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REVIEW

Revisiting the antigen markers of vector-borne parasitic diseases identified by immunomics: identification and application to disease control

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ABSTRACT

Introduction: Protein microarray is a promising immunomic approach for identifying biomarkers. Based on our previous study that reviewed parasite antigens and recent parasitic omics research, this article expands to include information on vector-borne parasitic diseases (VBPDs), namely, malaria, schistosomiasis, leishmaniasis, babesiosis, trypanosomiasis, lymphatic filariasis, and onchocerciasis.

Areas covered: We revisit and systematically summarize antigen markers of vector-borne parasites identified by the immunomic approach and discuss the latest advances in identifying antigens for the rational development of diagnostics and vaccines. The applications and challenges of this approach for VBPD control are also discussed.

Expert opinion: The immunomic approach has enabled the identification and/or validation of antigen markers for vaccine development, diagnosis, disease surveillance, and treatment. However, this approach presents several challenges, including limited sample size, variability in antigen expression, false-positive results, complexity of omics data, validation and reproducibility, and heterogeneity of diseases. In addition, antigen involvement in host immune evasion and antigen sensitivity/specificity are major issues in its application. Despite these limitations, this approach remains promising for controlling VBPD. Advances in technology and data analysis methods should continue to improve candidate antigen identification, as well as the use of a multiantigen approach in diagnostic and vaccine development for VBPD control.

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Immunoproteins; protein microarrays; parasitic diseases; serodiagnosis; vaccine candidates

1. Introduction

Vectors are living organisms that can transmit pathogens from person to person or from animals to humans. Most of these vectors are hematophagous arthropods, such as mosquitoes, ticks, sand flies, tsetse flies, and triatome bugs. Every year, there are more than 700,000 deaths globally caused by vector-borne diseases such as malaria, dengue, human African trypanosomiasis, leishmaniasis, Chagas disease, yellow fever, Japanese encephalitis, and onchocerciasis [1]. Vector-borne parasitic diseases (VBPDs) still play important roles in vector-borne infectious diseases and pose a serious threat to humankind globally [2,3]. These diseases include malaria, schistosomiasis, leishmaniasis, babesiosis, African trypanosomiasis, Chagas disease (American trypanosomiasis), onchocerciasis (river blindness), lymphatic filariasis and tungiasis. The disease, causative organism, vector, geographical distribution, and morbidity data are summarized in Table 1. The emergence of insecticide and drug resistance and ecological and climatic changes have aggravated the transmission and prevalence of VBPDs [3]. Despite the consistent efforts deployed in the control of malaria and other VBPDs, they remain major public health burdens worldwide, particularly in tropical and subtropical regions [4], in part due to the shortage of sensitive diagnostics and effective vaccines.

Over the past two decades, the development of genomics, proteomics and other 'omics' has led to a new generation of antigen discovery based on technologies such as functional genomics, immunomics, and systems biology [5,6]. The immunomic approach is a 21st century approach to vaccine development for complex pathogens [7]. This approach identifies diagnostic antigens in human pathogens, including vector-borne parasitic diseases. These antigens can be used for the development of diagnostic tools and vaccines [6]. The immunomic approach involves the identification of antigens expressed by a pathogen,

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Article highlights

- High -throughput (HTP) protein microarray is a promising approach for identifying biomarkers for vector-borne parasitic disease (VBPD) control.
- Antigen data were collected from articles published in English between April 2006 and December 2022 from PubMed and Web of Science using the following key words: 'immunomics,' 'immunoproteomics,' 'protein microarrays,' 'proteome array,' 'plasmodium,' 'leishmania,' 'tryromycin,' 'babesia,' 'antigens,' and 'vector-borne parasitic diseases.'
- A total of 192 published articles were screened, and the records were further sorted by the advanced key word 'antigens.' Seventy-seven records of protein microarray studies that focused on *Plasmodium*, *Schistosoma*, *Leishmania*, *Trypanosoma*, *Babesia*, and filaria worms were obtained.
- Although the immunomic approach has challenges, it enables the identification and/or validation of antigen markers for vaccine development, diagnosis, disease surveillance, and treatment.
- Advances in technology and data analysis methods are needed to improve candidate antigen identification. In addition, the combination of multiple antigens could be helpful for detecting antibodies against VBPD infections and developing vaccines for controlling VBPDs to accelerate elimination.

followed by the selection of the most promising antigens for further analysis. This approach has the potential to revolutionize the diagnosis and control of vector-borne parasitic diseases by identifying new diagnostic antigens and vaccine targets.

