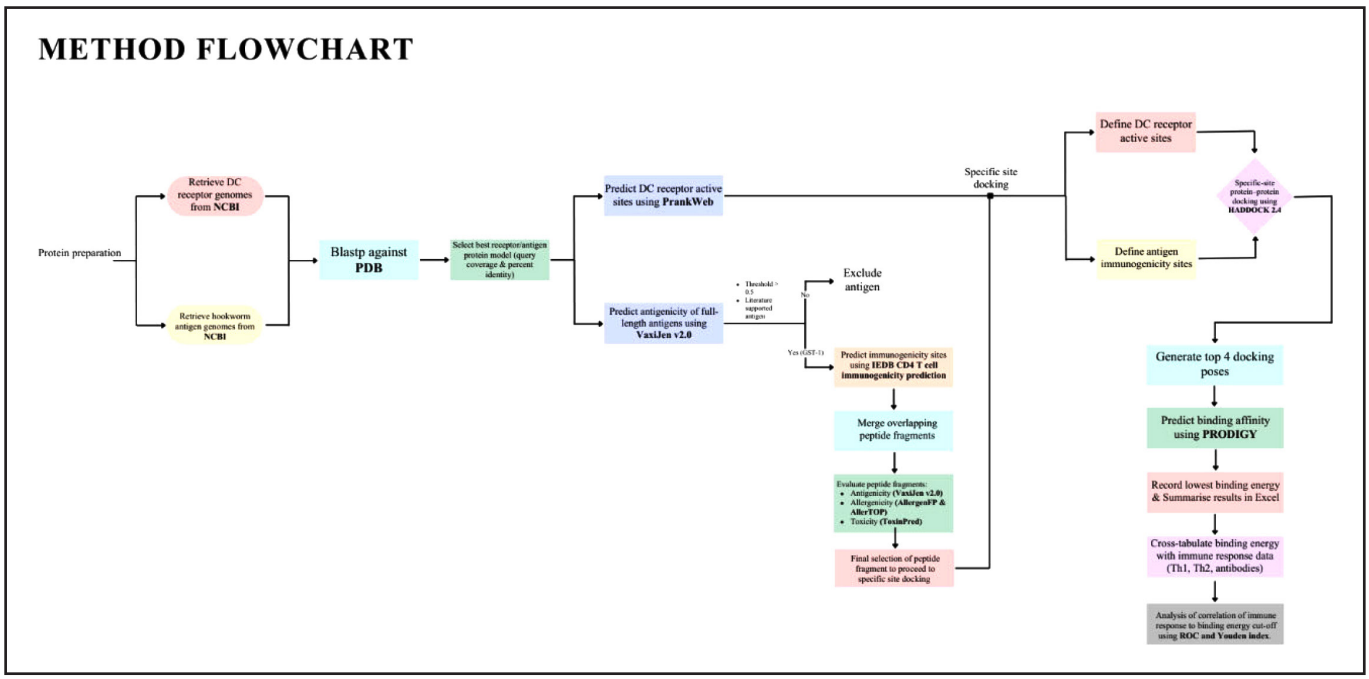


Figure 1. Method flowchart.

Determination of antigenicity of hookworm antigens

The capability of the hookworm antigens that trigger an immune response was defined as antigenicity. The antigenicity of the hookworm antigens was predicted via the VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with a threshold of 0.5 (Doytchinova & Flower, 2007a, 2007b, 2008). Antigens that were predicted as "probable antigens" and supported by the literature were selected for specific site docking.

Determination of immunogenicity site of hookworm antigens

The immunogenicity of selected hookworm antigens was predicted via the Immune Epitope Database (IEDB) analysis tool – CD4 T cell immunogenicity prediction (http://tools.iedb.org/CD4episcore/). The IEDB server analysed the peptide sequence and gave out immunogenicity scores for fragments. The fragments with overlapping peptides were combined as one fragment for allergenicity, toxicity, and specific site docking analysis.

Determination of antigenicity, allergenicity, toxicity, and antigenicity of peptide fragments

The antigenicity of the peptides was determined via the VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with a threshold of 0.5 (Doytchinova & Flower, 2007a, 2007b, 2008). The allergenicity of the peptide fragments of selected antigens was determined via AllergenFP v1.0 (https://ddg-pharmfac.net/AllergenFP/) and AllerTOP v. 2.0 (https://www.ddg-pharmfac.net/AllerTOP/) (Dimitrov *et al*., 2014a; Dimitrov *et al*., 2014b). The toxicity of the peptide fragments was determined via the ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/), and the allergenicity was determined via the VaxiJen v2.0 server (Doytchinova & Flower, 2007a, 2007b, 2008; Gupta *et al*., 2013).

Specific site docking

Specific site protein-protein docking

Specific site protein-protein docking was performed via a web-based server, HADDOCK 2.4 (https://wenmr.science.uu.nl/haddock2.4/), with the active site and immunogenicity site predicted in **2.1.2** and **2.1.5**. HADDOCK 2.4 gave out the best four binding poses of the protein-protein complex (Honorato *et al*., 2021).

Data analysis

The binding affinity of all four best poses of the protein-protein complexes was predicted via PRODIGY (https://wenmr.science.uu.nl/prodigy/). The lowest binding energy among the four complexes was recorded and summarised via Excel (Vangone & Bonvin, 2015; Xue *et al*., 2016). Results of binding energy were cross-tabulated to a literature search on immune responses via Th1, Th2 and antibody subtypes from clinical trial datasets to obtain the correlation of immune responses to binding energy cut-off. Analysis was performed using ROC and Youden index (Gupta *et al*., 2016).

RESULTS

Antigenicity Predictions

Table 1 shows the antigenicity of the hookworm antigens, and antigens that had a score above the threshold of 0.5 were considered as "probable antigens". Twelve hookworm antigens were selected for antigenicity prediction, and nine antigens were categorized as probable antigens, which were ASP-1, ASP-2, NAP-1, GST-1, PRX-1, MIF, NAP-5, HPI, GST-2 and GST-3. Based on the literature, GST-1 was selected for T cell epitopes, B cell epitopes, conserved regions, immunogenicity, allergenicity, and toxicity prediction.

Table 1. Antigenicity prediction of hookworm antigens

Antigen	Protein size (aa)	Antigen prediction
ES-2	102	0.2441 (Probable NON-**ANTIGEN**)
GST-2	206	0.4842 (Probable NON-**ANTIGEN**)
FAR-1	181	0.4858 (Probable NON-**ANTIGEN**)
PRX-1	196	0.5145 (Probable **ANTIGEN**)
GST-1	155	0.5475 (Probable **ANTIGEN**)
GST-3	206	0.5581 (Probable **ANTIGEN**)
ASP-1	425	0.5637 (Probable **ANTIGEN**)
NAP-5	90	0.5783 (Probable **ANTIGEN**)
MIF	119	0.6054 (Probable **ANTIGEN**)
NAP-1	102	0.6399 (Probable **ANTIGEN**)
HPI	181	0.7967 (Probable **ANTIGEN**)
ASP-2	217	0.8039 (Probable **ANTIGEN**)

Determination of T cell epitope, immunogenicity, allergenicity, and toxicity of selected hookworm antigens and specific site docking of predicted epitopes with DC receptors

Na-GST-1 was selected for immunogenicity, antigenicity, allergenicity, and toxicity prediction. Among the *Na*-GST-1 protein sequence, 8 epitopes were given with immunogenicity scores that were above the threshold (> 50%). The 8 epitopes were then grouped into 5 fragments due to the overlapping amino acids between the epitopes (Figure 2). The 5 peptides were then evaluated for their antigenicity via the VaxiJen v2.0 server, while the allergenicity was determined by AllergenFP v. 1.0 and AllerTOP v. 2.0. Among the 5 fragments, S1 – S4 were identified as probable antigen which had antigenicity scores above threshold (> 0.5) while S5 was predicted as probable non-antigen as the antigenicity was 0.2468 which did not exceed the threshold. Among the 5 peptides, S2 was identified as an allergen in AllerTOP v. 2.0. Besides, the 5 peptides were evaluated for their toxicity via ToxinPred, and all 5 fragments were identified as non-toxic. Table 2 summarises the predicted T cell epitopes, immunogenicity, allergenicity, and toxicity score of the Na-GST-1 peptides.

