Giardiasis is one of the most common gastrointestinal infections worldwide, mainly in developing countries. The etiological agent is the *Giardia lamblia* parasite. Giardiasis mainly affects children and immunocompromised people, causing symptoms such as diarrhea, dehydration, abdominal cramps, nausea, and malnutrition. In order to develop an effective vaccine against giardiasis, it is necessary to understand the host-*Giardia* interactions, the immunological mechanisms involved in protection against infection, and to characterize the parasite antigens that activate the host immune system. In this study, we identify and characterize potential T-cell and B-cell epitopes of *Giardia* immunogenic proteins by immunoinformatic approaches, and we discuss the potential role of those epitopes to stimulate the host’s immune system. We selected the main immunogenic and protective proteins of *Giardia* experimentally investigated. We predicted T-cell and B-cell epitopes using immunoinformatic tools (NetMHCII and BCPREDS). Variable surface proteins (VSPs), structural (giardins), metabolic, and cyst wall proteins were identified as the more relevant immunogens of *G. lamblia*. We described the protein sequences with the highest affinity to bind MHC class II molecules from mouse (I-A^{k} and I-A^{q}) and human (DRB1*03:01 and DRB1*13:01) alleles, as well as we selected promiscuous epitopes, which bind to the most common range of MHC class II molecules in human population. In addition, we identified the presence of conserved epitopes within the main protein families (giardins, VSP, CWP) of *Giardia*. To our knowledge, this is the first *in silico* study that analyze immunogenic proteins of *G. lamblia* by combining bioinformatics strategies to identify potential T-cell and B-cell epitopes, which can be potential candidates in the development of peptide-based vaccines. The bioinformatics analysis demonstrated in this
study provides a deeper understanding of the Giardia immunogens that bind to critical molecules of the host immune system, such as MHC class II and antibodies, as well as strategies to rational design of peptide-based vaccine against giardiasis.

Keywords: immunogenic, epitope, protection, vaccine, immunoinformatic

INTRODUCTION

Giardiasis is a highly prevalent foodborne gastrointestinal parasitic infection in developing countries, mainly affecting children and immunocompromised individuals. The clinical manifestations of giardiasis vary from asymptomatic to acute or chronic episodes characterized by severe diarrhea, accompanied with abdominal pain and intestinal lesions that lead to nutrient malabsorption syndrome and weight loss (Eckmann, 2003; Cedillo-Rivera et al., 2009; Ankarklev et al., 2010; Lujan and Svard, 2011; Lopez-Romero et al., 2015). Giardia lamblia is the etiological agent of giardiasis, a binucleated and flagellated protozoan that can infect humans and other mammals. G. lamblia has a simple life cycle, consisting of two different developmental stages defined by specific structural and biochemical features, wherein the cyst is the infective form, whereas the trophozoite is the proliferative form that colonizes the upper tract of small intestine (Lujan, 2006; Cedillo-Rivera et al., 2009; Ankarklev et al., 2010; Lopez-Romero et al., 2015).

The establishment of endoparasitic infections rely on the intricate molecular interaction between each specific stage of the life cycle of parasites and the immune responses of their hosts (Tedla et al., 2019; Smith et al., 2021). Generally, the integration of innate and adaptive immune responses defines the fate of parasitic infections, therefore immunocompetence, immunopolymorphism and immunological memory of the host are important for the resolution of parasitic infections (Lima and Lodoen, 2019; Mukherjee et al., 2019).

Several studies have reported the central role of the immune system in resolution of giardiasis by using different experimental approaches (Li et al., 2004; Ankarklev et al., 2010; Kamda et al., 2012; Dreesen et al., 2014; Grit et al., 2014; Lopez-Romero et al., 2015; Singer, 2016). The mechanism of pathogen clearance mainly depend on the processes mediated by adaptive effector cells, both B and T lymphocytes. Murine models of giardiasis have demonstrated that the establishment of humoral immunity could be implicated in resolution of infection (Singer and Nash, 2000; Eckmann, 2003; Velazquez et al., 2005). In addition, the role of mucosal and circulatory CD4+ T cells has been described as essential to collaborate with the activation of B cells and control murine giardiasis (Singer and Nash, 2000; Lujan, 2011; Singer, 2016). Interestingly, whilst CD4+ T cells are important effectors in giardiasis resolution, CD8+ T lymphocyte responses have been associated to the pathophysiological damage observed during G. lamblia infection, such as enterocyte ultrastructural alterations, representing a paradoxical challenge for immunotherapy against giardiasis (Scott et al., 2004; Lopez-Romero et al., 2015).

The development of effective vaccines against endoparasites is limited, partially due to the complex life-cycle of parasites and the mechanisms that have acquired to successfully overcome some immune responses, such as antigenic variation, and partially to the limitations of classical vaccine design strategies (Skwarczynski and Toth, 2016; Lima and Lodoen, 2019; Moormann et al., 2019; Autheman et al., 2021; Robleda-Castillo et al., 2021). At present, there are no approved vaccines for human use against giardiasis. However, the presence of immunogenic proteins in both, cyst and trophozoite forms of G. lamblia have been described by different approaches. Among the proteins of G. lamblia able to elicit immune responses are the variable surface proteins (VSP), heat shock proteins, lectins, cyst wall proteins (CWP) and cytoskeleton associated proteins, such as giardins and tubulins (Davids et al., 2006; Lopez-Romero et al., 2017; Quintero et al., 2017).

Nowadays, synthetic peptide-based vaccines are designed considering immunodominance, epitope structure, and adjuvants to stimulate and confer protection without the complete protein or pathogen administration (Skwarczynski and Toth, 2016; Malonis et al., 2020). Immunoinformatic analysis have been used to identify immunogenic antigens from medically important protozoa, such as Leishmania, Trypanosoma, and Plasmodium, which have been implemented in multi-peptide vaccines with high efficacy for the control of infection. For the malaria infection, the Mosquirix™ vaccine is currently in Clinical Trial Phase III (Teh-Poot et al., 2015; Cecilio et al., 2017; Laurens, 2020; Vakili et al., 2020).

Immunoinformatic analysis allows the identification of potential B-cell and T-cell epitopes pursued for the design of new peptide-based vaccine candidates, by combining proteomics and bioinformatics strategies. Potential B-cell epitopes are considered according to their surface accessibility, flexibility and physicochemical characteristics to interact with complementarity-determining regions (CDRs) in the antibody molecule, whereas T-cell lineal peptide epitopes are predicted based on their high-affinity binding to the major histocompatibility complex (MHC) class I and II molecules (Teh-Poot et al., 2015; Goodswen et al., 2017; Robleda-Castillo et al., 2021).

The aim of this study was to identify T-cell and B-cell epitopes within the immunogenic proteins of G. lamblia that induce a potential protective response against giardiasis, using immunoinformatic strategies (Figure 1). In addition, we analyzed and discussed the potential role of those epitopes to stimulate the host’s immune system, providing candidates for the development of peptide-based vaccines.
MATERIALS AND METHODS

Search and Selection of *Giardia* Immunogenic Proteins

The identification and selection of immunogenic antigens from *Giardia* was performed on the scientific platform NCBI (PubMed: http://www.ncbi.nlm.nih.gov/pubmed/) by filtering the results to the last 30 years, using several keywords to identify the potential articles, including: *Giardia lamblia*, immunogenic proteins, protection, immune response, vaccine, variant-surface proteins (VSPs), giardins, and cyst wall proteins (CWPs). Scientific papers were selected based on their evaluations of the humoral and cellular immune response activation by *Giardia* antigens, as well as in the in vitro and in vivo protection assays. The identified *G. lamblia* immunogens were categorized according to their functionality and location in the parasite as reported in web site Uniprot (https://www.uniprot.org/) and as reported in publications. The access numbers of the selected immunogens were located in GenBank and GiardiaDB. BLASTp analysis was performed between the assemblages of each protein.

CD4+ T-Cell Epitope Prediction

For MHC-II-binding epitopes, 15-mer long epitopes for each protein were predicted using NetMHCIIpan 3.2 server (http://www.cbs.dtu.dk/services/NetMHCIIpan-3.2/). We selected for T-cell epitopes prediction, the murine MHC class II molecules I-A^k^ and I-A^d^. Those MHC molecules are expressed on the C3H/He and BALB/c mouse models, respectively, which are mouse strains frequently used in giardiasis studies (Belosevic et al., 1984; Venkatesan et al., 1997; Larocque et al., 2003; Lee et al., 2014; Serradell et al., 2019; Garzon et al., 2020). The HLA-DRB1*03:01 and HLA-DRB1*13:01 human MHC class II molecules were selected due to their probable association with susceptibility to infection (AL-Khaliq et al., 2020; El-Beshbishy et al., 2020). The proteins Hen Egg-white Lysozyme (HEL) and ovalbumin (Ova) were used as control antigens for the epitope prediction of MHC class II alleles (I-A^k^ and I-A^d^, respectively). The predicted peptides were classified as strong and weak binders with a threshold percentile rank (% Rank) ≤ 2% and ≤ 10%, respectively. The non-binder peptides (> 10% rank) were not considered in the study. In addition, we performed a host homology analysis. We analyzed the homology of peptides with human proteins sequence (*Homo sapiens*, taxid:9606) and mouse (*Mus musculus*, taxid:10090). The immunodominant protein sequences of *Giardia* were subjected to BLASTp against non-redundant protein sequences (nr) database (Altschul et al., 1990), and complemented with Dynamic Vaxign analysis (Xiang and He, 2009; He et al., 2010). A selection of T-cell and B-cell peptide epitopes were screened in the alignments to identify homologs.
A percentage identity > 35% was set as a filter to consider homology in each epitope (Pertsemidis and Fondon, 2001).