The procedure of generating a high-throughput (HTP) protein microarray for antigen discovery has been described in our previous studies [6,8,9] and normally includes the following steps: (1) collection of serum samples, (2) selection of target genes/open reading frames (ORFs), (3) polymerase chain reaction (PCR) amplification of ORFs and preparation of linearized vector, (4) cloning of target sequences, (5) protein expression and immunoreactivity detection of His-tagged proteins, and (6) antibody profiling. With regard to profiling serological responses, immunoreactive antigens are statistically compared between infection-resistant and infection-susceptible host groups to select vaccine targets. Several potential vaccine candidates have been identified and tested for vaccine development [10–14], whereas antigens for diagnostics might be selected by comparing different serum samples from cohorts of exposed and unexposed individuals. For example, a biomarker identification proteomic array was established containing 992 validated and predicted *S. haematobium* proteins, which were screened using serum and urine antibodies from populations with epidemics in Gabon, Tanzania, and Zimbabwe. Arrayed antigens that were IgG-reactive predicted to be diagnostically informative, were then evaluated by ELISA using the same samples used to probe arrays, and samples from individuals residing in a low endemicity setting. Finally, two of these antigens, Sh-TSP-2 and MS3_01370, were screened and they could be used as sensitive, specific, and field-deployable diagnostics to support

Table 1. Notable vector-borne parasitic diseases that threaten global health.

Disease	Causative agents	Vector/ intermediate host	Geographical distribution	Point Prevalence[#]	DALYs*
Malaria	*Plasmodium falciparum*, *P. vivax*, *P. malaria*, *P. knowlesi*, *P. ovale*	Mosquitoes: *Anopheles* spp. (n = 30–40), e.g. *A. gambiae*, *A. stephensi*	Sub-Saharan Africa, Southeast Asia, South America	180,715,775	46,437,811
Schistosomiasis	*Schistosoma* spp., e.g. *S. japonicum*, *S. intercalatum*, *S. haematobium*, *S. mansoni*, *S. mekongi*, *S. guineensis*	Freshwater snails: *Oncomelania hupensis*, *Biomphalaria glabrata*, *Bulinus truncatus*	Southeast Asia, Africa, South America	139,967,778	1,638,072
Onchocerciasis	*Onchocerca volvulus*	Blackflies: *Simulium damnosum*, *S. neavei*, *S. ochraceum*, *S. exiguum*, *S. oyapockense*	Sub-Saharan Africa, Central and South America	19,063,570	1,230,433
Lymphatic filariasis	Filarial nematodes: *Brugia malayi*, *B. timori*, *Wuchereria bancrofti*	Mosquitoes: *Aedes* spp., *Culex* spp., *Anopheles* spp., *Mansonia* spp., *Coquillettida juxtamansonia*	Africa, Asia, South America	71,852,335	1,628,649
Chagas disease	*Trypanosoma cruzi*	Triatomines: *Triatoma dimidiate*, *Rhodnius prolixus*, *T. gerstaeckeri*, *T. infestans*, *Paratriatoma hirsute*,	Central and South America	6,469,284	275,377
Leishmaniasis	*Leishmania donovani*, *L. major*, *L. infantum*, *L. tropica*	Sand flies: *Phlebotomus sinensis*	Middle East, Central and South America, Asia, Southern Europe, East Africa	4,575,092	696,703
African Trypanosomiasis	*Trypanosoma brucei gambiense*, *T. b. rhodesiense*	Tsetse fly: *Glossina morsitans*, *G. fuscipes*, *G. palpalis*, *G. tachinoides*	Sub-Saharan Africa	3,768	82,615
Babesiosis	*Babesia* spp., e.g. *Babesia microti*, *B. divergens*, *B. duncani*	Ticks: *Ixodes scapularis*, *I. ricinus*	Mainly in Northeast United States, Asia, Europe	2,418*	8*α

[#]Prevalence and DALY data were retrieved from the Global Burden Disease results tool: Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Results Seattle, United States: Institute for Health Metrics and Evaluation (IHME), 2020. Available from https://vizhub.healthdata.org/gbd-results/.