Table 3 shows the heatmap of the binding affinity of GST-1 peptides against 15 DC receptors. The 15 DC receptors are separated into five major categories (i.e. pattern recognition receptors (PRRs), antigen uptake and presentation receptors, phagocytic receptors,

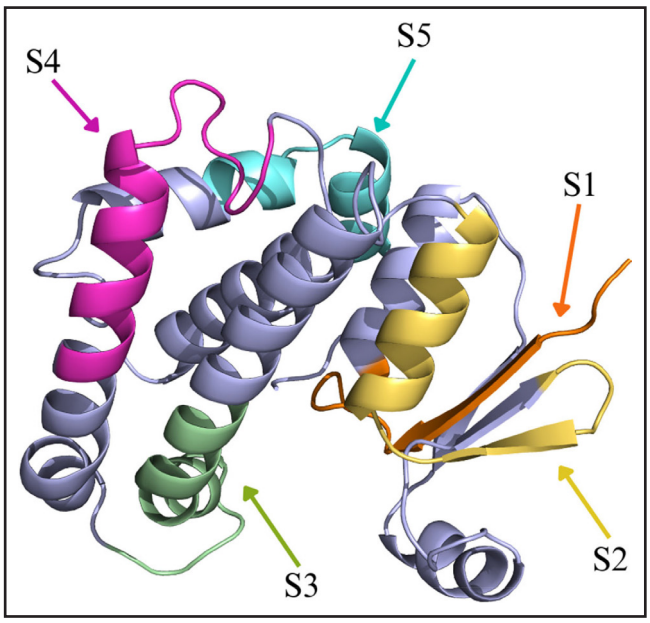


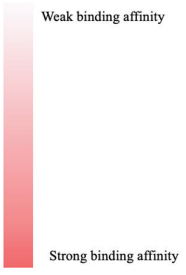
Figure 2. Schematic representation of the *Na*-GST-1 protein sequence highlighting five immunogenic fragments.

Table 2. Prediction of T cell epitope, immunogenicity, allergenicity, and toxicity of *Na*-GST-1 peptide fragments

Peptide fragments	Protein description	Peptide	Start	End	Immunogenicity Score (%)	Antigenicity	AllergenFP v.1.0	AllerTOP v.2.0	Toxicity
S1	GST-1	MVHYKLTYFAIRGAG	1	15	83.05	0.5936 (Probable **ANTIGEN**)	NO	NO	NT
S2		VDGKQLAQSLAICRY	56	70	98.99	0.5236 (Probable **ANTIGEN**)	NO	YES	NT
		LAQSLAICRYLARQF	61	75	91.58				
S3		SDYRVEIKSFFYTVI	96	110	93.54	0.6685 (Probable **ANTIGEN**)	NO	NO	NT
		EIKSFFYTVIGMREG	101	115	91.99				
S4		KFFGFITKFLKKSPS	131	145	75.27	1.0680 (Probable **ANTIGEN**)	NO	NO	NT
		ITKFLKKSPSGFLVG	136	150	61.78				
S5		EKIRAIPKLKKWIET	186	200	81.15	0.2468 (Probable **NON-ANTIGEN)**	NO	NO	NT

Table 3. Binding affinity of the epitopes against different DC receptors

DC receptor		Binding affinity (kcal/mol)				
Pattern recognition receptor (PRRs):	**TLR2**	-13.4	-12.3	-11.6	-11.0	-15.0
	TLR7	-12.2	-12.5	-12.4	-10.7	-12.8
	TLR3	-12.3	-11.9	-10.8	-9.2	-9.9
	TLR4	-10.3	-12.2	-11.2	-10.1	-10.2
	TLR9	-10.7	-9.8	-10.1	-9.6	-9.3
Antigen Uptake and Presentation Receptors:	**DEC-205**	-10.5	-12.2	-12.2	-10.5	-11.9
	DC-SIGN	-8.8	-8.7	-8.6	-9.6	-8.8
Phagocytic Receptors:	**CD206**	-10.2	-8.9	-13.2	-9.9	-10.4
	CD14	-9.3	-9.1	-9.8	-9.8	-8.5
Immune Modulatory Receptors:	**CD36**	-10.8	-13.3	-12.7	-9.6	-12.6
	BDCA-2	-9.3	-9.1	-8.4	-9.1	-7.9
Specialized Antigen Uptake Receptors:	**DCIR**	-9.4	-8.9	-10.5	-8.5	-8.9
	Clec9A	-10.3	-9.7	-10.7	-8.3	-9.5
	Clec10A	-11.1	-10.3	-9.5	-9.7	-8.4
	Langerin	-8.4	-9.5	-8.0	-8.4	-9.3
		S1	**S2**	**S3**	**S4**	**S5**
				Epitopes		



immune modulatory receptors, and specialized antigen uptake receptors) based on different roles and functions. As no universally accepted binding energy cutoff exists for defining immunogenic relevance in DC receptor–antigen docking studies, results were evaluated comparatively rather than using an absolute threshold. A colour gradient was applied to visualize relative binding strengths across receptors. Based on the indicator beside the heatmap, the darker the color indicated the stronger the binding affinity between the peptides and the DC receptors. From Table 3, S1, S4 and S5 had a stronger binding affinity with TLR2, while S2 had a stronger binding affinity with CD36 and S3 with CD206. Moreover, based on the color distribution in Table 3, S5 had the strongest binding affinity with TLR2 among all the peptides. Besides, binding affinities between the epitopes and DC receptors were mainly accumulated on PRRs (i.e. TLR2, TLR7, TLR3 and TLR4) and antigen uptake and presentation receptors (i.e. DEC-205). However, other receptors needed to be highlighted due to the relatively strong binding affinity with the multiple epitopes, which were CD36. Furthermore, according to Table 3, S4 had relatively weaker binding affinity among the epitopes.

Analysis of Prediction

Tabulation of the potential antibody responses with binding energy showed a wide range of immune responses based on binding energy (Table 4). The cutoff was determined from the mean of binding energy, between -7.9 to 11.79 kcal/mol, that elicited an immune response based on positive outcome, or lowest energy binding (kcal/mol), except for IgG4.

Table 4. The calculated binding energy with possible IgG subtypes outcome

Antibody	Min Cutoff	Max Cutoff	Mean
IgG1	–15.0	–8.0	–10.42
IgG2a	–11.9	–9.6	–11.79
IgG3	–15.0	–8.0	–10.04
IgG4	–11.9	–7.9	–7.9

DISCUSSION

Reverse vaccinology remains a very important tool for design and discovery of antigens for vaccine development. Evaluation of new tools and method are based on existing data especially that has advanced to clinical trials. Therefore, GST-1 has been actively investigated as a vaccine candidate due to its significant role in the blood-feeding pathway of hookworms (Diemert *et al*., 2017). Vaccination with recombinant GST-1 in hamsters and dogs demonstrated a reduction in worm burden and fecal egg counts (Zhan *et al*., 2005). Subsequently, the recombinant GST-1 vaccine progressed to clinical trials in the United States and Brazil (Diemert *et al*., 2017). These trials confirmed the vaccine's safety and tolerability, as well as an increased level of antigen-specific IgG following vaccination (Diemert *et al*., 2017). the suitability of GST-1 was selected as model to evaluate the interaction with dendritic cell receptors during infection or during vaccination could provide insights to the immune responses. Limited with just *in silico* tools, this study provides the insights to predictive reverse vaccinology and will require further evaluation *in silico* and *in vivo* for validation purposes.

The five regions GST-1 (S1-S5) were predicted for their antigenicity. Among them, S5 was predicted to be non-antigenic while S2 was identified as allergenic. In consideration of the limitation in sensitivity of the prediction tools (Doytchinova & Flower, 2008) and the high immunogenicity score predicted for S2 and S5, it was still selected for further investigation. The binding interaction between DC surface receptors and selected immunogenic fragments of GST-1 is currently an investigational attempt to model functional immunology. It is important to recognize that docking affinity does not directly imply functional immune signalling.

Dendritic cells act as intermediaries between innate and adaptive immunity by recognizing, capturing, processing, and presenting helminth ES products to T cells (Motran *et al*., 2018). Antigen-presenting cells (APCs) are central to innate immune responses, recognizing a broad range of molecular patterns on pathogens, known as pathogen-associated molecular patterns (PAMPs), through TLRs, C-type lectin receptors (CLRs), or NOD-like receptors (NLRs) (Venugopal *et al*., 2009; Motran *et al*., 2018).

In this study, it was observed that the regions S1, S4, and S5 of *Na*-GST-1 demonstrated stronger binding affinity to DC receptors compared to other regions, with S5 showing the strongest binding. These findings suggest that TLR2 plays a significant role in recognizing *Na*-GST-1. Activation of dendritic cells through TLR2 can lead to the production of both Th1-associated cytokines, such as IL-12p70, and TNF-α, and Th2-associated cytokines, such as IL-10 (Toebak *et al*., 2006). Th2-type immune responses are typically characterized by three key attributes: wound repair, inflammation, and resistance to helminths (Rajasekaran *et al*., 2017). A study reporting the safety and immunogenicity profile of another hookworm antigen, *Na*-ASP-2, showed that vaccination with *Na*-ASP-2 induced elevated levels of antigen-specific IgE, which led to allergic symptoms (urticaria) in three participants (Diemert *et al*., 2012). This suggests that individuals previously exposed to hookworm infection may have pre-existing *Na*-ASP-2-specific IgE (Diemert *et al*., 2012). Upon immunization, epitopes on *Na*-ASP-2 may be recognized by these IgE antibodies, potentially triggering adverse allergic reactions (Diemert *et al*., 2012). Therefore, the level the antigen-specific IgE is one of the important safety criteria to evaluate a suitable vaccine candidate. In studies conducted in the US and Brazil, adults who received *Na*-GST-1/Alhydrogel did not exhibit detectable levels of IgE, indicating that the antigen does not induce sensitization during hookworm infection (Diemert *et al*., 2017). This supports the safety profile observed for *Na*-GST-1, suggesting it does not elicit allergic responses (Diemert *et al*., 2017). Moreover, the clinical trial data on the hookworm vaccine showing a similar ratio of IgG1 and IgG3 may be further investigated through this docking profile, where the strong binding affinity to TLR2 is known to preferentially induce a Th2 response in dendritic cells and influence antibody production through IL-4–mediated IgG1 class switching (Re & Strominger, 2001; Li *et al*., 2021). Therefore, the strong binding affinity of *Na*-GST-1 to TLR2 may contribute to both the safety and immunogenicity of this antigen and *in vitro* studies will be important to validate this.