**Prediction of Promiscuous Peptides for MHC Class II Alleles**

The analyses of epitopes with promiscuous binding to a variety of MHC class II alleles permit a greater chance of the CD4+ T cells stimulation and allow to propose ideal epitopes for a clinically effective vaccine. The identification of T-cell epitopes with promiscuous binding to MHC class II alleles was determined with the Tepitool analysis resource from the IEDB (Paul et al., 2016) (http://tools.iedb.org/tepitool/). The predictions were done by using the consensus method (Wang et al., 2008; Wang et al., 2010) which employs SMM_align, NN_align, Combinatorial library, Sturniolo methods and NetMHCIIpan (Nielsen et al., 2008; Karosiene et al., 2013). A pre-selected reference panel of 26 alleles was employed and only the peptide epitopes binding at least 50% of the alleles were selected as promiscuous (Greenbaum et al., 2011). By default, Tepitool selects the epitopes with a percentile rank ≤ 20 as promiscuous. The input sequences of the proteins were those determined as the strongest binders for murine I-Á², I-Á¹ alleles, HLA-DRB1*03:01 and HLA-DRB1*13:01.

**B-Cell Epitope Prediction**

Linear/continuous B-cell epitopes for secreted or extracellular proteins were identified using BCPrep method in BCPreDS server which is based on support vector machine (SVM) that uses string kernels (http://ailab-projects1.ist.psu.edu:8080/BCPreDS/predict.html) (El-Manzalawy et al., 2008). We used the following parameters for prediction, 80% specificity and a cutoff score > 0.6. Epitopes with a length of 16-mer and 18-mer were selected for the study since most B-cell epitopes are between 15 to 25 long amino acids (Potocnakova et al., 2016), also better accuracy percentages are obtained with peptide windows of 16 amino acids in length (El-Manzalawy et al., 2008).

**Epitope Conservation Analysis**

To identify the percentage of conservation of the epitopes in the sequences of the proteins classified within the families, giardins, VSPs, and CWPs, the FASTA sequences of proteins were selected for a multi-alignment in T-coffee (https://www.ebi.ac.uk/Tools/msa/tcoffee/) and Boxshade webserver (https://embnet.vital-it.ch/software/BOX_form.html). The conservancies of strong T-cell epitope and B-cell epitopes previously predicted were identified by IEDB epitope conservancy analysis tool (http://tools.iedb.org/conservancy/). The conservancy of epitope sequence was assigned at > 60% for giardins and CWPs, and > 50% for VSPs. Every T-cell and B-cell epitopes that was filtered by the threshold, was subjected to cross-reactivity analysis (mouse and human).

**RESULTS**

**Giardia Immunogenic Proteins Selection**

To identify the *Giardia* immunogens, which have been described in the scientific literature, a screening search (last 30 years) of articles was performed. A total of 29 research articles were selected, wherein 29 proteins with potential high immunogenicity were reported (Table 1). The selected immunogens, mainly belong to WB and GS/M-83 -H7 strains, representative of *Giardia* A and B assemblages (genetic groups), respectively. The proteins presented a homology (id%) > 78% between assemblages, unlike for VSPs, due the expressed VSPs are different between the trophozoites of assemblages A and B. (Franzén et al., 2009). Proteins were classified based on their location and function (Figure 2). Out of the 29 immunogenic proteins identified, 3 proteins correspond to cyst wall proteins (CWP 1, CWP 2, and CWP 3), 11 proteins are structural proteins located mainly in the ventral disc and cytoskeleton, such as giardins, tubulin, SALP, 21.2 protein, and GHSP-115.

In addition, 5 proteins have metabolic functions in *Giardia*, such as arginine deiminase (ADI), ornithine carbamoyl transferase (OCT), fructose-bisphosphate aldolase (FBA), uridine phosphorylase (UPL), and enolase. Among the intracellular proteins, we also found the *Giardia* Trophozoite Antigens (GTA-1 and GTA-2) and the binding immunoglobulin protein (BIP). Other immunogens in the study correspond to 7 variants-specific surface proteins (VSPs). Most of the scientific papers (more than 90%) selected during the screening search performed in the present study were focused on evaluating the immunogenicity of *Giardia* proteins by analyzing the antibody-mediated immune response. Only a few have evaluated its ability to activate cellular immune responses. The immunological assays reported in those papers have been performed using human samples, and animal models susceptible to giardia infection (mice, gerbils, kittens, and puppies). Some of those articles have reported the protective capacity of certain immunogens, such as α-1 giardin, α-11 giardin, 21.2 protein, UPL-1, VSP9B10, VSP1267, VSPH7 and CWP 2 (Larocque et al., 2003; Palm et al., 2003; Serradell et al., 2018; Davids et al., 2019; Serradell et al., 2019).

**T-Cell and B-Cell Epitopes From Giardia Immunogenic Proteins**

The cellular and humoral immune responses have an important role in the clearance of giardiasis. CD4+ helper T lymphocytes are involved in the activation of the effector mechanisms against *Giardia*. CD4+ cells are activated by dendritic cells, as well as by B lymphocytes through the MHC II-peptide presentation, for this reason, we initially identified T-cell epitopes from *Giardia* immunogenic proteins. T-cell epitopes that had an affinity to the murine MHC class II I-Á² and I-Á¹ molecules, as well as to the human MHC class II HLA-DRB1*03:01 and HLA-DRB1*13:01 were identified. We used the NetMHCIIpan server for T-cell epitope prediction. Out of the 29 proteins that were subjected to prediction, a total of 354 strong binder peptides and 1,298 weak binder peptides were predicted (Figure 3). The subsequent analyzes were focused on strong peptides. We recorded the first 5 epitopes of each protein with the highest affinity to the MHC class II molecules I-Á², I-Á¹, HLA-DRB1*03:01 and HLA-DRB1*13:01 (Tables S1, S2). Then, we selected the 20 peptide epitopes with the strongest binding affinity to each MHC
### TABLE 1 | Immunogenic proteins of *Giardia lamblia*.

| No. | Protein | Assemblages | Id % | Location | Length (amino acids) | References |
|-----|---------|-------------|------|----------|----------------------|------------|
|     |         |             |      |          |                      |            |
|     | Structural proteins |             |      |          |                      | 1.α-1 gardin* | A 99% Ventral disc 295 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2006; Feliziani et al., 2011; Jenikova et al., 2011; Feng et al., 2016; Radunovic et al., 2017; Davids et al., 2019) |
|     |         | B           |      |          |                      |            |
|     |         |             |      |          |                      | 2.α-2 gardin* | A 81% Ventral disc 296 (Palm et al., 2003; Davids et al., 2019) |
|     |         | B           |      |          |                      | 3.α-7.1 gardin | A Ventral disc 295 (Palm et al., 2003; Tellez et al., 2005) |
|     |         |             |      |          |                      | 4.α-7.3 gardin | A Ventral disc 295 (Palm et al., 2003; Tellez et al., 2005) |
|     |         |             |      |          |                      | 5.α-11 gardin* | A Ventral disc 295 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2019) |
|     |         |             |      |          |                      | 6.β-gardin*  | A 100% Cytoskeleton 272 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2019) |
|     |         | B           |      |          |                      | 7. SALP-1     | A 99% Ventral disc 255 (Palm et al., 2003) |
|     |         | B           |      |          |                      | 8.21.1 protein* | A 95% Ventral disc 786 (Davids et al., 2019) |
|     |         |             |      |          |                      | 9.α-Tubulin   | A 100% Cytoskeleton 754 (Palm et al., 2003; Davids et al., 2006) |
|     |         | B           |      |          |                      | 10.β-Tubulin  | A Cytoskeleton 447 (Palm et al., 2003) |
|     |         |             |      |          |                      | 11. GHSP-115  | A Intracellular 1039 (Bae et al., 2009) |
|     |         |             |      |          |                      | Metabolic proteins |
|     |         |             |      |          |                      | 12. ADI*       | A 89% Intracellular 580 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2008) |
|     |         | B           |      |          |                      | 13. OCT*      | A 97% Intracellular 327 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2019) |
|     |         | B           |      |          |                      | 14. FBA*      | A 97% Intracellular 323 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2019) |
|     |         |             |      |          |                      | 15. UPL-1      | A 95% Intracellular 310 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2019) |
|     |         |             |      |          |                      | 16. Enolase*   | A 95% Intracellular 445 (Palm et al., 2003; Tellez et al., 2005; Davids et al., 2006; Jenikova et al., 2011) |
|     |         |             |      |          |                      | Variable-specific surface proteins |
|     |         |             |      |          |                      | 17. VSP9B10*   | A Membrane/Intracellular 739 (Palm et al., 2003; Rivero et al., 2010; Cabrera-Licona et al., 2017; Serradell et al., 2018; Serradell et al., 2019) |
|     |         |             |      |          |                      | 18. VSP1267*  | A Membrane/Intracellular 596 (Palm et al., 2003; Rivero et al., 2010; Cabrera-Licona et al., 2017; Serradell et al., 2018; Serradell et al., 2019) |
|     |         |             |      |          |                      | 19. VSP AS8   | A Membrane/Intracellular 616 (Hjøllo et al., 2018) |
|     |         |             |      |          |                      | 20. TSA 417   | A Membrane 713 (Reiner & Gillin, 1991; Palm et al., 2003; Rivero et al., 2010) |
|     |         |             |      |          |                      | 21. VSPH7*    | B Membrane 557 (Stäger et al., 1997; Stäger et al., 1998; Bierz et al., 2001; Bierz et al., 2003; Serradell et al., 2018) |
|     |         |             |      |          |                      | 22. VSP5      | B Membrane/Intracellular 171 (Hjøllo et al., 2018) |
|     |         |             |      |          |                      | 23. VSP5G8    | B Membrane 607 (Quintero et al., 2017; Garzon et al., 2020) |
|     |         |             |      |          |                      | Heat Shock Proteins |
|     |         |             |      |          |                      | 24. BIP       | A 99% ER/ESV 662 (Lee et al., 2014; Lopez-Romero et al., 2017) |
|     |         | B           |      |          |                      | 25. CWP 1      | A 88% ESV 241 (Lujan et al., 1995; Abdul-Wahid & Faubert, 2008; Ma’ayeh et al., 2017) |