DALYs, disability-adjusted life years. * Data found were those of the United States of America only (Source: Centers for Disease Control and Prevention (CDC). Surveillance for babesiosis — United States, 2019. Annual Summary. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC, 2021).α Cases of deaths reported rather than DALYs.

schistosomiasis control and elimination initiatives, with particular focus on post-elimination surveillance [15].

HTP protein microarrays provide a unique opportunity to explore and analyze humoral immune responses to vector-transmitted organisms such as *P. falciparum* (Pf) [8,16–18], *P. vivax* (Pv) [19,20], *S. japonicum* [21–23], *S. mansoni* [24], *L. infantum* [25], *B. microti* [26–28], *T. cruzi* [29,30], and several other human parasitic pathogens, including *Echinococcus multilocularis*, *E. granulosus* [31], *Necator americanus* [32], and *Toxoplasma gondii* [33–35]. This study reviews the applications of protein microarrays in the identification and testing of candidate antigens associated with malaria, schistosomiasis, leishmaniasis, babesiosis, African trypanosomiasis, Chagas disease, onchocerciasis, and lymphatic filariasis. Advances in the validation of antigen markers and research priorities are discussed to suggest candidates for the rational development of effective diagnostics and vaccines. Finally, the application of the immunomic approach for VBPD control is also reviewed.

2. High-throughput protein microarrays and antigen identification/discovery

Protein chip arrays were first developed to study HTP gene expression, and commercial antibody screening on chip-size protein microarrays was performed approximately 25 years ago [36]. Protein chip 'fabrication' was improved from 2001–2006 to elucidate enzymatic analysis and protein interactions [37,38] and has become a suitable assay for determining the serodiagnosis of infectious diseases. The first human parasite-based HTP protein microarray was generated against serum from malaria-infected individuals harboring Pf antigens in 2006 [39], which provided a significant understanding of humoral immune responses to malaria parasites. Two years later, the approach was performed more efficiently by profiling antibody responses to Pf antigens [17]. This effort ultimately provided general knowledge on receptor – ligand interactions to inform immune-dominant antigens [6,9].

Protein microarray is an attractive alternative method, especially for high-throughput studies or studies that focus on members of protein families, as it represents an ideal high-throughput platform that can simultaneously study antibody responses to hundreds of proteins and provides insights into natural immunity development [40]. Moreover, proteomics offers a major advantage in the discovery of new biomolecular targets because of its ability to simultaneously characterize proteomes and subproteomes without any prior knowledge of the nature of proteins [41]. Overall, the HTP protein microarray is an up-and-coming research field that studies aspects of immune responses and identifies antigen candidates in conjunction with traditional and conventional approaches such as mass spectrometry, reverse immunogenetics, 2D gel electrophoresis, western blotting, and enzyme-linked immunosorbent assay (ELISA).

3. Identification of antigen markers of VBPDs using protein microarrays

Related articles were searched in the PubMed and Web of Science databases with the following keywords: 'immunomics' OR 'immunoproteomics' OR 'protein microarrays' OR 'proteome array' AND 'plasmodium' OR 'leishmania' OR 'babesia' OR 'trypanosoma' OR 'onchocerca' OR 'brugia' OR 'wuchereria.' A total of 192 published articles were screened, and the records were further sorted by the advanced key word 'antigens.' A total of 77 records of protein microarray studies focused on *Plasmodium*, *Schistosoma*, *Leishmania*, *Trypanosoma*, *Babesia*, and *Filaria* were obtained. The final criteria for selection were based on the following: (1) published research articles on pathogens causing VBPDs and (2) involved antigen identification via protein microarrays, while unrelated articles were excluded. Finally, a list of 64 articles published between 2006 and 2022 was retained. The VBPDs for which antigen markers have been identified and/or tested using protein microarrays include malaria, schistosomiasis, leishmaniasis, babesiosis, trypanosomiasis, onchocerciasis, and filariasis (Figure 1a,b).