Studies have demonstrated that TLR2-deficient bone marrow-derived cells (BMDCs) infected with schistosomula antigen (SSA) and soluble egg antigen (SEA) fail to produce IL-10, while TLR4-deficient BMDCs exhibit high levels of IL-10 production along with the upregulation of TLR2 transcription (Gao *et al*., 2012). These findings indicate that TLR2 plays a vital role in enhancing Th2 response during helminth infection, whereas TLR4 appears to play a minor role in producing IL-10 (Gao *et al*., 2012; Rajasekaran *et al*., 2017). The observations of Gao *et al*. (2012) are supported by findings from Layland *et al*. (2007) and Ferreira *et al*. (2007), which showed a decrease in IL-10 production in TLR2-deficient mice. Besides, signalling through TLR2 or TLR4 can impair the ability of dendritic cells to produce IL-12, causing them to polarize toward a Th2 immune response (Motran *et al*., 2018). The response elicited depends on the type of pathogen molecules recognized by TLRs (Motran *et al*., 2018). Generally, TLR4 recognizes LPS, leading to the production of IL-12 and the induction of a Th1 immune response

(Kerepesi *et al*., 2007). However, some studies have shown that TLR4 also plays a role in recognizing helminth antigens, causing dendritic cells to shift toward a DC2 phenotype, which is important in mediating a Th2 immune response (Kerepesi *et al*., 2007). For example, bacterial LPS recognized by TLR4 typically activates the mitogen-activated protein kinases (MAPKs), particularly p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), which are associated with a strong Th1 response (Thomas *et al*., 2003). However, helminth antigens like LNFPIII from Schistosoma, recognized by TLR4, trigger only ERK phosphorylation, leading to increased IL-4 and decreased IFNγ production, which favors a Th2 response (Thomas *et al*., 2003). The increased level of IL-4 promotes the production of IgG1, IgG4, and IgE, which shows a pattern similar to that observed in clinical trials (Lee & Rosenberg, 2013; Diemert *et al*., 2017). Thus, the binding of *Na*-GST-1 to TLR4 may elicit a response similar to that of Schistosoma, leading to the suppression of the Th1 response and the activation of the Th2 response.. In this study, *Na*-GST-1 exhibited strong binding affinity to TLR2 and TLR4, suggesting that this strong interaction may induce a Th2-dominant immune response.

Besides TLR2 and TLR4, TLR3 and TLR7 are involved in recognizing double-stranded and single-stranded RNA, respectively (Venugopal *et al*., 2009). These receptors mediate immature dendritic cell (iDC) maturation through MyD88-independent and MyD88-dependent pathways, respectively (Aksoy *et al*., 2005; Venugopal *et al*., 2009). Based on this *in silico* model, *Na*-GST-1 protein cannot be directly recognized by TLR3 and TLR7 because these receptors are specialized for recognizing nucleic acid. The previous reports showed that during the initial stages of infection, TLR3 and TLR7 may be capable of recognizing the mRNA of *Na*-GST-1. The *Na*-GST-1 mRNA can be detected in numerous life stages of the hookworm, such as L1, L2, L3, adult worms, and eggs (Zhan *et al*., 2005). In contrast, the GST-1 protein has only been detected in adult hookworm somatic extracts and adult ES products (Zhan *et al*., 2005). This finding suggests that *Na*-GST-1 mRNA may not be translated during the non-blood-feeding stages of the hookworm life cycle or is expressed at a very low level that could not be detected (Zhan *et al*., 2005). While TLR3 and TLR7 are unlikely to directly bind *Na*-GST-1 protein, evidence from other studies suggests that the binding of helminth ES products with TLR3 during infections may enhance the survival of the helminths. Donnelly *et al*. (2010) reported that TLR3 may be degraded by the helminth ES products, resulting in the inactivation of the MyD88-independent TRIF-dependent signalling pathways and suppression of the Th1 immune response. For instance, the parasitic flatworm *Fasciola hepatica* secretes *Fasciola hepatica* Cathepsin L1 (*Fhe*CL1), a protease involved in tissue degradation and immunosuppression, which contributes to parasite survival (Donnelly *et al*., 2010). *Fhe*CL1 has been shown to target and degrade TLR3 in macrophages, thereby impairing their activation and downregulating Th responses (Donnelly *et al*., 2010). In the present study, the stronger binding affinity observed between TLR3 and *Na*-GST-1 suggests that *Na*-GST-1 may interact with TLR3 in a similar manner, potentially leading to its degradation. These findings indicate that signalling through TLR3 can modulate both Th1 and Th2 immune responses, suggesting that TLR3 *Na*-GST-1 may act as an inhibitor of TLR3, disrupting its function and contributing to the downregulation of the Th1 responses and a shift towards Th2 bias. The impact of TLR3 loss on immune polarization is further supported by findings from Joshi *et al*. (2008), who reported that TLR3-deficient mice exhibited increased levels of Th2-associated cytokines and chemokines such as IL-17, IL-4 and CCL11 and a decrease in Th1-associated factors such as IL-12, CXCL9 and CXCL10. The cytokines and chemokines that drive the switch to a Th2 immune response subsequently lead to the production of IgE and class switching of IgG to the IgG1 and IgG4 subclasses (Kraemer *et al*., 2022).

Additionally, TLR7 has been shown to possess structural features to bind with a wide range of TLR7 agonists beyond single-stranded RNA (Talukdar *et al*., 2021; Bzówka *et al*., 2023). These include guanosine-, imidazolequinoline-, benzimidazole- pyrimidine-, benzoxazole-, and indoline-based agonists (Talukdar *et al*., 2021; Bzówka *et al*., 2023). A study on an alum-adsorbed TLR7 agonist reported that binding of the TLR7 agonist to TLR7 decreased the levels of Th2-like cytokines (e.g., IL-4 and IL-13) and increased the levels of Th1-like cytokines (e.g., IFN-γ), which in turn resulted in IgG2a and IgG2b isotype switching (Buonsanti *et al*., 2016). This finding is further supported by a study on a hepatitis B virus (HBV) therapeutic vaccine using a TLR7 agonist, T7-EA, which demonstrated that the TLR7 agonist induces an antigen-specific Th1 immune response by increasing the levels of IFN-α, IFN-γ, IL-6, TNF-α, CCL2, and CCL4, subsequently leading to the production of IgG2a (Hu *et al*., 2020). The Th1 immune response may contribute to early protection against the parasite; however, a prolonged Th1 response can lead to chronic helminth infection and a strong inflammatory reaction that is detrimental to the host. Therefore, the Th1 response is usually downregulated by the subsequent Th2 immune response (Moreau & Chauvin, 2010; Cortés *et al*., 2017). The ability of TLR7 to bind with diverse TLR7 agonists suggests the possibility that *Na*-GST-1 may be one of the possible TLR7 agonists that have not been discovered yet, and the stronger binding affinity between TLR7 and *Na*-GST-1 might induce a unique immune response pathway. Further investigation is required to elucidate the potential role of *Na*-GST-1 as a TLR7 agonist and its impact on immune response.

Besides PRRs, DEC-205, a member of C-type lectin receptors, plays a significant role in antigen recognition, processing, and presentation (Shrimpton *et al*., 2009). Targeting antigens to DEC-205 has been shown to enhance both humoral and cellular immune responses (Lakhrif *et al*., 2018). For example, the specific binding of *Toxoplasma gondii* antigen SAG1 on DEC-205 resulted in effective MHC class II antigen presentation, which significantly augmented CD4+ T cell immune response (Lakhrif *et al*., 2018). This response predominantly involved the production of Th1-associated cytokines, such as interferon-gamma (IFN-γ), and IL-2, along with the generation of antibodies (i.e. IgG2a and nasal IgA) (Lakhrif *et al*., 2018). Moreover, when DEC-205 stimulation was combined with TLR activation, it elicited an even stronger CD4+ T cell immune response (Lakhrif *et al*., 2018). In our study, we observed a strong binding affinity between DEC 205 and *Na*-GST-1, suggesting that the interaction between *Na*-GST-1 and DEC-205 may activate a signalling pathway that promotes Th1-dominant immune response. This activation could play a protective role for the host against hookworm infection.

CD206, also known as mannose receptor (MR), is a member of the C-type lectin receptor family that particularly recognizes collagen, sulfated sugars, and sugars terminating in D-mannose, L-fucose, or N-acetylglucosamine (Paveley *et al*., 2011). CD206 has been shown to play a vital role in recognizing parasitic ES products. A deficiency in CD206 can result in increased secretion of antigen-specific IFNγ and reduced production of IL-4, thereby decreasing the Th2 response and enhancing the Th1 response (Paveley *et al*., 2011). Furthermore, both *in vitro* and *in vivo* studies conducted by Everts *et al*. (2012) demonstrated that Th2 polarization of DCs by omega-1, a component of schistosome eggs, was disrupted when CD206 was either blocked or absent on DCs. Based on this, the strong binding affinity observed between *Na*-GST-1 peptides against CD206 suggests that the interaction may promote the polarization of DCs via CD206, potentially inducing a Th2-dominant immune response.