(Continued)
class II molecule analyzed (Tables 2, 3). The strong binders showed a similar percentile rank to the main immunodominant epitope (48-63) of the hen egg-white lysozyme (HEL) (Nelson et al., 1992; Velazquez et al., 2002) and to the peptide (323-339) of ovalbumin (OVA) (McFarland et al., 1999) (Table S3). Both peptide sequences have a high affinity binding to I-Ak and I-Ad alleles, respectively. Due to the high affinity with MHC class II molecules and the capacity to activate the cellular immune response, the binding registers of HEL and OVA peptides have been highly characterized and used as study models (McFarland et al., 1999; Bevaart et al., 2004; Dissanayake et al., 2005; Lovitch and Unanue, 2005; Landais et al., 2009; Strong and Unanue, 2011). Several sequences of giardins, UPL-1, ADI, GTA-1 and enolase showed high binding affinity for murine and human MHC II alleles (Tables 2, 3). Additionally, a criterion for selection of T-cell epitopes was that they should be promiscuous. Since MHC class II alleles have different binding specificities, selection of peptides that bind to several MHC

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### TABLE 1 | Continued

| No. | Protein | Assemblages | Id % | Location       | Length (amino acids) | References                                                                 |
|-----|---------|-------------|------|-----------------|----------------------|----------------------------------------------------------------------------|
| 26  | CWP 2   | A           | 88%  | Cyst            | 362                  | (Lujan et al., 1995; Laroque et al., 2003; Abdul-Wahid & Faubert, 2008; Lee et al., 2009; Feng et al., 2016; Radunovic et al., 2017) |
|     |         | B           |      | Cyst            | 363                  |                                                                            |
| 27  | CWP3    | A           | 78%  | Cyst            | 247                  | (Lujan et al., 1995)                                                     |
|     |         | B           |      | Cyst            | 242                  |                                                                            |
| Others | GTA-1  | A           | 100% | Intracellular   | 181                  | (Palm et al., 2003)                                                      |
|      |         | B           |      | Intracellular   | 181                  |                                                                            |
| 29  | GTA-2   | A           | 95%  | Intracellular   | 225                  | (Palm et al., 2003; Davids et al., 2006)                                  |
|     |         | B           |      | Intracellular   | 225                  |                                                                            |

Id %: percentage identity between G. lamblia assemblages A and B (BLAST analysis).

Giardia immunogenic proteins present in the secretome.

Immunogenic proteins that induce protection against giardiasis.

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FIGURE 2 | Schematic representation of cellular localization of immunogenic proteins of G. lamblia. A total of 29 proteins have been reported as immunogenic antigens in the cyst and trophozoite of G. lamblia. Immunogenic proteins were classified based on their location and function in structural proteins, metabolic proteins, heat shock proteins (HSPs), variable-specific surface proteins (VSPs), and cyst wall proteins (CWPs). Proteins were located in ventral disc, plasma membrane, cytoskeleton, intracellular and secretome/extracellular of the parasite. CWPs can also be found in encystation-specific secretory vesicles (ESV).
variants can allow the designing of vaccines to achieve a broad allelic coverage and protect against infection. We used the cut-off values of Tepitool to be binding to \( \geq 50\% \) of the MHC class II alleles more frequently in the world population, we found that 26 peptide sequences were highly promiscuous epitopes (Table 4). These data were used as screening for subsequent analyzes.

B-cell linear epitopes of \textit{Giardia} immunogens were identified by using BCPRED tool. A total of 535 B-cell epitopes were

| Protein/Assemblage | Position | Epitope (15 mer) | Affinity (nM) | % Rank | Protein/Assemblage | Position | Epitope (15 mer) | Affinity (nM) | % Rank |
|--------------------|----------|-----------------|---------------|--------|--------------------|----------|-----------------|---------------|--------|
| 1 \( \alpha \)-11- giardin/A,B | 221 | IAHYNNLAPARAVAY | 3636.76 | 0.01 | UPL 1*/B | 236 | AVHMSAHAIALAQRK | 35.06 | 0.02 |
| 2 \( \alpha \)-2- giardin/A,B | 173 | YISSFMAGVPPEEYK | 4529.08 | 0.02 | Enolase/\( \alpha \),B | 2 | EAPSTIAKAKMII | 40.24 | 0.03 |
| 3 GHSP-115/A,B | 354 | LLNEAARALPLPSY | 4967.03 | 0.04 | UPL 1*/A | 236 | AVYMAHAIALAQRK | 40.3 | 0.03 |
| 4 ADI*/B | 381 | PTIDFKASPAYS | 5194.2 | 0.05 | \( \alpha \)-11- giardin/B | 223 | HFYNLAPARAVAYF | 40.47 | 0.03 |
| 5 \( \alpha \)-11- giardin/A | 224 | YHLLRGATAAAGRA | 5456.24 | 0.09 | \( \alpha \)-11- giardin*/A,B | 14 | QHLLRGATAAAGRA | 42.4 | 0.04 |
| 6 \( \alpha \)-7.1- giardin*/A,B | 14 | QHLLRGATAAAGRA | 4529.08 | 0.02 | GHSP-115/A,B | 354 | LLNEAARALPLPSY | 4967.03 | 0.04 |
| 7 GTA-1*/B | 100 | LEMLSAPNLMSA | 5487.86 | 0.1 | \( \alpha \)-7.1- giardin/A,B | 89 | SAKLMAAAKATEIK | 45.63 | 0.06 |
| 8 \( \alpha \)-11- giardin/B | 224 | FYNLAPARAVAYF | 5635.09 | 0.12 | \( \alpha \)-11- giardin*/A,B | 2 | EAPSTIAKAKMII | 40.24 | 0.03 |
| 9 UPL 1*/B | 236 | AVHMSAHAIALAQRK | 5650.66 | 0.12 | \( \alpha \)-11- giardin/A,B | 223 | HFYNLAPARAVAYF | 40.47 | 0.03 |
| 10 GHSP-115/A,B | 516 | SDELQAARAIAEAKL | 5886.75 | 0.17 | \( \alpha \)-7.1- giardin/A,B | 91 | KKLMAAAKATEKAL | 57.02 | 0.12 |
| 11 \( \beta \)-tubulin 1/A,B | 272 | PLSRGSQVYRAL | 5938.82 | 0.12 | GTA-1*/B | 100 | LEMLSAPNLMSA | 57.55 | 0.05 |
| 12 \( \beta \)-giardin/A,B | 135 | QIAHNDIAAALRKE | 6091.83 | 0.25 | GTA-2/B | 20 | WNEIRATKVMVES | 70.45 | 0.25 |
| 13 ADI*/B | 101 | KYEFHSGARITPKM | 6095.59 | 0.25 | \( \alpha \)-2- giardin/A,B | 176 | SFMAGVPPEEYKSN | 6227.85 | 0.3 |
| 14 \( \alpha \)-7.3- giardin/A,B | 31 | KORAEHAHAARATG | 6319.49 | 0.4 | BIP/\( \alpha \),B | 152/167 | EKTIAAATIPFAY | 70.8 | 0.25 |
| 15 \( \alpha \)-2- giardin/A,B | 31 | KORAEHAHAARATG | 6319.49 | 0.4 | ADI/B | 380 | QPTIDFKASPAYS | 71.52 | 0.25 |
| 16 BIP/A,B | 396 | DEAAVWGAAVOSIL | 6320.86 | 0.4 | GHSP-115/A,B | 518 | ELOAARAEKLLA | 72.82 | 0.25 |
| 17 CPA 1/A | 91 | YLSNLNSLAAPEG | 6432.84 | 0.4 | \( \alpha \)-tubulin 1/A,B | 326 | KDNNAAAVIKHTRT | 76.23 | 0.3 |
| 18 UPL 1*/A | 236 | AVHMSAHAIALAQRK | 6457.37 | 0.4 | ADI/B | 123 | YKRRYKLASSLRNLV | 78.29 | 0.4 |
| 19 ADI*/B | 381 | PTIDFKASPAYS | 6704.04 | 0.4 | \( \alpha \)-tubulin 1/A,B | 326 | KDNNAAAVIKHTRT | 76.23 | 0.3 |
| 20 GTA-2/A,B | 396 | NASYHCACAFQDSIR | 6686.76 | 0.5 | \( \alpha \)-7.1- giardin/A,B | 86 | RNSSAKLMAAAKAT | 79.17 | 0.4 |