3.1. Protein microarrays for identifying malaria antigens

Studies on *Plasmodium* have profiled antigen-specific antibodies associated with anti-disease immunity in Papua New Guinea-exposed children [42]. Other investigations have identified and validated serological biosignatures using serum samples from individuals from endemic areas in the western Kenyan highlands and at three field sites in Rourkela, Nadiad and Chennai, India, as well as in Yunnan Province, China [8,16,43]. Recently, a study revealed that natural protective antibody responses against

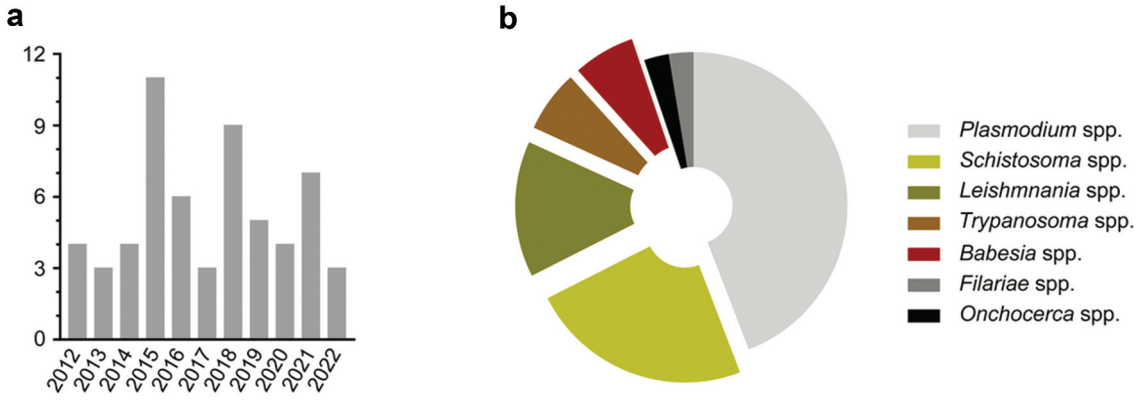

Figure 1. Immunomics-based studies related to vector-borne parasitic diseases (VBPDs). (A) Immunomic studies related to the identification of VBPD antigens from 2012 to 2022. (B) Study repartition of antibody responses to VBPDs via the immunomic approach from 2012 to 2022.

individuals experimentally challenged with Pf sporozoites were associated with new target candidates [44].

High-throughput protein microarrays have contributed to the development of numerous *Plasmodium* candidate antigens, as well as to the discovery of new antigens that were not identified by traditional methods [8,19,45–47]. In our previous studies [19,48], we identified 169 highly immunoreactive antigens, 12 of which were well-characterized Pv vaccine antigen candidates; the remaining 157 have not been reported previously. In a further study that profiled natural antibody responses to Pf antigens, we identified 30 highly immunogenic merozoite antigens, including 10 well-known Pf blood-stage vaccine candidates, and reported the first seven proteins and two hypothetical proteins associated with immunogenicity [8].

In a recent study, two new Pf antigens, star-related lipid transfer protein (START) and protein disulfide isomerase 8 (PDI8), were detected by immunomicIgGscreening and may be used to differentially diagnose subclinical malaria in children [49]. Peptide microarrays have also been applied to identify epitope candidates, which defined correlates of vaccine protection and measured strain-specific vaccine-induced antibodies [50]. Moreover, the Pf circumsporozoite protein (PfCSP) [51], Pf apical membrane antigen 1(PfAMA1) [52,53] and Pf merozoite surface proteins 1 and 3 (PfMSP1 and PfMSP3) [54,55] have been validated by protein microarrays and have undergone clinical development. Among the Pv antigens that have been validated by this approach, PvCSP, the leading vaccine candidate, has been assessed in clinical trials [56]. It is important to recall that in P. vivax infections, hypnozoites persist in the liver for long durations in dormant forms (latent liver stage) and remain undetectable with common diagnostics. Eight antigen markers capable of classifying individuals with vivax infections who may harbor hypnozoites were recently developed in malaria-endemic regions of Thailand, Brazil, and the Solomon Islands [57]. Nevertheless, two potential biomarker candidates, TF (serotransferrin) and HPX (hemopexin), were identified by the immunoproteomic approach for knowlesi malaria infection [58]. Plasmodium knowlesi was identified as the fifth major malaria parasite that regularly infects humans [59]. It can cause severe clinical symptoms and even lead to mortality as a result of hyperparasitemia in a short period of time. The study improved the current understanding of humoral immune responses to knowlesi malaria infection and identified potential biomarkers for *P. knowlesi* infection [58] (Table 2).