Moreover, multiple *Na*-GST-1 epitopes demonstrated strong binding affinity to CD36. CD36 is a member of the class B scavenger receptor family that plays diverse roles, such as facilitating parasite sequestration in host organs, modulating immune responses,

and aiding in the phagocytic clearance of parasites (Thylur *et al*., 2017). Additionally, CD36 functions as a PRR for DCs, mediating the internalization of parasitic antigens for processing and presentation via myeloid dendritic cells (mDCs) and promoting pro-inflammatory cytokines via plasmacytoid dendritic cells (pDCs) (Bachmann *et al*., 2022). During Plasmodium falciparum infection, CD36 on dendritic cells (DCs) recognizes the infected erythrocytes (PfIEs) displaying PfEMP1 proteins, triggering the production of pro-inflammatory cytokines (Bachmann *et al*., 2022).

Furthermore, blocking CD36 on human DCs or using CD36-deficient murine DCs resulted in reduced internalization of *Pf*IEs, which display the *Pf*EMP1, along with decreased pro-inflammatory cytokines production, reduced parasite clearance, increased parasitemia, and elevated malaria severity and mortality (Gowda *et al*., 2013; Patel *et al*., 2007). A study investigating the role of CD36 in malaria immunity reported that malaria infection skews the immune response towards Th1 (Thylur *et al*., 2017). In their findings, at 3 and 5 days post-infection, the levels of Th1 cytokines (i.e., IFN-γ and IL-12) were significantly higher in wild-type mice compared to CD36-deficient mice (Thylur *et al*., 2017). However, by 10 and 17 days post-infection, there was no significant difference in cytokine levels between wild-type and CD36-deficient mice (Thylur *et al*., 2017). This suggests that CD36 plays an important role in regulating Th1-related cytokine production and inducing the Th1 immune response during the early phase of infection (Thylur *et al*., 2017). In this study, the strong binding affinity between CD36 and *Na*-GST-1 suggests that CD36 may play an important role in the internalization of *Na*-GST-1 by DCs, thus inducing production of type-1 cytokines and leading to the development of a Th1 immune response, and parasite sequestration in the early stage of hookworm infection.

Aside from the DC receptors discussed above (i.e. TLR 2, 3, 4, 7, DEC-205, CD206, and CD36), the weaker binding affinities observed between *Na*-GST-1 and other DC receptors (TLR9, DC-SIGN, BDCA-2, CD14, DCIR, CLEC9A, CLEC10A, and Langerin) suggest that these receptors may not play major roles in recognizing *Na*-GST-1 during hookworm infection. However, they could contribute to minor or indirect signalling pathways during the infections. For instance, TLR9 is able to recognize CpG DNA sequences found in bacterial and viral DNA, as well as parasitic CpG DNA from *Leishmania* major (Hkima Abou Fakher *et al*., 2009; Hennessy *et al*., 2010). DC-SIGN functions as an antigen-presenting receptor on iDCs, internalizing antigens and presenting them to T cells (van Die *et al*., 2003; Vázquez-Mendoza *et al*., 2013). BDCA-2 serves as a marker for pDC and is potentially involved in ligand internalization, processing and presentation (Dzionek *et al*., 2001). CD14 acts as a co-receptor that enhances TLR signalling by facilitating the engagement of TLRs with adaptor proteins (Granucci & Zanoni, 2013). Some specialized antigen uptake receptors, like DCIR and Clec9A, may have negative effects, including hyperinflammation through the recognition of self-antigens (Piva *et al*., 2012; Maglinao *et al*., 2013). Additionally, Clec10A and langerin have been shown to contribute to the stimulation of Th1 and Th2 immune responses (van Vliet *et al*., 2005; Kautz-Neu *et al*., 2011). However, due to their weak binding affinity to *Na*-GST-1, they are unlikely to serve as the primary functional receptors in recognizing *Na*-GST-1.

Table 5 summarizes the possible immune responses, cytokines, and antibody subtypes that may be induced when *Na*-GST-1 binds to dendritic cell (DC) receptors, based on current research in helminth infection studies. The table shows that the binding of *Na*-GST-1 to DC receptors tends to induce both Th1 and Th2 immune responses *in silico*, which is consistent with previous findings (Oliveira *et al*., 2025). In that study, significant levels of IgG1 and IgG2a were observed, indicating IgG responses associated with both Th1 and Th2 activation (Oliveira *et al*., 2025). This mixed Th1/Th2 response observed across different DC receptors suggests that *Na*-GST-1 may be capable of inducing a mixed immune profile. A mixed Th1/Th2 response is desirable, as it may help prevent chronic inflammation and autoimmunity caused by dominant Th1 responses, as well as allergic reactions driven by dominant Th2 responses (Infante-Duarte & Kamradt, 1999). The predictions made in this study suggest that *Na*-GST-1 is a promising vaccine candidate for eliciting a mixed Th1/Th2 response. Furthermore, the DC receptors predicted to have strong binding affinity with *Na*-GST-1 warrant further investigation in future studies.

In the US and Brazil studies, adults receiving *Na*-GST-1/Alhydrogel, had similar levels of IgG1 and IgG3 observed, while no IgG2 and IgG4 were detected (Diemert *et al*., 2017). Our study attempts to evaluate the utilization of an *in silico* model for the improvement of the evaluation of vaccine antigens in the future and improve the designs and development strategies under the reverse vaccinology approach. The strong binding affinity to TLR2 is known to preferentially induce a Th2 profile in DC and can affect antibody response through production of IL-4 and IgG1 class switching (Re & Strominger, 2001; Li *et al*., 2021). Meanwhile, TLR3 induces a Th1-type profile through intermediary release of IL-12 or interferon-alpha (IFN-α) that contributes to IgG3 class switching (Netea *et al*., 2005). It is hypothesized that strong bindings to these receptors, TLR3, TLR4 and TLR7, enhance production of IL-12 and IFN-γ, leading to IgG3 class switching.

Regarding the absence of IgG2 and IgG4 in clinical trial data, IgG4 is typically associated with long-term antigen exposure and immune tolerance, with IL-10 as the key driver for IgG4 class switching. In the present model, TLR2, CD206 and DC-SIGN can lead to IL-10 production; however, particularly for weak binding of CD206 and DC-SIGN, which may be insufficient to trigger IL-10, therefore, the absence of IgG4. Our docking method also showed that the potential role of only S3, which had the strongest binding to CD206, could be used as a tool to understand the activation of anti-inflammatory responses or neutralizing responses.

TLR9 can recognize the CpG DNA sequences of *Leshmania major* and activate the DCs, subsequently triggering Th-1 dominant response or $CD11c^{high}$ DC, thus IFN-γ production and cytotoxic function in natural killer (NK) cells (Schleicher *et al*., 2007; Liese *et al*., 2008; Hkima Abou Fakher *et al*., 2009). Following this, if coupled with IL-10, IgG class switch can occur. TLR9 can regulate isotype switching to IgG2a through B cell interaction (He *et al*., 2004; Jegerlehner *et al*., 2007). Based on this information, the lack of IgG2 described in the trials, and the affinity of GST-1 to TLR 9, could potentially indicate the moderate responses that may not be sufficient to trigger isotype switching, including the lack of IL-10 presence to contribute to this immune environment.

PAMP recognition by specific receptors is a crucial event in activation and DC and initiation of adaptive immune responses (Banchereau & Steinman, 1998). While this may be highly hypothetical, the utilization of binding energy to predict the outcome of immunology is still exploratory. The present work suggests that the binding energy for each IgG subtype could differ from one to another to confer a functional prediction; however, the mean cut-off for IgG1, IgG2 and IgG3 was found to be approximately -10 kcal/mol. Using these outcomes, future researchers can consider applying a lower cutoff to evaluate their design and modifications accordingly to improve the outcomes. Additionally, this work is limited to *in silico* and only utilises a small number of receptors involved in the immunogenicity pathways; therefore, more *in vitro* and *in vivo* work will be necessary to ensure this method can be applied in the future.