The 20 epitopes with the highest affinity to I-A\( ^{\alpha} \) and I-A\( ^{\alpha} \) MHC class II were selected. Epitopes were organized according to % rank of affinity. Epitopes with conserved prediction with murine (*) and human (ǂ) MHC class II molecules.
TABLE 3 | Strong binder epitopes of *G. lamblia* to *HLA* class-II molecules.

| Protein/Assemblage | Position | Epitope (15 mer) | Affinity (nM) | % Rank |
|---------------------|----------|------------------|----------------|--------|
| 1 α-2- giardin/A  | 4        | LSQIADMKQAIDAK   | 24.31 0.03     |        |
| 2 α-2- giardin/B  | 4        | LSQIADMKQAIDAK   | 24.48 0.03     |        |
| 3 α- tubulin 1/A,B | 112      | KEIVDLVLDRVRKL   | 28.47 0.06     |        |
| 4 ADI/A,B         | 88       | EREULMGQALSKY    | 29.02 0.07     |        |
| 5 ADI/B           | 495      | SREGADVHKLYQKL   | 29.86 0.07     |        |
| 6 GHSP-115/A,B    | 847      | LARLRLRLDESPLA   | 30.95 0.08     |        |
| 7 FBA/B           | 249      | IKCVKINDSSRMAMT  | 31.77 0.09     |        |
| 8 FBA/A           | 249      | VCKINVDSRSSRAMT  | 32.4 0.1       |        |
| 9 CWP 1/B         | 60       | NINNIALDLSMSLT   | 36.35 0.15     |        |
| 10 α-11- giardin/B| 274      | WGVMRIDDISRFQSGK | 37.88 0.17     |        |
| 11 FBA/A,B        | 251      | KINVDSRSSRAMTGA  | 39.88 0.25     |        |
| 12 BIP/A,B        | 416/431  | HDVLIDVTPLTGLI   | 40.31 0.25     |        |
| 13 OCT/B          | 18       | KELMLDaVSRALMKK  | 41.1 0.25      |        |
| 14 CWP 1/A,B      | 60       | NINNIALDLSMSLT   | 42.32 0.25     |        |
| 15 BIP/A,B        | 102/117  | YKVINKDGRPPVQLS  | 42.34 0.25     |        |
| 16 α-11- giardin/A| 274      | WGVMRIDDISRFQSGK | 44.39 0.3      |        |
| 17 GHSP-115/A,B   | 153      | KAMISDHEKTALIUA  | 48.05 0.4      |        |
| 18 α-7.3- giardin/A,B | 64 | LMMVLVDDEEDVRCR  | 48.53 0.4      |        |
| 19 21.1 protein/A,B| 355      | NQAFFKVDLNLMSKT  | 49.96 0.4      |        |
| 20 α-7.1- giardin/A,B | 157 | LMMVLVDDEEDVRCR  | 50.29 0.5      |        |

The 20 epitopes with the highest affinity to *HLA* class-II DRB1*03:01 and DRB1*13:01 were selected.

Epitopes were organized according to % rank of affinity. Epitopes with conserved prediction with murine (*) and human (†) MHC class II molecules.

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### Giardins, VSPs, and CWPs Have Conserved T-Cell and B-Cell Epitopes

Among the immunogenic proteins identified on *Giardia*, there are three families highly characterized in the parasite, giardins, VSPs, and CWPs. It was of our interest to know whether those *Giardia* protein families conserved the predicted T-cells and B-cells epitopes. A multiple alignment of those three protein families was carried out and the epitopes that had > 60% conservation for the giardins and CWPs, and > 50% conservation for the VSPs were located. Giardins present 11 T-cell and 10 B-cell conserved epitopes (Table 6). The T-cell epitopes 3, 7, and 9 have amino acid residues shared with the B-cell epitopes 2, 6, and 4, respectively (Figure 4A).

Regarding the VSPs, we identified 5 T-cell and 6 B-cell conserved epitopes (Table 7), which are found at the C-terminal amino acid residues. The number 1 T-cell epitope was conserved in seven proteins. In addition, the T-cell epitope 3 was the only one that overlaps with the numbers 2 and 3 of B-cell epitopes (Figure 4B). In the CWP family, we identified 8 and 7 T-cell and B-cell conserved epitopes, respectively (Table 8). Several T-cell and B-cell epitopes overlap in CWPs as T-cell epitope 1 (159-173 aa) with B-cell epitope 4 (164-181 aa) (Figure 4C).

### DISCUSSION

Vaccine development has evolved over the years since Edward Jenner introduced the smallpox vaccine in 1796. Nowadays, in order to generate specific and safe vaccines with fewer side effects, extensive research is needed to design vaccines. In the initial phases, it is necessary to understand pathophysiology of infection, the pathogen-host relationship, as well as also to identify and characterize the immunodominant antigens that can generate immunity. Each research focused on those aspects supports the design of effective and safe vaccines for the population. At present, there is no vaccine for human giardiasis. Therefore, in this study, several T-cell and B-cell epitopes of *G. lamblia* immunogens were identified, which presented different immunogenic characteristics, some T-cell epitopes were promiscuous with strong binding affinity to MHC class II molecules, epitopes without homology to the hosts and conserved among protein families.
The *G. lamblia* antigens shown in Table 1 are molecules that have been experimentally characterized as immunogens. In addition to the physicochemical properties, other characteristics contribute to the immunogenicity of a molecule.

i) **Foreignness of the immunogen:** there must be a degree of phylogenetic difference between the candidate molecule and the host to avoid self-reactivity (Crumpton, 1974). In the present study, several amino acid regions of *G. lamblia* immunogens (tubulin, enolase, BIP, CWP and VSP) showed some degree of homology with human and mouse molecules, ubiquitous proteins in eukaryotic cells. Although, those peptides could potentially cause an autoimmune or allergenic reaction, suggesting the necessity to do additional studies to evaluate the safety of those predicted peptides.

ii) **Exposure to the immune system:** *Giardia* is a non-invasive parasite of the intestinal mucosa, therefore extracellular proteins play an important role in stimulating the immune system (Troeger et al., 2007; Cotton et al., 2011). The antigen location is crucial for easy recognition.
by the immune response, which is why surface proteins have been targets for vaccine development (Serradell et al., 2016; Abdi et al., 2019; Uwase et al., 2020). *Giardia* proteins that are located in the cytoskeleton, ventral disc, membrane, and proteins of secretome (proteins with an asterisk in Table 1) can have greater accessibility for the immune system, activating an efficient antibody-mediated response, as well as antigen uptake and presentation by antigen-presenting cells (Kaufmann and Hess, 1999; Foged et al., 2005; Mora and Telford, 2010). iii) Chemical stability and conservation of proteins: Giardins are a large group of structural proteins that are divided into alpha, beta, and gamma, there are 21 genes for alpha-giardins that are conserved in assemblages A and B (Feliziani et al., 2011). CWP 1, CWP 2, and CWP 3 have around 60% identity in a sequence of 7.3- giardin 94 TDTLLTTPYEYARK 0.977 No No
29 -Tubulin 57 VNCACNDKGDEKKRMRR 1.000 No No
30 OCT 243 WMSKHTKEOEKARL 0.978 Yes (37 %) Yes (37 %)
31 OCT 242 WMSKHTKEOEKARL 0.978 No No
32 UPL-1 48 VIKFRAPRPRFTTITG 0.971 No No