3.2. Protein microarrays for identifying schistosomiasis antigens

Schistosomiasis threat is classified second behind malaria among the VBPDs. Antigen screening for serodiagnostics and vaccines against the disease has also been intensively performed, and parasite-specific immunodominant targets have been identified for clinical development. Immunomics-based studies have validated a panel of potential antigens and have identified novel antigenic targets for schistosomiasis [11,21,22,24,72–74]. In a previous study that highlighted an integrated immunoproteomic approach for the analysis of *S. japonicum* tegument proteins, we reported for the first time 30 highly immunoreactive tegument antigens [22]. Several of the antigens that we validated showed strong immunogenicity (Table 3), of which the recombinant 28-kDa glutathione S-transferase of *S. haematobium* (rSh28GST), *S. mansoni* tetraspanin 2 (Sm-TSP-2), and S. japonicum associated-protein 23 (Sj23) antigens have progressed to clinical trials [12,77]. Very recently, an integrated immunomic approach was used to identify novel antibody markers for the diagnosis of urogenital schistosomiasis is caused by *S. haematobium*. From the list of 253 candidates that were screened, the authors identified two targets, Sh-TSP-2 and MS3_01370, which are considered to have high application potential [15].

3.3. Protein microarrays for identifying leishmaniasis antigens

Leishmaniasis remains a major public health problem throughout the tropics, and serodiagnostics and vaccines are strongly needed in this regard. The 35 Mb genome of *Leishmania* was sequenced in late 2002. It contains approximately 8,500 genes, and it is assumed that the genome will translate into more than 10,000 proteins [78]. These major research advances could eventually allow rapid screening for specific parasite genotypes and assist in diagnostic and epidemiological studies. Immunomics-based studies have validated a panel of potential antigens and identified novel targets for leishmaniasis (Table 4). An immunoproteomic approach was recently performed in Brazil using visceral leishmaniasis patient sera, as well as sera from Chagas disease patients and healthy endemic controls. The same study identified 29 and 21 valid sequences in the promastigote and amastigote stages of *Leishmania* and *Trypanosoma* parasites, respectively [25]. In another study in Brazil, a novel *L. infantum* hypothetical protein called LiHyGwas identified. The antigen can be used for accurate serum diagnosis of visceral leishmaniasis in dogs and humans and can serve as a potential prognostic marker of human disease [83].

3.4. Protein microarrays for identifying babesiosis antigens

Babesiosis is caused by the invasion of erythrocytes by *Babesia* parasites. *Babesia microti* is considered a major etiological agent of emerging human babesiosis, a VBPD transmitted by ticks. To identify pathogen-encoded factors involved in host-parasite interactions, Silva and colleagues screened 174 *B. microti* proteins to construct a proteome array that included several predicted parasite secretory proteins. The authors used the immunoproteomic approach and identified several novel antigens that trigger strong host immune responses during the onset of infection [84]. Moreover, antibody responses to various *B. microti* BmGPI antigens were detected in both humans and animal reservoirs, and BmGPI12 was identified as the best biomarker of infection because of its high sensitivity and specificity when used in a microarray antibody assay [28]. Therefore, BmGP112 is a promising candidate biomarker for the detection of *B. microti* antibodies that might be useful in blood screening to prevent transfusion-transmitted babesiosis. Recently, we used antisera from BALB/c mice infected with *B. microti* to screen diagnostic antigens of *B. microti* by selecting 204 target sequences from homologous proteins

Table 2. List of records obtained from PubMed and web of science searches for malaria antigen screening via protein microarrays.