Table 5. Summary of immune responses, cytokines and antibody subtypes induced by DC receptors

DC receptor	Th cell response induced	Cytokines Induced	Antibody response trigger	Reference
TLR2	Th2	IL-4 IL-15 IL-9 IL-10 IL-13	IgG1 IgG3 IgE	(Re & Strominger, 2001; Gao *et al*., 2012; Mukherjee *et al*., 2016; Diemert *et al*., 2017; Rajasekaran *et al*., 2017; Li *et al*., 2021)
TLR3	Th2/Th17 (Degradation of TLR3 lead to suppression of Th1)	IL-4 IL-17 CCL11	IgG1 IgG4 IgE	(Joshi *et al*., 2008; Kraemer *et al*., 2022)
TLR4	Th2 (In the case of helminth infection)	IL-4	IgG1 IgG4 IgE	(Thomas *et al*., 2003; Lee & Rosenberg, 2013)
TLR7	Th1 (In the context of TLR7 agonist)	IFN-α IFN-γ IL-6 TNF-α CCL2 CCL4	IgG2a IgG2b	(Hennessy *et al*., 2010; Buonsanti *et al.*, 2016; Hu *et al*., 2020)
TLR9	Th1	IFN-γ	IgG1 IgG3	(Bhattacharya *et al*., 2023; Diemert *et al*., 2024)
DEC-205	Th1	IFN-γ IL-2	IgG2a Nasal IgA	(Lakhrif *et al*., 2018)
DC-SIGN	Th2/Reg	Strong IL6, and IL8, IL10 and IL12	IgG1 Some IgG3, IgE	(McRae *et al*., 2015; Tiburcio *et al*., 2021)
CD206	Th2	IL-4 IL-13 IL-10	IgG1 IgE	(Paveley *et al*., 2011; Henry *et al*., 2017)
CD14	Th2/Treg	Low IL-12 output and weak T-cell priming	IgG4 IgE	(Fujiwara *et al*., 2009)
CD36	Th1	Early stage of infection: – IFN-γ – IL-12 Later stage of infection: – IL-10	Early stage of infection: – IgG2b – IgG2c Later stage of infection: – IgG1	(Gracie & Bradley, 1996; Kraemer *et al*., 2022; Thylur *et al*., 2017)
BDCA-2	Th1	IFN-a	IgG4 IgE	(Manurung *et al*., 2024)
DCIR	Th2/Treg	Increased in IL-10	IgG4 IgE	(Tsuboi *et al*., 2012; Rivera-Toledo *et al*., 2024)
Clec9A	TFH	DNGR-1 signal promotes cross presentation to CD8+ cells and IL21 Tfh	IgG1 (possibly)	(Cueto *et al*., 2020)
Clec10A	Th2/Threg skew	Upregulate IL-10	IgG4 IgE	(Gaze *et al*., 2012)
Langerin	Th1/Tfh	IL-12	IgG1 IgG3	(Loukas & Prociv, 2001; Kumkate *et al*., 2007)

CONCLUSION

This study investigated the antigenicity and immunogenicity of *Na*-GST-1 and its potential interactions with dendritic cell (DC) receptors using an *in silico* framework. The findings indicate that *Na*-GST-1 shows comparatively stronger predicted binding affinity with several DC receptors, including TLR2, TLR3, TLR4, TLR7, DEC-205, CD206, and CD36, relative to other receptors examined. These interactions suggest a potential role for these receptors in early antigen recognition and processing at the molecular level. Importantly, these observations are predictive in nature and do not imply direct functional activation of downstream immune pathways.

The results provide preliminary insight into how *Na*-GST-1 may engage multiple DC receptors, supporting its plausibility as a candidate antigen for further investigation. While molecular docking cannot directly infer immune polarization or antibody responses,

the binding trends observed offer a rationale for prioritizing *Na*-GST-1 in subsequent experimental studies. Future validation may include dendritic cell activation assays, antigen uptake analyses, and cytokine profiling to experimentally assess immune outcomes.

Hookworm infection remains a major public health burden, particularly in low- and middle-income regions, and emerging concerns regarding anthelmintic resistance highlight the need for alternative control strategies. Within this context, the present work contributes early-stage *in silico* evidence that may inform rational vaccine research pipelines and guide downstream *in vitro* and *in vivo* investigations aimed at elucidating immunological mechanisms involved in hookworm infection.

Despite the insights generated from the computational analyses, certain methodological and data-related constraints should be considered. Several limitations of the present study should be acknowledged.

1. The analyses are based entirely on in silico predictions, including antigenicity, immunogenicity, and protein–protein docking. While multiple complementary computational tools were employed to improve robustness, these approaches cannot substitute for experimental validation and do not directly reflect functional immune responses in biological systems.

2. The immunogenicity comparisons used for downstream correlation analyses were derived from published clinical trial datasets. These data are influenced by multiple experimental variables, most notably the use of adjuvants, which are known to substantially modulate immune responses, including T-helper polarisation and antibody subclass distribution. As a result, the observed associations between predicted binding affinity and reported immune responses should be interpreted with caution, as they may reflect adjuvant-driven effects rather than antigen–receptor interactions alone.

3. Interpretations related to antibody responses, including IgG and its subclasses, are inherently limited in an in silico docking context. Predicted binding affinity between *Na*-GST-1 and DC receptors does not directly equate to antibody production, magnitude, or class switching. Therefore, no causal inference regarding IgG responses can be made based on the current analyses, and any observed trends should be considered exploratory rather than confirmatory.

4. The availability of structural data and curated immunological datasets constrained the scope of the analysis. Some DC receptors and antigen variants could not be evaluated comprehensively due to limited structural or experimental information.

Taken together, these limitations underscore that the findings presented here are hypothesis-generating and intended to support early-stage decision-making. Experimental validation will be essential to confirm the functional relevance of the predicted interactions and to clarify the immunological pathways involved.

ACKNOWLEDGEMENT

This study was supported under the project “Development of Vaccines Using mRNA Technology Against Neglected Tropical Diseases (DEVARNA)” under the Ministry of Science, Technology and Innovation Malaysia (MOSTI) Strategic Research Fund (Code: SRF0821163APP/ MOSTI003-2021SRF). This work was conducted within a research environment supported by the Ministry of Higher Education Malaysia through the Higher Institution Centre of Excellence (HICoE) programme (MO002–2019; TIDREC-2023).

Conflict of Interest Statement

The author declares that they have no conflict of interest.

REFERENCE

Abuzeid, A.M.I., Zhou, X., Huang, Y. & Li, G. (2020). Twenty-five-year research progress in hookworm excretory/secretory products. *Parasites & Vectors* **13**: 136. https://doi.org/10.1186/s13071-020-04010-8

Adegnika, A.A., de Vries, S.G., Zinsou, F.J., Honkepehedji, Y.J., Dejon Agobé, J.-C., Vodonou, K.G., Bikangui, R., Bouyoukou Hounkpatin, A., Bache, E.B., Massinga Loembe, M. *et al*. (2021). Safety and immunogenicity of co-administered hookworm vaccine candidates *Na*-GST-1 and *Na*-APR-1 in Gabonese adults: A randomised, controlled, double-blind, phase 1 dose-escalation trial. *The Lancet Infectious Diseases* **21**: 275-285. https://doi.org/10.1016/S1473-3099(20)30288-7

Aksoy, E., Zouain, C.S., Vanhoutte, F., Fontaine, J., Pavelka, N., Thieblemont, N., Willems, F., Ricciardi-Castagnoli, P., Goldman, M., Capron, M. *et al*. (2005). Double-stranded RNAs from the Helminth Parasite *Schistosoma* Activate TLR3 in Dendritic Cells*. *Journal of Biological Chemistry* **280**: 277-283. https://doi.org/10.1074/jbc.M411223200

Bachmann, A., Metwally, N.G., Allweier, J., Cronshagen, J., del Pilar Martinez Tauler, M., Murk, A., Roth, L.K., Torabi, H., Wu, Y., Gutsmann, T. *et al*. (2022). CD36 – A host receptor necessary for malaria parasites to establish and maintain infection. *Microorganisms* **10**: 2356. https://doi.org/10.3390/microorganisms10122356

Baker, J.M., Trinies, V., Bronzan, R.N., Dorkenoo, A.M., Garn, J.V., Sognikin, S. & Freeman, M.C. (2018). The associations between water and sanitation and hookworm infection using cross-sectional data from Togo’s national deworming program. *PLOS Neglected Tropical Diseases* **12**: e0006374. https://doi.org/10.1371/journal.pntd.0006374

Banchereau, J. & Steinman, R.M. (1998). Dendritic cells and the control of immunity. *Nature* **392**: 245-252. https://doi.org/10.1038/32588

Bethony, J., Loukas, A., Smout, M., Brooker, S., Mendez, S., Plieskatt, J., Goud, G., Bottazzi, M., Zhan, B., Wang, Y. *et al*. (2005). Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB Journal/ : Official Publication of the Federation of American Societies for Experimental Biology* **19**: 1743-1745. https://doi.org/10.1096/fj.05-3936fje

Bhattacharya, P., Gannavaram, S., Ismail, N., Saxena, A., Dagur, P.K., Akue, A., KuKuruga, M. & Nakhasi, H.L. (2023). Toll-like Receptor-9 (TLR-9) Signaling is crucial for inducing protective immunity following immunization with genetically modified live attenuated Leishmania parasites. *Pathogens* **12**: 534. https://doi.org/10.3390/pathogens12040534

Buonsanti, C., Balocchi, C., Harfouche, C., Corrente, F., Galli Stampino, L., Mancini, F., Tontini, M., Malyala, P., Bufali, S., Baudner, B. *et al*. (2016). Novel adjuvant Alum-TLR7 significantly potentiates immune response to glycoconjugate vaccines. *Scientific Reports* **6**: 29063. https://doi.org/10.1038/srep29063