**Table 5** | Predicted B-cell immunodominant epitopes of *G. lamblia* proteins.

| Protein | Position | Epitope(16 or 18 mer) | Score | Human homology | Mouse homology |
|---------|----------|-----------------------|-------|----------------|---------------|
| 1 β-giardin | 238 | DREKAEKKEADKVKVN | 1.000 | No | No |
| 2 SALP-1 | 216 | NPAEDEAENAG | 1.000 | No | No |
| 3 21.1 protein | 24 | AIPPRAGSTNLAGDTG | 1.000 | No | No |
| 4 α-Tubulin | 438 | ETGDLGEDGEDMEEDA | 1.000 | No | No |
| 5 β-Tubulin | 430 | VDGEERVEEEEDFGDE | 1.000 | No | No |
| 6 GHSP-115 | 790 | SVQPSTTIVSEEGSD | 1.000 | No | No |
| 7 FBA | 274 | PEKFKDRPLGEPGDRD | 1.000 | No | No |
| 8 VSP1810 | 125 | AOGYVFPQADASHOS | 1.000 | No | No |
| 9 VSP1267 | 543 | TDGTSDNSGNIGDOTST | 1.000 | No | No |
| 10 VSP AS8 | 546 | CAPPAGSSPVTQCVYQQ | 1.000 | No | No |
| 11 TSA 417 | 113 | CTEAAPQYFAPVGAAN | 1.000 | No | No |
| 12 VSPH7 | 371 | ARAAPPGSTPDKTNGVCT | 1.000 | No | No |
| 13 VSP5 | 114 | SCAPPPTPPGPPVTQY | 1.000 | Yes (37 %) | No |
| 14 VSP5G8 | 505 | CATCTTAATCTCSTQAD | 1.000 | Yes (37 %) | Yes (37 %) |
| 15 BIP | 488 | LNDIPPRSTPQEVTF | 1.000 | Yes (83 %) | Yes (83 %) |
| 16 VSPG 564 | 24 | SYCSWGTSCDSNNVN | 0.992 | No | No |
| 17 α-11- giardin | 8 | PEKVAILEAKNESSV | 0.999 | No | No |
| 18 Enolase 6 CO | 220 | QDEGFGAPNFADPEVP | 0.998 | Yes (56 %) | Yes (56 %) |
| 19 CWP 1 | 29 | YDATDGANWKNLWLS | 0.998 | No | No |
| 20 α-1.1- giardin | 57 | TYSPPRTTRARCGK | 0.997 | No | No |
| 21 ADI | 80 | VLSEASPAERVLMDQ | 0.996 | No | No |
| 22 CWP 2 | 29 | YDATDGANWKNLWLS | 0.996 | No | No |
| 23 CWP 2 | 48 | SYCSWGTSCDSNNVN | 0.992 | No | No |
| 24 α-1- giardin | 148 | RVSPPGSPDEAQRLD | 0.991 | No | No |
| 25 CWP 3 | 25 | FYSDTSGANWMPNWNL | 0.987 | No | No |
| 26 OCT | 243 | MSYHTKEOEKARL 0.985 | No | No |
| 27 OCT | 242 | WMSKHTKEOEKARL 0.978 | Yes (37 %) | Yes (37 %) |
| 28 α-2- giardin | 235 | VNCACNDKGDEKKRMRR | 0.977 | No | No |
| 29 α-7.3- giardin | 94 | TDTLLTTPYEYARK | 0.977 | No | No |
| 30 CWP 3 | 25 | QFYSDTSGANWKLNNW | 0.975 | No | No |
| 31 GTA-1 | 162 | RSIIRLCPPVSDAEVEVE | 0.974 | No | No |
| 32 UPL-1 | 48 | VIKFRAPRPRFTTITG | 0.971 | No | No |

Bold and underline letters correspond to dipeptide regions related to activation of specific-isotype antibody response.

*Giardia* immunogens has mainly focused on IgA and IgG humoral response, perhaps due to the accessibility and feasibility of *in vitro* immunological assays, together with the evaluation in experimental animals. Infected mice with *G. lamblia* have demonstrated the establishment of humoral immunity around the third to fifth week post-infection, which could be implicated in the resolution of infection (Singer and Nash, 2000; Eckmann, 2003; Velazquez et al., 2005). We identified 24 immunodominant B-cell epitopes from immunogenic proteins of *Giardia* by bioinformatic analysis. Several studies have demonstrated the high immunogenicity of excretory/secretory proteins of *Giardia* (Palm et al., 2003; Hanek et al., 2011; Jiménez et al., 2014). The metabolic proteins ADI, OCT, and enolase were recognized by serum from patients with acute giardiasis (Palm et al., 2003). In other microorganisms, the immunological role of those proteins has been evaluated. Enolase has a protecive role in candidiasis (Montagnoli et al., 2004). OCT activates an antibody response in *Streptococcus suis* infection, and it is involved in reducing pathogenicity factors (Wang et al., 2020). VSPs are highly expressed on the membrane of *Giardia* trophozoite and are involved in the antigenic variation of the parasite. Although the mechanisms that induce antigenic switching are unknown, it is hypothesized that anti-VSP antibodies could stimulate the VSP switching. Several studies indicate the high effectiveness of VSPs to activate an antibody-mediated response in infected humans and animals (Stäger et al., 1998; Hjollo et al., 2018; Serradell et al., 2018), as well as the effector mechanisms of anti-VSP antibodies...
against trophozoites, such as cytotoxicity, opsonization, and neutralization (Nash and Aggarwal, 1986; Stäger et al., 1997; Rivero et al., 2010).

The clearance of *Giardia* infection requires humoral and cellular immune mechanisms. In *Giardia*, there is little research focused on characterizing the cellular response, however, it is known that CD4+ T lymphocytes play an important role in infection. CD4-deficient mice treated with an anti-CD4 antibody could not clear the infection, as well as CD4+ T cells deficiency is related to chronic giardiasis (Heyworth et al., 2010).

### Table 6 | Epitope conservation of giardins family.

#### T-cell epitope

| Predicted epitope          | Protein match | Epitope sequence | Position | Identity (%) | Host-homology >35% |
|---------------------------|---------------|------------------|----------|--------------|---------------------|
|                           | α-11 giardin/A | IAHYNLAPARAVAY     | 221-235  | 100          | No                  |
|                           | α-11 giardin/B | IAHYNLAPARAVAY     | 221-235  | 100          | No                  |
|                           | α-11 giardin/A | WGVMDIDIISSRFQSK   | 274-288  | 93.33        | No                  |
|                           | α-11 giardin/B | WGVMDIDIISSRFQSK   | 274-288  | 100          | No                  |
|                           | ε-7.1 giardin  | GQRAEHAAFRAGQ      | 124-138  | 80           | No                  |
|                           | ε-7.3 giardin  | GQRAEHAAFRAGQ      | 31-45    | 100          | No                  |
|                           | α-7.1 giardin  | LMMVLDEIDVRRC      | 157-171  | 93.33        | No                  |
|                           | α-7.3 giardin  | LMMVLDEIDVRRC      | 64-78    | 100          | No                  |
|                           | α-1 giardin    | YLIDFFGTVPSEYR     | 173-187  | 100          | No                  |
|                           | α-2 giardin/B  | YLIDFFGTVPSEYR     | 173-187  | 100          | No                  |
|                           | α-1 giardin    | KHYAKFYCDMGTIE     | 263-277  | 60           | No                  |
|                           | α-2 giardin/A  | KHYAKFYCDMGTIE     | 263-277  | 100          | No                  |
|                           | α-2 giardin/B  | KHYAKFYCDMGTIE     | 263-277  | 66.67        | No                  |
|                           | α-1 giardin    | DEKRMRRTIMMVVOK     | 244-258  | 100          | No                  |
|                           | α-2 giardin/A  | DEKRMRRTIMMVVOK     | 244-258  | 100          | No                  |
|                           | α-2 giardin/B  | DEKRMRRTIMMVVOK     | 244-258  | 100          | No                  |
|                           | α-1 giardin    | HYGNLAKDIRMTNSK     | 268-282  | 93.33        | No                  |
|                           | α-1 giardin    | RPIAEARKQNGKSI      | 187-201  | 100          | No                  |
|                           | α-2 giardin/A  | RPIAEARKQNGKSI      | 187-201  | 100          | No                  |
|                           | α-2 giardin/B  | RPIAEARKQNGKSI      | 187-201  | 100          | No                  |
|                           | α-11 giardin/A | VVLATPDERKLQAQ      | 97-111   | 100          | No                  |
|                           | α-11 giardin/B | VVLATPDERKLQAQ      | 97-111   | 93.33        | No                  |

#### B-cell epitope

| Predicted epitope          | Protein match | Epitope sequence | Position | Identity (%) | Host-homology >35% |
|---------------------------|---------------|------------------|----------|--------------|---------------------|
|                           | α-1 giardin    | RVSRRPGSPEDEAQLRD| 143-163  | 100          | No                  |
|                           | α-2 giardin/B  | RVSRRPGSPEDEAQLRD| 143-163  | 93.75        | No                  |
|                           | α-1 giardin    | INCAONDKGDREKRMR | 235-250  | 100          | No                  |
|                           | α-2 giardin/A  | INCAONDKGDREKRMR | 235-250  | 93.75        | No                  |
|                           | α-2 giardin/B  | INCAONDKGDREKRMR | 235-250  | 100          | No                  |
|                           | α-1 giardin    | AKASYAGKELPDIKK  | 39-56    | 100          | Yes (61%)          |
|                           | α-2 giardin/A  | AKASYAGKELPDIKK  | 39-56    | 72.22        | No                  |
|                           | α-2 giardin/B  | AKASYAGKELPDIKK  | 39-56    | 66.67        | No                  |
|                           | α-1 giardin    | AEFAKNGKSIQAIAT  | 190-207  | 100          | No                  |
|                           | α-2 giardin/B  | AEFAKNGKSIQAIAT  | 190-207  | 100          | No                  |
|                           | α-1 giardin    | AFCRSARINNAOQDAELK| 236-253  | 100          | No                  |
|                           | α-2 giardin/A  | AFCRSARINNAOQDAELK| 143-160  | 94.44        | No                  |
|                           | α-2 giardin/B  | AFCRSARINNAOQDAELK| 143-160  | 94.44        | No                  |
|                           | α-1 giardin    | AEYAAFRANGKTASEY  | 127-144  | 100          | No                  |
|                           | α-3 giardin    | AEYAAFRANGKTASEY  | 127-144  | 100          | No                  |
|                           | α-7.3 giardin  | AHDFAAFAATGKTSETSEY| 34-51   | 83.33        | No                  |
|                           | α-7.3 giardin  | AHDFAAFAATGKTSETSEY| 34-51   | 83.33        | No                  |
|                           | α-1 giardin    | ALCCONATLHCPARGAAY| 309-326  | 100          | No                  |
|                           | α-3 giardin    | ALCCONATLHCPARGAAY| 216-233  | 100          | No                  |
|                           | α-7.3 giardin  | ALCCONATLHCPARGAAY| 216-233  | 100          | No                  |
|                           | α-7.3 giardin  | ALCCONATLHCPARGAAY| 216-233  | 100          | No                  |
|                           | α-7.3 giardin  | ALCCONATLHCPARGAAY| 216-233  | 100          | No                  |

Red letters correspond to amino acids residues other than the predicted epitope.