Species	Disease	Antigen candidate identified/validated, description	References
Pf	Malaria	MSP1,2,3,10 (merozoite surface protein 1,2,3), GPI-AP	[8,10,16,18,20,39,43,60,61]
		LSA1,3 (liver-stage antigen1, 3)	[10,16–18,39,45,46,60,62]
		AMA1 (apical membrane antigen 1), moving junction	[8,17,18,39,45,50,63]
		EBA-175 (erythrocyte-binding antigen), EBL family	[8,10,16,17,45,46]
		CSP (circumsporozoite protein), virulence	[18,39,45,64]
		SSP2/TRAP, hypothetical	[17,39,45,49]
		STARP (sporozoite threonine asparagine-rich protein)	[16,18,45,46,64]
		PfEMP1 (Pf erythrocyte membrane protein 1)	[18,20,45,46,60,65]
		SERA5 (serine repeat antigen 5)	[20,45,46]
		RALP1 (rhoptry-associated leucine zipper-like protein)	[8]
		MRSP2 (MSP7-like protein)	[8,20]
		ETRAMP11,14 (early transcribed membrane protein 11,14)	[8,10,20]
		Conserved *Plasmodium* protein, hypothetical	[8,10,18,20,45,46,64]
		D18(Protein disulfide isomerase)	[49]
		PTP5 (EMP1-traffificking protein)	[43]
		PfEMP1 (VAR)erythrocyte membrane protein 1	
		ApiAP2 (transcription factor with AP2 domain(s))	
		Transmembrane emp24 domain containing protein	
		Asparagine-rich antigen	
		Conserved protein, unknown function	
		SYN6 (SNARE protein, putative)	
		HSP70 (heat shock protein 70)	
Pv	Malaria	MSP1 (merozoite surface protein 1), GPI-AP	[44,48,66,67]
		AMA1 (apical membrane antigen 1)	[48,68]
		PvCSP (circumsporozoite protein)	[69]
		Pv12, GPI-anchored protein, Cys6 family	[44,48,57,70]
		MSP7, 8, 10 (merozoite surface protein 7,8,10),	[43,44,48,68,69,71]
		GPI-anchored proteins	[19,43,48]
		ETRAMP11 (early transcribed membrane protein)	[43,48]
		Hypothetical	[43]
		WD, Gbeta-repeat domain containing protein	[57]
		Protein transport protein SFT2, putative	
		Transcription factor with AP2 domain(s), putative (ApiAP2)	
		Plasmodium exported protein, unknown function	
		Major blood stage surface antigen Pv200	
		Zinc finger protein, putative	
		Hypothetical, predicted Pf homolog liver stage antigen 3	
		RBP2b (reticulocyte binding protein 2b)	
		RAMA (rhoptry-associated membrane antigen)	
		MSP1–19 (merozoite surface protein1–19)	
		Pv-fam-a (tryptophan-rich antigen)	
		PvEBPII (erythrocyte-binding protein II)	
		MSP3.10 (merozoite surface protein 3.10)	
Pk	Malaria	TF (serotransferrin)	[58]
		HPX (hemopexin)	

between *B. microti* and *B. bovis* genome sequences from the PiroplasmaDB (http://piroplasmadb.org) using protein arrays. In this study, we identified 10 (5.9%, 10/169) highly immunoreactive proteins, most (80%, 8/10) of which had not been characterized before [26]. These data suggest that these immunoreactive proteins could potentially be useful as candidate antigens for the development of diagnostics for babesiosis. In a further study, we applied protein microarrays and screened 87 targeted proteins in the plasma of an infected mouse model [27]. Later, six antigens were selected, and short synthetic peptides were screened via microarray platforms; five antigenic peptides were identified from *B. bovis* (ApBb) and further validated via ELISA [85]. Finally, biomarkers for disease progression have been identified using immunoreactivity profiles, which represent useful information for the rational development of diagnostics and vaccines in the future. Table 5 provides a list of *Babesia* antigens identified and/or validated using protein microarrays.