Bzówka, M., Bagrowska, W. & Góra, A. (2023). Recent advances in studying toll-like receptors with the use of computational methods. *Journal of Chemical Information and Modeling*, **63**: 3669-3687. https://doi.org/10.1021/acs.jcim.3c00419

Cortés, A., Muñoz-Antoli, C., Esteban, J.G. & Toledo, R. (2017). Th2 and Th1 responses: Clear and hidden sides of immunity against intestinal helminths. *Trends in Parasitology* **33**: 678-693. https://doi.org/10.1016/j.pt.2017.05.004

Cueto, F.J., del Fresno, C. & Sancho, D. (2020). DNGR-1, a dendritic cell-specific sensor of tissue damage that dually modulates immunity and inflammation. *Frontiers in Immunology* **10**: 3146. https://doi.org/10.3389/fimmu.2019.03146

Diemert, D., Campbell, D., Brelsford, J., Leasure, C., Li, G., Peng, J., Zumer, M., Younes, N., Bottazzi, M.E., Mejia, R. *et al*. (2018). Controlled human hookworm infection: Accelerating human hookworm vaccine development. *Open Forum Infectious Diseases* **5**: ofy083. https://doi.org/10.1093/ofid/ofy083

Diemert, D.J., Freire, J., Valente, V., Fraga, C.G., Talles, F., Grahek, S., Campbell, D., Jariwala, A., Periago, M.V., Enk, M. *et al.* (2017). Safety and immunogenicity of the Na-GST-1 hookworm vaccine in Brazilian and American adults. *PLoS Neglected Tropical Diseases* **11**: e0005574. https://doi.org/10.1371/journal.pntd.0005574

Diemert, D.J., Pinto, A.G., Freire, J., Jariwala, A., Santiago, H., Hamilton, R.G., Periago, M. V., Loukas, A., Tribolet, L., Mulvenna, J. *et al*. (2012). Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: Implications for the development of vaccines against helminths. *Journal of Allergy and Clinical Immunology* **130**: 169-176.e6. https://doi.org/10.1016/j.jaci.2012.04.027

Diemert, D.J., Zumer, M., Bova, M., Gibbs-Tewary, C., Malkin, E.M., Campbell, D., Hoeweler, L., Li, G., Bottazzi, M.E., Hotez, P. J. *et al*. (2024). Randomized, observer-blind, controlled Phase 1 study of the safety and immunogenicity of the Na-GST-1/Alhydrogel hookworm vaccine with or without a CpG ODN adjuvant in hookworm-naıve adults. *PLOS Neglected Tropical Diseases* **18**: e0012788. https://doi.org/10.1371/journal.pntd.0012788

Dimitrov, I., Bangov, I., Flower, D.R. & Doytchinova, I. (2014a). AllerTOP v.2 – A server for *in silico* prediction of allergens. *Journal of Molecular Modeling* **20**: 2278. https://doi.org/10.1007/s00894-014-2278-5

Dimitrov, I., Naneva, L., Doytchinova, I. & Bangov, I. (2014b). AllergenFP: Allergenicity prediction by descriptor fingerprints. *Bioinformatics* **30**: 846-851. https://doi.org/10.1093/bioinformatics/btt619

Donnelly, S., O'Neill, S.M., Stack, C.M., Robinson, M.W., Turnbull, L., Whitchurch, C. & Dalton, J.P. (2010). Helminth cysteine proteases inhibit TRIF-dependent activation of macrophages via degradation of TLR3. *Journal of Biological Chemistry* **285**: 3383-3392. https://doi.org/10.1074/jbc.M109.060368

Doytchinova, I.A. & Flower, D.R. (2007a). Identifying candidate subunit vaccines using an alignment-independent method based on principal amino acid properties. *Vaccine* **25**: 856-866. https://doi.org/10.1016/j.vaccine.2006.09.032

Doytchinova, I.A. & Flower, D.R. (2007b). VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics* **8**: 4. https://doi.org/10.1186/1471-2105-8-4

Doytchinova, I. & Flower, D. (2008). Bioinformatic approach for identifying parasite and fungal candidate subunit vaccines. *The Open Vaccine Journal* **1**: 22-26. https://doi.org/10.2174/1875035400801010022

Dzionek, A., Sohma, Y., Nagafune, J., Cella, M., Colonna, M., Facchetti, F., Günther, G., Johnston, I., Lanzavecchia, A., Nagasaka, T. *et al*. (2001). BDCA-2, a novel plasmacytoid dendritic cell–specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon α/β induction. *The Journal of Experimental Medicine* **194**: 1823-1834.

Everts, B., Hussaarts, L., Driessen, N.N., Meevissen, M.H.J., Schramm, G., van der Ham, A.J., van der Hoeven, B., Scholzen, T., Burgdorf, S., Mohrs, M. *et al*. (2012). Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *Journal of Experimental Medicine* **209**: 1753-1767. https://doi.org/10.1084/jem.20111381

Ferreira, K.S., Bastos, K.R., Russo, M. & Almeida, S.R. (2007). Interaction between Paracoccidioides brasiliensis and pulmonary dendritic cells induces interleukin-10 production and toll-like receptor–2 expression: Possible mechanisms of susceptibility. *The Journal of Infectious Diseases* **196**: 1108-1115. https://doi.org/10.1086/521369

Fujiwara, R.T., Cannado, G.G.L., Freitas, P.A., Santiago, H.C., Massara, C.L., Carvalho, O. dos S., Corrça-Oliveira, R., Geiger, S.M. & Bethony, J. (2009). Necator americanus infection: A possible cause of altered dendritic cell differentiation and eosinophil profile in chronically infected individuals. *PLoS Neglected Tropical Diseases* **3**: e399. https://doi.org/10.1371/journal.pntd.0000399

Gao, Y., Zhang, M., Chen, L., Hou, M., Ji, M. & Wu, G. (2012). Deficiency in TLR2 but not in TLR4 impairs dendritic cells derived IL-10 responses to schistosome antigens. *Cellular Immunology* **272**: 242-250. https://doi.org/10.1016/j.cellimm.2011.10.007

Gaze, S., McSorley, H.J., Daveson, J., Jones, D., Bethony, J.M., Oliveira, L.M., Speare, R., McCarthy, J.S., Engwerda, C.R., Croese, J. *et al*. (2012). Characterising the mucosal and systemic immune responses to experimental human hookworm infection. *PLOS Pathogens* **8**: e1002520. https://doi.org/10.1371/journal.ppat.1002520

Gowda, N.M., Wu, X., Kumar, S., Febbraio, M. & Gowda, D.C. (2013). CD36 contributes to malaria parasite-induced pro-inflammatory cytokine production and NK and T cell activation by dendritic cells. *PLOS ONE* **8**: e77604. https://doi.org/10.1371/journal.pone.0077604

Gracie, J.A. & Bradley, J.A. (1996). Interleukin-12 induces interferon-γ-dependent switching of IgG alloantibody subclass. *European Journal of Immunology* **26**: 1217-1221. https://doi.org/10.1002/eji.1830260605

Granucci, F. & Zanoni, I. (2013). Role of CD14 in host protection against infections and in metabolism regulation. *Frontiers in Cellular and Infection Microbiology* **3**: 32. https://doi.org/10.3389/fcimb.2013.00032

Gupta, N., Chaudhry, R. & Thakur, C.K. (2016). Determination of cutoff of ELISA and immunofluorescence assay for scrub typhus. *Journal of Global Infectious Diseases* **8**: 97-99. https://doi.org/10.4103/0974-777X.188584

Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Consortium, O.S.D.D. & Raghava, G.P.S. (2013). In silico approach for predicting toxicity of peptides and proteins. *PLOS ONE* **8**: e73957. https://doi.org/10.1371/journal.pone.0073957

Gutiérrez-Kobeh, L., Rodríguez-González, J., Argueta-Donohué, J., Vázquez-López, R., Wilkins-Rodríguez, A.A., Gutiérrez-Kobeh, L., Rodríguez-González, J., Argueta-Donohué, J., Vázquez-López, R. & Wilkins-Rodríguez, A.A. (2018). Role of dendritic cells in parasitic infections. In *Dendritic Cells*. IntechOpen. https://doi.org/10.5772/intechopen.79491

He, B., Qiao, X. & Cerutti, A. (2004). CpG DNA induces IgG class switch DNA Recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-101. *The Journal of Immunology* **173**: 4479-4491. https://doi.org/10.4049/jimmunol.173.7.4479

Hennessy, E.J., Parker, A.E. & O'Neill, L.A.J. (2010). Targeting Toll-like receptors: Emerging therapeutics? *Nature Reviews Drug Discovery* **9**: 293-307. https://doi.org/10.1038/nrd3203

Henry, E.K., Inclan-Rico, J.M. & Siracusa, M.C. (2017). Type 2 cytokine responses: Regulating immunity to helminth parasites and allergic inflammation. *Current Pharmacology Reports* **3**: 346-359. https://doi.org/10.1007/s40495-017-0114-1

Hkima Abou Fakher, F., Rachinel, N., Klimczak, M., Louis, J. & Doyen, N. (2009). TLR9-dependent activation of dendritic cells by DNA from Leishmania major favors Th1 cell development and the resolution of Lesions1. *The Journal of Immunology* **182**: 1386-1396. https://doi.org/10.4049/jimmunol.182.3.1386