Bold and underline letters correspond to dipeptide regions related to activation of specific-isotype antibody response.
In this study, 26 epitopes are proposed to activate CD4+ cells due to their high affinity to several MHC class II molecules. First, four MHC class II alleles were chosen, the MHC class II I-A^k and I-Ad that are expressed in mice widely used as model for giardiasis, as well as the HLA-DRB1*03:01 and HLA-DRB1*13:01 alleles that are related to an increased risk of *G. lamblia* infection (AL-Khaliq et al., 2020; El-Beshbishi et al., 2020). MHC class II molecules are expressed in dendritic cells and B lymphocytes, which are chemotactically attracted by trophozoite-stimulated epithelial cells (Roxström-Lindquist et al., 2005). Dendritic cells pre-stimulated with *Giardia* antigens can confer IL-6-dependent protection, which has been related to B-lymphocyte growth and T-cell differentiation (Weaver et al., 2006; Kamda et al., 2012). Proinflammatory chemokines, including TNF-α, and B lymphocyte activating interleukins, such as IL-4 and IL-5 belong to the chemokine profile described in *Giardia* infection (Cotton et al., 2015; Serradell et al., 2018). Additionally, an increase in IL-17 producing CD4+ cells from infected patients with *Giardia* (Saghaug et al., 2015). Interleukin IL-17 has been associated with IgA production and infection control (Dann et al., 2015). Although we focused the analysis on the strong epitopes classified by the NetMHCII algorithm, we did not disregard sequences with low affinity to MHC class II for future tests, due peptides with a low binding affinity can activate effective T-cell response, as the HEL 20-35 peptide (Nelson et al., 1992; Velazquez et al., 2002).

In this study, we identified conserved epitopes among giardins, VSPs, and CWPs. T-cell and B-cell epitopes overlap in some amino acid residues. Responses between B and T cells are closely linked for the development of an effective immune response. B cells as an antigen presenting cell can recognize antigens through the BCR, as well as present T-dependent antigens through the binding of peptides to MHC class II molecules. T-helper cells recognize peptide-MHC class II sequences.
complex and send activation signals to the B cell (Shimoda and Koni, 2007; Akkaya et al., 2019). Those pathways promote processes such as the isotype switch, affinity maturation and immunological memory, necessary in the development of protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses. Does it remain to be evaluated whether these epitopes can be generated naturally? Are they resistant to antigen processing? if the antibodies generated are protective immune responses.

The proteins described in this study have been proven to be immunogenic, however, only few of them have been evaluated in protection assays. Prior to consider an immunogen as a vaccine candidate, it is crucial to demonstrate its protective capacity by using experimental models. The proteins α-1 giardin, α-11 giardin, 21.2 protein, UPL-1, VSP9B10, VSP1267, VSPH7 and CWP 2 have shown to induce a protective immune response against infection by *G. lamblia* (Table 1) when administered orally or intraperitoneally. Mice and Mongolian gerbils were commonly used as animal models in protection assays, although

| Table 7 | Epitope conservation of VSP family. |
|---|---|
| **T- cell epitope** | | |
| Predicted epitope | Protein match | Epitope sequence | Position | Identity (%) | Host- homology >35% |
| | | | | | Human | Mouse |
| 1 | LSTGAIAQIVSVAAPV | VSP 5G8 | LSSTGAIAQIVSVAAPV | 574-588 | 80 | No |
| 2 | SRCNTGVVPINGQCA | VSP9B10 | SRCNTGVVPINGQCA | 51-65 | 100 | Yes (40%) |
| 3 | PVLCYLVQDSASWNLN | VSP 5G8 | PVLCYLVQDSASWNLN | 557-571 | 100 | No |
| 4 | VAVLQIARAACTPG | VSP 5G8 | VAVLQIARAACTPG | 8-22 | 100 | No |
| 5 | QAAQGYFVPPGADASHQS | VSP9B10 | QAAQGYFVPPGADASHQS | 123-137 | 100 | No |
| 6 | CATCCTAAGCTSTCAD | VSP 5G8 | CATCCTAAGCTSTCAD | 505-520 | 100 | Yes (37%) |
| 7 | AGQGYFVPPGADASHQS | VSP9B10 | AGQGYFVPPGADASHQS | 125-140 | 100 | No |
| 8 | SCAPTPPQGVTAYCV | VSP 5G8 | SCAPTPPQGVTAYCV | 547-562 | 100 | No |
| 9 | PGSTVCATPTGGTCT | VSP 5G8 | PGSTVCATPTGGTCT | 438-463 | 100 | Yes (37%) |
| 10 | KGATASDCTACPAGRA | VSP 5G8 | KGATASDCTACPAGRA | 329-347 | 100 | Yes (43%) |
| 11 | TDCAPGATVSGSGVSGS | VSPH7 | TDCAPGATVSGSGS | 272-287 | 100 | Yes (62%) |

*Red letters correspond to amino acids residues other than the predicted epitope. Bold and underline letters correspond to dipeptide regions related to activation of specific-isotype (IgG and IgA) antibody response.*
### TABLE 8 | Epitope conservation of CWP family.

#### T-cell epitopes

| Predicted epitope | Protein match | Epitope sequence | Position | Identity (%) | Host- homology >35% |
|-------------------|---------------|------------------|----------|--------------|---------------------|
|                   |               |                  |          |              | Human | Mouse |
| 1 LKEHLDCNQLTGDV | CWP 1/A       | LKEHLDCNQLSGTV  | 159-173  | 86.67        | Yes (60%) Yes (53%) |
|                   | CWP 1/B       | LKEHLDCNQLTGDV  | 159-173  | 100          | Yes (60%) Yes (60%) |
|                   | CWP 2/A       | LKEHLDCNELLGDV  | 159-173  | 93.33        | Yes (53%) Yes (40%) |
|                   | CWP 2/B       | LKEHLDCNELLGDV  | 159-173  | 93.33        | Yes (53%) Yes (40%) |
| 2 YLSNNSLAGAIPGL | CWP 1/A       | YLSNNSLAGAIPGL  | 91-105   | 100          | Yes (53%) Yes (53%) |
|                   | CWP 1/B       | YLSNNSLAGAIPGL  | 91-105   | 73.33        | Yes (46%) Yes (40%) |
|                   | CWP 2/A       | YLSNNSLAGAIPGL  | 91-105   | 66.67        | Yes (46%) Yes (46%) |
|                   | CWP 2/B       | YLSNNSLAGAIPGL  | 91-105   | 73.33        | Yes (46%) Yes (46%) |
| 3 DLSDMSTGAIPENI | CWP 1/A       | DLSDMSTGAIPENI  | 67-81    | 86.67        | Yes (46%) Yes (46%) |
|                   | CWP 1/B       | DLSDMSTGAIPENI  | 67-81    | 100          | No Yes (64%) |
|                   | CWP 2/A       | DLSDMSTGAIPENI  | 67-81    | 73.33        | No Yes |
|                   | CWP 2/B       | DLSDMSTGAIPENI  | 67-81    | 80           | No No |
| 4 LTNLOYLIINKAGLT| CWP 1/A       | LTNLOYLIINKAGLT | 108-122  | 86.67        | No Yes (46%) |
|                   | CWP 1/B       | LTNLOYLIINKAGLT | 108-122  | 100          | Yes (40%) (40%)
|                   | CWP 2/A       | LTNLOYLIINKAGLT | 108-122  | 80           | Yes (53%) Yes (43%) |
|                   | CWP 2/B       | LTNLOYLIINKAGLT | 108-122  | 80           | No Yes (46%) |
| 5 IPECICDLTHMMFWY| CWP 1/A       | IPECICDLTHMMFWY | 125-139  | 80           | No No |
|                   | CWP 1/B       | IPECICDLTHMMFWY | 125-139  | 80           | No No |
|                   | CWP 2/A       | IPECICDLTHMMFWY | 125-139  | 100          | No No |
|                   | CWP 2/B       | IPECICDLTHMMFWY | 125-139  | 100          | No No |
| 6 IEIGYGLADAOHDL | CWP 3/A       | IEIGYGLADAOHDL  | 8-22     | 66.67        | No No |
|                   | CWP 3/B       | IEIGYGLADAOHDL  | 9-23     | 100          | No No |
| 7 WKSNNWLADVSYCS | CWP 2/A       | WKSNNWLADVSYCS  | 37-51    | 86.67        | No No |
|                   | CWP 2/B       | WKSNNWLADVSYCS  | 37-51    | 100          | Yes (53%) No |
| 8 GNARSRSAVARPTARA| CWP 2/A      | GNARSRSAVARPTARA | 321-335  | 100          | No No |
|                  | CWP 2/B       | GNARSRSAVARPTARA | 321-335  | 73.33        | No No |