Honorato, R.V., Koukos, P.I., Jiménez-García, B., Tsaregorodtsev, A., Verlato, M., Giachetti, A., Rosato, A. & Bonvin, A.M.J.J. (2021). Structural biology in the Clouds: The WeNMR-EOSC ecosystem. *Frontiers in Molecular Biosciences* **8**: 729513. https://doi.org/10.3389/fmolb.2021.729513

Hoogerwerf, M.-A., Janse, J.J., Kuiper, V.P., Schuijlenburg, R. van, Kruize, Y.C., Sijtsma, J.C., Nosoh, B.A., Koopman, J.-P.R., Verbeek-Menken, P.H., Westra, I.M. *et al*. (2023). Protective efficacy of short-term infection with Necator americanus hookworm larvae in healthy volunteers in the Netherlands: A single-centre, placebo-controlled, randomised, controlled, phase 1 trial. *The Lancet Microbe* **4**: e1024-e1034. https://doi.org/10.1016/S2666-5247(23)00218-5

Hu, Y., Tang, L., Zhu, Z., Meng, H., Chen, T., Zhao, S., Jin, Z., Wang, Z. & Jin, G. (2020). A novel TLR7 agonist as adjuvant to stimulate high quality HBsAg-specific immune responses in an HBV mouse model. *Journal of Translational Medicine* **18**: 112. https://doi.org/10.1186/s12967-020-02275-2

Infante-Duarte, C. & Kamradt, T. (1999). Th1/Th2 balance in infection. *Springer Seminars in Immunopathology* **21**: 317-338. https://doi.org/10.1007/BF00812260

Jakubec, D., Skoda, P., Krivak, R., Novotny, M. & Hoksza, D. (2022). PrankWeb 3: Accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Research* **50**: W593-W597. https://doi.org/10.1093/nar/gkac389

Jegerlehner, A., Maurer, P., Bessa, J., Hinton, H.J., Kopf, M. & Bachmann, M.F. (2007). TLR9 signaling in B cells determines class switch recombination to IgG2a. *The Journal of Immunology* **178**: 2415-2420. https://doi.org/10.4049/jimmunol.178.4.2415

Jendele, L., Krivak, R., Skoda, P., Novotny, M. & Hoksza, D. (2019). PrankWeb: A web server for ligand binding site prediction and visualization. *Nucleic Acids Research* **47**: W345-W349. https://doi.org/10.1093/nar/gkz424

Joshi, A.D., Schaller, M.A., Lukacs, N.W., Kunkel, S.L. & Hogaboam, C.M. (2008). TLR3 modulates immunopathology during a Schistosoma mansoni egg-driven Th2 response in the lung. *European Journal of Immunology* **38**: 3436-3449. https://doi.org/10.1002/eji.200838629

Kautz-Neu, K., Noordegraaf, M., Dinges, S., Bennett, C.L., John, D., Clausen, B.E. & von Stebut, E. (2011). Langerhans cells are negative regulators of the anti-Leishmania response. *Journal of Experimental Medicine* **208**: 885-891. https://doi.org/10.1084/jem.20102318

Kerepesi, L.A., Hess, J.A., Leon, O., Nolan, T.J., Schad, G.A. & Abraham, D. (2007). Toll-like receptor 4 (TLR4) is required for protective immunity to larval *Strongyloides stercoralis* in mice. *Microbes and Infection* **9**: 28-34. https://doi.org/10.1016/j.micinf.2006.10.003

Koopman, J.P.R., Driciru, E. & Roestenberg, M. (2021). Controlled human infection models to evaluate schistosomiasis and hookworm vaccines: Where are we now? *Expert Review of Vaccines* **20**: 1369-1371. https://doi.org/10.1080/14760584.2021.1951244

Kraemer, L., McKay, D.M., Russo, R.C. & Fujiwara, R.T. (2022). Chemokines and chemokine receptors: Insights from human disease and experimental models of helminthiasis. *Cytokine & Growth Factor Reviews* **66**: 38-52. https://doi.org/10.1016/j.cytogfr.2022.05.002

Krivák, R. & Hoksza, D. (2018). P2Rank: Machine learning based tool for rapid and accurate prediction of ligand binding sites from protein structure. *Journal of Cheminformatics* **10**: 39. https://doi.org/10.1186/s13321-018-0285-8

Kumkate, S., Jenkins, G.R., Paveley, R.A., Hogg, K.G. & Mountford, A.P. (2007). CD207+ Langerhans cells constitute a minor population of skin-derived antigen-presenting cells in the draining lymph node following exposure to *Schistosoma mansoni*. *International Journal for Parasitology* **37**: 209-220. https://doi.org/10.1016/j.ijpara.2006.10.007

Lakhrif, Z., Moreau, A., Hérault, B., Di-Tommaso, A., Juste, M., Moiré, N., Dimier-Poisson, I., Mévélec, M.-N. & Aubrey, N. (2018). Targeted delivery of Toxoplasma gondii antigens to dendritic cells promote immunogenicity and protective efficiency against toxoplasmosis. *Frontiers in Immunology* **9**: 317. https://doi.org/10.3389/fimmu.2018.00317

Layland, L.E., Rad, R., Wagner, H. & da Costa, C.U.P. (2007). Immunopathology in schistosomiasis is controlled by antigen-specific regulatory T cells primed in the presence of TLR2. *European Journal of Immunology* **37**: 2174-2184. https://doi.org/10.1002/eji.200737063

Lee, J.J. & Rosenberg, H.F. (2013). Eosinophils: mediators of host–parasite interactions. In: Eosinophils in Health and Disease, Lee, J.J. & Rosenberg, H.F. (editors.). London: Academic Press, pp. 301-327. https://doi.org/10.1016/B978-0-12-394385-9.00010-9

Li, Y., Chen, Q., Ji, W., Fan, Y., Huang, L., Chu, C. & Zhou, W. (2021). TLR2 deficiency promotes IgE and inhibits IgG1 class-switching following ovalbumin sensitization. *Italian Journal of Pediatrics* **47**: 162. https://doi.org/10.1186/s13052-021-01088-3

Liese, J., Schleicher, U. & Bogdan, C. (2008). The innate immune response against *Leishmania* parasites. *Immunobiology* **213**: 377-387. https://doi.org/10.1016/j.imbio.2007.12.005

Loukas, A. & Prociv, P. (2001). Immune Responses in hookworm infections. *Clinical Microbiology Reviews* **14**: 689-703. https://doi.org/10.1128/CMR.14.4.689-703.2001

Maglinao, M., Klopfleisch, R., Seeberger, P.H. & Lepenies, B. (2013). The C-Type lectin receptor DCIR is crucial for the development of experimental cerebral malaria. *The Journal of Immunology* **191**: 2551-2559. https://doi.org/10.4049/jimmunol.1203451

Manurung, M.D., Sonnet, F., Hoogerwerf, M.-A., Janse, J.J., Kruize, Y., Bes-Roeleveld, L. de, König, M., Loukas, A., Dewals, B.G., Supali, T. *et al*. (2024). Controlled human hookworm infection remodels plasmacytoid dendritic cells and regulatory T cells towards profiles seen in natural infections in endemic areas. *Nature Communications* **15**: 5960. https://doi.org/10.1038/s41467-024-50313-0

McRae, K.M., Stear, M.J., Good, B. & Keane, O.M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology* **37**: 605-613. https://doi.org/10.1111/pim.12290

Mendez, S., D' Samuel, A., Antoine, A.D., Ahn, S. & Hotez, P. (2008). Use of the air pouch model to investigate immune responses to a hookworm vaccine containing the Na-ASP-2 protein in rats. *Parasite Immunology* **30**: 53-56. https://doi.org/10.1111/j.1365-3024.2007.00994.x

Moreau, E. & Chauvin, A. (2010). Immunity against helminths: Interactions with the host and the intercurrent infections. *BioMed Research International* **2010**: 428593. https://doi.org/10.1155/2010/428593

Motran, C.C., Silvane, L., Chiapello, L.S., Theumer, M.G., Ambrosio, L.F., Volpini, X., Celias, D.P. & Cervi, L. (2018). Helminth infections: Recognition and modulation of the immune response by innate immune cells. *Frontiers in Immunology* **9**: 664. https://doi.org/10.3389/fimmu.2018.00664

Mukherjee, S., Karmakar, S. & Babu, S.P.S. (2016). TLR2 and TLR4 mediated host immune responses in major infectious diseases: A review. *The Brazilian Journal of Infectious Diseases* **20**: 193-204. https://doi.org/10.1016/j.bjid.2015.10.011

Netea, M.G., Van der Meer, J.W.M., Sutmuller, R.P., Adema, G.J. & Kullberg, B.-J. (2005). From the Th1/Th2 paradigm towards a Toll-Like Receptor/T-Helper Bias. *Antimicrobial Agents and Chemotherapy* **49**: 3991-3996. https://doi.org/10.1128/AAC.49.10.3991-3996.2005

Oliveira, A.S.D., Versteeg, L., Briggs, N., Adhikari, R., Villar, M.J., Redd, J.R., Hotez, P., Bottazzi, M.E. & Pollet, J. (2025). Altering the intracellular trafficking of Necator americanus GST-1 antigen yields novel hookworm mRNA vaccine candidates. *PLOS Neglected Tropical Diseases* **19**: e0012809. https://doi.org/10.1371/journal.pntd.0012809