#### B- cell epitope

| Predicted epitope | Proteins match | Epitope sequence | Position | Identity (%) | Host- homology >35% |
|-------------------|---------------|------------------|----------|--------------|---------------------|
|                   |               |                  |          |              | Human | Mouse |
| 1 YDATDGANWKTNWLS | CWP 1/A       | YDATDGANWKTNWLS  | 29-44    | 100          | No No |
|                   | CWP 1/B       | YDATDGANWKTNWLS  | 29-44    | 93.75        | No No |
|                   | CWP 2/A       | YDATDGANWKTNWLT  | 29-44    | 81.25        | No No |
|                   | CWP 2/B       | YDATDGANWKTNWL.A | 29-44    | 81.25        | No No |
|                   | CWP 3/A       | YDATDGANQMPNWLQ  | 26-41    | 75           | No No |
|                   | CWP 3/B       | YDATDGANQMPNWLQ  | 27-42    | 81.25        | No No |
| 2 SYCSWTGTDSDNINV | CWP 1/A       | SYCSWTGTDSDNINV  | 47-62    | 62.50        | No No |
|                   | CWP 2/A       | SYCSWTGTDSDNINV  | 47-62    | 100          | No No |
|                   | CWP 2/B       | SYCSWTGTDSDNINV  | 47-62    | 100          | No No |
|                   | CWP 3/B       | DYCWCWTVGSCDNDINV | 45-60    | 68.75        | No No |
| 3 LOINNAGLTGDIPEC | CWP 1/A       | LOINNAGLTGDIPEC  | 114-129  | 81.25        | No Yes (40%) |
|                   | CWP 1/B       | LOINNAGLTGDIPEC  | 114-129  | 81.25        | Yes (37%) Yes (37%) |
|                   | CWP 2/A       | LOINNAGLTGDIPEC  | 114-129  | 100          | Yes (43%) Yes (37%) |
|                   | CWP 2/B       | LOINNAGLTGDIPEC  | 114-129  | 100          | Yes (50%) Yes (37%) |
| 4 LDQCQLTGDVPgLMTLP | CWP 1/A    | LDQCQLTGDVPgLMTLP | 164-181  | 88.89        | Yes (61%) Yes (44%) |
|                   | CWP 1/B       | LDQCQLTGDVPgLMTLP | 164-181  | 100          | Yes (61%) Yes (44%) |
|                   | CWP 2/A       | LDQCQLTGDVPgLMTLP | 164-181  | 72.22        | Yes (44%) No |
|                   | CWP 2/B       | LDQCQLTGDVPgLMTLP | 164-181  | 72.22        | Yes (44%) No |
| 5 TTDCDYCTALPPTNCPPTT | CWP 1/A  | TTDCDYCTALPPTNCPPTT | 210-227  | 50           | Yes (38%) Yes (39%) |
|                   | CWP 1/B       | TTDCDYCTALPPTNCPPTT | 210-227  | 61.11        | No No |
|                   | CWP 2/A       | TTDCDYCTALPPTNCPPTT | 211-228  | 100          | No No |
|                   | CWP 2/B       | TTDCDYCTALPPTNCPPTT | 211-228  | 94.44        | No No |
| 6 AGCSNHONCNOCERXTCC | CWP 3/A  | AGCSNHONCNOCERXTCC | 205-220  | 75           | No No |
|                   | CWP 3/B       | AGCSNHONCNOCERXTCC | 206-221  | 100          | No No |
| 7 CNARSGNOCOKAKSNMN | CWP 2/A       | CNARSGNOCOKAKSNMN | 252-269  | 100          | No No |
|                   | CWP 2/B       | CNARSGNOCOKAKSNMN | 252-269  | 100          | No No |

Red letters correspond to amino acids residues other than the predicted epitope. 
Bold and underline letters correspond to dipeptide regions related to activation of specific-isotype (IgG and IgA) antibody response.
other Giardia-susceptible animals, such as cats and dogs have also been used (Serradell et al., 2016). Currently, there is no human or dog effective vaccine against G. lamblia. In 1999, Fort Dodge Animal Health developed a vaccine based on killed disrupted trophozoites (GiardiaVax), which attenuated giardiasis symptoms, produced antibodies, and reduced the shedding cysts to 30% and 5% in vaccinated kitten and puppies respectively (Olson et al., 2000). GiardiaVax was also tested on Meriones unguiculatus, showing protection in 33% of the mice at the third day post-infection, the rest of the vaccinated group cleared the infection at seventh day (Jiménez-Cardoso et al., 2002). However, other studies differ in the vaccine efficacy. It was reported that the Giardia parasite persisted by week 28 in vaccinated cats with three doses of GiardiaVax (Stein et al., 2003), as well as in dogs, no differences were found in the elimination of cysts between the control and vaccinated group (Anderson et al., 2004). First-generation vaccines, such as the whole pathogen vaccine are characterized by generating no or low cell-mediated response and can also generate adverse effects such as hypersensitivity (Jiskoot et al., 2019). In recent years, protection strategies for clinically relevant pathogens have been focused on the peptide- and epitope-based vaccines (Table S4). Initially, in silico analysis facilitate the identification of T- and B-cell epitopes, and which can significantly reduce time and cost of research. Peptide-based vaccines can generate an effective and targeted immune response if the proper adjuvants and delivery system are considered. In gastrointestinal infections such as giardiasis several mucosal adjuvants can be used, such as choleric toxin, which increase the permeability of the intestinal epithelium promoting the antigen-uptake by immune cells (Rhee et al., 2012).

Validation strategies for the effectiveness of a peptide-based vaccine can be completed with additional in silico and experimental assays. In several viral pathogens, IFN-γ response activation is evaluated, due to the importance of this cytokine in effector mechanisms. Additionally, 3-D modeling and molecular docking are performed for the multi-epitope vaccine constructs. All subunit- and epitope-based vaccines shown in Table S4 have high protection efficacy in their respective diseases, as well as induced specific humoral and cellular responses. The advances in vaccines design of parasites show methodological strategies for the antigens characterization that can be implemented in Giardia studies. Likewise, Giardia shares some characteristics with other protozoa. Giardia presents antigenic variation, characteristic of the differential expression of variable surface proteins (VSP). Plasmodium and Trypanosoma are other parasites that express variable surface antigens (Borst and Ulbert, 2001; Kyes et al., 2007). Heat shock proteins are highly conserved molecules, in Leishmania which have been described as immunomodulatory proteins as well as have been used as components of vaccines (Lopez-Romero et al., 2017). Although there is little research on the immunological characterization of Giardia HSPs, studies have described the BIP protein as an immunogenic protein (Lee et al., 2014). We believe that more studies are needed to analyze the similarities among immunogenic antigens of Giardia and other pathogens, as well as the immune responses that may activate.

Our study is restricted by limiting immunoinformatic analyses to G. lamblia immunogenic proteins. At present, proteins are the molecules most characterized at the immunological level, however, different types of antigens may contribute to elicit immune responses during G. lamblia infection. Trophozoites of G. lamblia are able to activate innate immune responses, such as the complement system through the lectin pathway, after the recognition of surface N-acetylglucosamine (GlcNAc) by mannose-binding lectin (MBL). Interestingly, specific surface glycoconjugate antigens, glycosylphosphatidylinositol (GPI) and lipophosphoglycan (LPG) have been described as important inducers of immune responses during parasitic infection with Trypanosoma spp, Leishmania spp, Plasmodium falciparum, Cryptosporidium y Entamoeba histolytica (Ropert and Gazzinelli, 2000; Priest et al., 2003; Wong-Baeza et al., 2010). Based on this information, it is necessary to address future analyses at the molecular basis under the immune responses elicited by GlcNAc and other glycoconjugate antigens present in trophozoites and cysts of G. lamblia, in addition, the immunogenic role of post-translational modifications, such as glycosylation in VSPs, should be fully analyzed.

The present study describes a global approach to the identification of immunodominant and protective antigens of Giardia, being the first study to determine potential T-cell and B-cell immunogenic epitopes predicted by immunoinformatic tools as candidates for a vaccine against Giardia infection. For effective vaccine development against Giardia, it is necessary to consider several factors: i) the inclusion of conserved and variable protein sequences from the most common Giardia assemblages in humans (A and B); ii) the activation of the immune mechanisms of innate and adaptive response, considering the relationship between the parasite, gut mucosal immune system, microbiota, and the tolerogenic environment; iii) the use of proper adjuvants; iv) administration routes to guarantee an immune response in mucosa. For future studies, in vitro and in vivo assays are required to verify the effectiveness and protective role of T-cell and B-cell epitopes in giardiasis. These results obtained in the present study suggest that experimental administration of a multi-epitope vaccine constructed on basis of immunoinformatic approach could provide an effective prophylactic strategy against Giardia.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

TG performed and is involved in immunoinformatic analyses, wrote the manuscript, and prepared all figures. GL-R and
DO-T performed in silico assays and analyzed the data. EA contributed to the writing and editing of the manuscript. AG-E contributed to the writing and editing of the manuscript. RR-Z contributed to the library searches and assembling relevant literature. CV designed and supervised the project, revised the manuscript, and was responsible for the funding. All authors contributed to the article and approved the submitted version.

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**REFERENCES**

Abdi, R. D., Dunlap, J. R., Gillespie, B. E., Ensermu, D. B., Almeida, R. A., and Dego, O. K. (2019). Comparison of Staphylococcus Aureus Surface Protein Extraction Methods and Immunogenicity. *Helioy 5* (10), e02528. doi: 10.1016/j.hellyon.2019.e02528

Abdul-Wahid, A., and Faubert, G. M. (2004). Similarity in Cyst Wall Protein (CWP) Trafficking Between Encysting Giardia Duodenalis Trophozoites and CWP-Expressing Human Embryonic Kidney-293 Cells. *Biochem. Biophys. Res. Commun.* 324 (3), 1069–1080. doi: 10.1016/j.bbrc.2004.09.167

Abdul-Wahid, A., and Faubert, G. (2008). Characterization of the Local Immune Response to Cyst Antigens During the Acute and Elimination Phases of Primary Murine Giardiasis. *Int. J. Parasitol.* 38 (6), 691–703. doi: 10.1016/j.ijpara.2007.10.004

Akaya, M., Kwak, K., and Pierce, S. K. (2019). B Cell Memory: Building Two submitted version. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

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Vertebrate and Invertebrate Digestive Tracts 11, 680555. doi: 10.3389/fcimb.2021.680555

Stäger, S., Feith, R., Gottstein, B., and Müller, N. (1997). Giardia Lamblia Variant Surface Protein H7 Stimulates a Heterogeneous Repertoire of Antibodies Displaying Differential Cytological Effects on the Parasite. Mol. Biochem. Parasitol. 85 (1), 113–124. doi: 10.1016/S0168-6651(96)02818-6

Stäger, S., Gottstein, B., Sager, H., Jungi, T. W., and Müller, N. (1998). Influence of Antibodies in Mother’s Milk on Antigenic Variation of Giardia Lamblia in the Murine Mother-Offspring Model of Infection. Infect. Immun. 66 (4), 1287–1292. doi: 10.1128/IAI.66.4.1287-1292.1998

Stein, J. E., Radecki, S. V., and Lappin, M. R. (2003). Efficacy of Giardia Vaccination in the Treatment of Giardiasis in Cats. J. Am. Vet. Med. Assoc. 222 (11), 1548–1551. doi: 10.2460/javma.2003.222.1548

Strong, B. S.I., and Unanue, E. R. (2011). Presentation of Type B Peptide-MHC Complexes From Hen Egg White Lysozyme by TLR Ligands and Type I IFNs Independent of H2-DM Regulation. J. Immunol. (Baltimore Md.: 1950) 187 (5), 2193–2201. doi: 10.4049/jimmunol.1100152

Sun, C. H. J., McCaffery, M., Reiner, D. S., and Gillin, F. D. (2003). Mining the Giardia Lamblia Genome for New Cyst Wall Proteins. J. Biol. Chem. 278 (24), 21701–21708. doi: 10.1074/jbc.M302023200

Tedia, M. G., Every, A. L., and Scheerlinck, J. P. Y. (2019). Investigating Immune Responses to Parasites Using Transgenesis. Parasit. Vectors 12 (1), 1–14. doi: 10.1186/s13071-019-3550-4

Teh-Poot, C., Tzec, R. J., Arjona, E., Martinez-Vega, P., Jesus Ramirez-Sierra, M., Rosado-Vallado, M., and Dumonteil, E. (2015). From Genome Screening to Creation of Vaccine Against Trypanosoma Cruzi by Use of Immunoinformatics. J. Infect. Dis. 211 (2), 258–266. doi: 10.1093/infdis/jiu418

Téllez, A., Palm, D., Weiland, M., Alemán, J., Winiecka-Krusnell, J., Linder, E., et al. (2005). Secretory Antibodies Against Giardia Intestinalis in Lactating Nicaraguan Women. Parasite Immunol. 27 (5), 163–169. doi: 10.1111/j.1365-3024.2005.00758.x

Troger, H., Joerg Epple, H., Schneider, T., Wahnschaffe, U., Ullrich, R., Burchard, Tedla, M. G., Every, A. L., and Scheerlinck, J. P. Y. (2019). Investigating Immune Responses to Parasites Using Transgenesis. Parasit. Vectors 12 (1), 1–14. doi: 10.1186/s13071-019-3550-4

Uwase, J., Chu, R., Kassegne, K., Lei, Y., Shen, F., Fu, H., et al. (2020). Immunogenicity Analysis of Conserved Fragments in Plasmodium Ovale Species Merozoite Surface Protein 4. Malaria J. 19 (1), 1–11. doi: 10.1186/s12936-020-01727-7

Vakili, B., Nezafat, N., Zare, B., Erfani, N., Akbari, M., Ghasemi, Y., et al. (2020). A New Multi-Epitope Peptide Vaccine Induces Immune Responses and Protection Against Leishmania Infantum in BALB/c Mice. Med. Microbiol. Immunol. 209 (1), 69–79. doi: 10.1007/s00430-019-00640-7

Velazquez, C., Beltran, M., Ontiveros, N., Rascon, L., Figueroa, D. C., Granados, A. J., et al. (2005). Giardia Lamblia Infection Induces Different Secretory and Systemic Antibody Responses in Mice. Parasite Immunol. 27 (9), 351–356. doi: 10.1111/j.1365-3024.2005.00793.x

Velazquez, C., Vidasly, I., Der Van Drift, K., Gross, M. L., and Unanue, E. R. (2002). Chemical Identification of a Low Abundance Lysozyme Peptide Family Bound to I-A K Histocompatibility Molecules. J. Biol. Chem. 277 (45), 42514–42522. doi: 10.1074/jbc.M203162009

Venkatapurapu, P., Finch, R. G., and Finnie, D. L. (1997). A Comparison of Mucosal Inflammatory Responses to Giardia Muris in Resistant B10 and Susceptible BALB/c Mice. Parasite Immunol. 19 (3), 137–143. doi: 10.1046/j.1365-3024.1997.00189.x

Wang, P., Sidney, J., Dow, C., Mothé, B., Sette, A., and Peters, B. (2008). A Systematic Assessment of MHC Class II Peptide Binding Predictions and Evaluation of a Consensus Approach. PloS Comput. Biol. 4 (4), e1000048. doi: 10.1371/journal.pcbi.1000048

Wang, P., Sidney, J., Kim, Y., Sette, A., Lund, O., Nielsen, M., et al. (2010). Peptide Binding Predictions for HLA DR, DP and DQ Molecules. BMC Bioinformatics 11 (1), 568. doi: 10.1186/1471-2105-11-568

Wang, Y., Yi, L., Sun, L. Y., Liu, Y. C., Wen, W. Y., Li, X. K., et al. (2020). Identification and Characterization of a Streptococcus Suis Immunogenic Ornithine Carbamoyltransferase Involved in Bacterial Adherence. J. Microbiol. Immunol. Infect. 53 (2), 234–239. doi: 10.1016/j.jmii.2018.05.004

Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavioli, M., and Murphy, K. M. (2006). Th17: An Effector CD4 T Cell Lineage With Regulatory T Cell Ties. Immunity 24 (6), 677–688. doi: 10.1016/j.immuni.2006.06.002

Weiland, M. E.L., Palm, J. E.D., Griffiths, W. J., McCaffery, J. M., and Svärd, S. G. (2003). Characterisation of Alpha-1 Giardin: An Immunodominant Giardia Lamblia Annexin With Glycosaminoglycan-Binding Activity. Int. J. Parasitol. 33 (12), 1341–1351. doi: 10.1016/S0020-7519(03)00201-7

Wong-Baeza, I., Alcántara-Hernández, M., Mancilla-Herrera, I., Ramírez-Saldivar, I., Arriaga-Pizano, L., Ferat-Osorio, E., et al. (2010). The Role of Lipopeptidophosphoglycan in the Immune Response to Entamoeba Histolytica. J. Biomed. Biotechnol. 2010, 254521. doi: 10.1155/2010/254521

Xiang, Z., and He, Y. (2009). Vaxign: A Web-Based Vaccine Target Design Program for Reverse Vaccinology. Proc. Vaccinol. 1 (1), 23–29. doi: 10.1016/j.provac.2009.07.005

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