Patel, S.N., Lu, Z., Ayi, K., Serghides, L., Gowda, D.C. & Kain, K.C. (2007). Disruption of CD36 impairs cytokine response to Plasmodium falciparum glycosylphosphatidylinositol and confers susceptibility to severe and fatal malaria in Vivo1. *The Journal of Immunology* **178**: 3954-3961. https://doi.org/10.4049/jimmunol.178.6.3954

Paveley, R.A., Aynsley, S.A., Turner, J.D., Bourke, C.D., Jenkins, S.J., Cook, P.C., Martinez-Pomares, L. & Mountford, A.P. (2011). The Mannose Receptor (CD206) is an important pattern recognition receptor (PRR) in the detection of the infective stage of the helminth *Schistosoma mansoni* and modulates IFNγ production. *International Journal for Parasitology* **41**: 1335-1345. https://doi.org/10.1016/j.ijpara.2011.08.005

Piva, L., Tetlak, P., Claser, C., Karjalainen, K., Renia, L. & Ruedl, C. (2012). Cutting edge: Clec9A+ dendritic cells mediate the development of experimental cerebral malaria. *The Journal of Immunology* **189**: 1128-1132. https://doi.org/10.4049/jimmunol.1201171

Rajasekaran, S., Anuradha, R. & Bethunaickan, R. (2017). TLR specific immune responses against helminth infections. *Journal of Parasitology Research* **2017**: 6865789. https://doi.org/10.1155/2017/6865789

Re, F. & Strominger, J.L. (2001). Toll-like Receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *Journal of Biological Chemistry* **276**: 37692-37699. https://doi.org/10.1074/jbc.M105927200

Rivera-Toledo, E., Fernández-Rojas, M.A., Santiago-Olivares, C., Cruz-Rivera, M., Hernández-Bautista, V., Ávila-Horta, F., Flisser, A. & Mendlovic, F. (2024). Transcriptome profiling of macrophages persistently infected with human respiratory syncytial virus and effect of recombinant Taenia solium calreticulin on immune-related genes. *Frontiers in Microbiology* **15**: 1402589. https://doi.org/10.3389/fmicb.2024.1402589

Sarkar, E.R., Sikder, S., Giacomin, P. & Loukas, A. (2024). Hookworm vaccines: Current and future directions. *Expert Review of Vaccines* **23**: 1029-1040. https://doi.org/10.1080/14760584.2024.2410893

Schleicher, U., Liese, J., Knippertz, I., Kurzmann, C., Hesse, A., Heit, A., Fischer, J.A.A., Weiss, S., Kalinke, U., Kunz, S. *et al*. (2007). NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs. *The Journal of Experimental Medicine* **204**: 893-906. https://doi.org/10.1084/jem.20061293

Shrimpton, R.E., Butler, M., Morel, A.-S., Eren, E., Hue, S.S. & Ritter, M.A. (2009). CD205 (DEC-205): A recognition receptor for apoptotic and necrotic self. *Molecular Immunology* **46**: 1229-1239. https://doi.org/10.1016/j.molimm.2008.11.016

Taban, Q., Mumtaz, P.T., Masoodi, K. Z., Haq, E. & Ahmad, S.M. (2022). Scavenger receptors in host defense: From functional aspects to mode of action. *Cell Communication and Signaling* **20**: 2. https://doi.org/10.1186/s12964-021-00812-0

Talukdar, A., Ganguly, D., Roy, S., Das, N. & Sarkar, D. (2021). Structural evolution and translational potential for agonists and antagonists of endosomal Toll-like receptors. *Journal of Medicinal Chemistry* **64**: 8010-8041. https://doi.org/10.1021/acs.jmedchem.1c00300

Thomas, P.G., Carter, M.R., Atochina, O., Da'Dara, A.A., Piskorska, D., McGuire, E. & Harn, D.A. (2003). Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-Like Receptor 4-Dependent mechanism 1. *The Journal of Immunology* **171**: 5837-5841. https://doi.org/10.4049/jimmunol.171.11.5837

Thylur, R.P., Wu, X., Gowda, N.M., Punnath, K., Neelgund, S.E., Febbraio, M. & Gowda, D.C. (2017). CD36 receptor regulates malaria-induced immune responses primarily at early blood stage infection contributing to parasitemia control and resistance to mortality. *Journal of Biological Chemistry* **292**: 9394-9408. https://doi.org/10.1074/jbc.M117.781294

Tiburcio, R., Melo, L.D., Nunes, S., Barbosa, A.L.A., de Oliveira, E.C., Suarez, M., Borges, V.M., Tavares, N. & Brodskyn, C.I. (2021). DC-SIGN mediates the interaction between neutrophils and Leishmania amazonensis-Infected dendritic cells to promote DC maturation and parasite elimination. *Frontiers in Immunology* **12**: 750648. https://doi.org/10.3389/fimmu.2021.750648

Toebak, M.J., Moed, H., Von Blomberg, M.B.E., Bruynzeel, D.P., Gibbs, S., Scheper, R.J. & Rustemeyer, T. (2006). Intrinsic characteristics of contact and respiratory allergens influence production of polarizing cytokines by dendritic cells. *Contact Dermatitis* **55**: 238-245. https://doi.org/10.1111/j.1600-0536.2006.00918.x

Tong, C., Liang, Y., Han, X., Zhang, Z., Zheng, X., Wang, S. & Song, B. (2023). Research progress of dendritic cell surface receptors and targeting. *Biomedicines* **11**: 1673. https://doi.org/10.3390/biomedicines11061673

Tsuboi, H., Matsuo, N., Iizuka, M., Tsuzuki, S., Kondo, Y., Tanaka, A., Moriyama, M., Matsumoto, I., Nakamura, S. & Sumida, T. (2012). Analysis of IgG4 class switch-related molecules in IgG4-related disease. *Arthritis Research & Therapy* **14**: R171. https://doi.org/10.1186/ar3924

van Die, I., van Vliet, S.J., Nyame, A.K., Cummings, R.D., Bank, C.M.C., Appelmelk, B., Geijtenbeek, T.B.H. & van Kooyk, Y. (2003). The dendritic cell–specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* **13**: 471-478. https://doi.org/10.1093/glycob/cwg052

van Vliet, S.J., van Liempt, E., Saeland, E., Aarnoudse, C.A., Appelmelk, B., Irimura, T., Geijtenbeek, T.B.H., Blixt, O., Alvarez, R., van Die, I. *et al*. (2005). Carbohydrate profiling reveals a distinctive role for the C-type lectin MGL in the recognition of helminth parasites and tumor antigens by dendritic cells. *International Immunology* **17**: 661-669. https://doi.org/10.1093/intimm/dxh246

Vangone, A. & Bonvin, A.M. (2015). Contacts-based prediction of binding affinity in protein–protein complexes. *eLife* **4**: e07454. https://doi.org/10.7554/eLife.07454

Vázquez-Mendoza, A., Carrero, J.C. & Rodriguez-Sosa, M. (2013). Parasitic infections: A role for C-Type Lectins Receptors. *BioMed Research International* **2013**: 456352. https://doi.org/10.1155/2013/456352

Venugopal, P.G., Nutman, T.B. & Semnani, R.T. (2009). Activation and regulation of Toll-Like Receptors (TLRs) by helminth parasites. *Immunologic Research* **43**: 252-263. https://doi.org/10.1007/s12026-008-8079-0

Wang, D., Sun, B., Feng, M., Feng, H., Gong, W., Liu, Q. & Ge, S. (2015). Role of scavenger receptors in dendritic cell function. *Human Immunology* **76**: 442-446. https://doi.org/10.1016/j.humimm.2015.03.012

Wong, M.T.J., Anuar, N.S., Noordin, R. & Tye, G.J. (2023). Soil-transmitted helminthic vaccines: Where are we now? *Acta Tropica* **239**: 106796. https://doi.org/10.1016/j.actatropica.2022.106796

Xiao, S., Zhan, B., Xue, J., Goud, G.N., Loukas, A., Liu, Y., Williamson, A., Liu, S., Deumic, V. & Hotez, P. (2008). The evaluation of recombinant hookworm antigens as vaccines in hamsters (Mesocricetus auratus) challenged with human hookworm, Necator americanus. *Experimental Parasitology* **118**: 32-40. https://doi.org/10.1016/j.exppara.2007.05.010

Xue, L.C., Rodrigues, J.P., Kastritis, P.L., Bonvin, A.M. & Vangone, A. (2016). PRODIGY: A web server for predicting the binding affinity of protein–protein complexes. *Bioinformatics* **32**: 3676-3678. https://doi.org/10.1093/bioinformatics/btw514

Zhan, B., Liu, S., Perally, S., Xue, J., Fujiwara, R., Brophy, P., Xiao, S., Liu, Y., Feng, J., Williamson, A. *et al*. (2005). Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm Ancylostoma caninum. *Infection and Immunity* **73:** 6903-6911. https://doi.org/10.1128/IAI.73.10.6903-6911.2005