3.5. Protein microarrays for identifying trypanosomiasis antigens

Chagas disease is caused by the parasitic hemoflagellate *T. cruzi*. It is a lifelong and debilitating disease endemic to the Americas. It is estimated that six to seven million people have been infected, with approximately 50,000 new cases each year [87,92]. Despite this alarming situation, the lack of available vaccines and effective drugs means that the main control strategy for Chagas disease still relies on the prevention of parasite transmission. Significant targets have been identified to improve the serological diagnosis of Chagas disease (Table 5). A high-density peptide chip, a highly multiplexed platform based on next-generation sequencing, helped identify 2,031 disease-specific peptides and 97 novel parasite antigens. These data set the stage for high-throughput biomarker discovery screenings and proteome-wide studies of immune responses against *Trypanosoma* pathogens [30].

Table 3. List of records obtained from PubMed and web of science searches for schistosomiasis antigen screening via protein microarrays.

Species	Disease	Antigen candidate identified/validated, description	References
Sj	Schistosomiasis	Butyl-cholinesterase	[74]
		Sj-TST-26 (tetraspanin), a homolog of human TST-33	[74–76]
		Microsomal glutathione-S-transferase (GST)	[74,75]
		Calpain	[11,21,23,72,76]
		Extracellular superoxide dismutase (Cu–Zn)	[22]
		Sj23, 29 (*S. japonicum* membrane-associated protein 23,29)	[22]
		STIP1 (homology and U-Box-containing protein 1)	[75]
		PPase (ADP-ribose pyrophosphatase)	[75]
		Hypothetical/guanylate kinase associated	[23]
		SjTHBS1 (thrombospondin 1), hypothetical	[23,75]
		Hypothetical protein	
		Sj-L6 L-1 (Ly-6-like protein 1)	
Sm	Schistosomiasis	Sm-TSP-2 (*S. mansoni* tetraspanin)	[11,72]
		Smp80 (calpain)	[11]
		Sm14, fatty acid-binding protein (FABP) family	[11]
		Sm-CaM-2,3 (*S. mansoni* calmodulin 2,3)	[11]
		Sm-TSP-3 (25-kDa integral membrane protein, Tetraspanin 3)	[11,23,24,72]
		Sm29 (*S. mansoni* membrane-associated protein 29)	[11,24,72,75,76]
Sh	Schistosomiasis	Acetylcholinesterase, Tetraspanin-33	[74]
		rSh28GST, glutathione S-transferase	[15]
		Facilitated glucose transporter member 1	[15]
		Sodium/potassium transporting ATPase subunit	
		Calpain	
		Sh-TSP-2(Tetraspanin 2)	
		MS3_01370(CD63 antigen)	

Sj, *Schistosoma japonicum*; Sm, *Schistosoma mansoni*; Sh, *Schistosoma haematobium*.

Table 4. List of records obtained from PubMed and web of science searches for leishmaniasis antigen screening via protein microarrays.

Species	Disease	Antigen candidate identified/validated, description	References
Li	Visceral leishmaniasis or Cutaneous leishmaniasis	Actin	[79–81]
		Endonuclease III	[25,79]
Lp	Mucosal leishmaniasis	GTP-binding protein	[79]
		Heat shock proteins, HSP70 , HSP 83–1	[82]
		Eukaryotic initiation factor 5a, putative	
		Parafagellar rod protein 1D	
		Cytochrome oxidase subunit IV, putative	
		MORN repeat-containing protein 1	
		Translation elongation factor 1-beta, putative	
		Tryparedoxin peroxidase the paraflagellar rod proteins (PFR)	
		cell surface protease leishmanolysin (GP63)	
		GRP78	
		CAS/CSE/importin domain protein	
		Endonuclease III	
		Paraflagellar rod protein 1C,1D	
		Hypothetical protein	
		RNA-binding protein	
		Proteasome activator protein	
		Peroxidoxin	
		Alpha-tubulin, Beta-tubulin	
		Pyridoxal kinase	
		Elongation factor 1-beta,2	
		ALBA-domain protein 1	
		GTP-binding protein	
		IQ calmodulin-binding motif containing protein	
		Poly(A) polymerase	
		Calpain-like cysteine peptidase	
		Cytochrome oxidase subunit IV	
		Right-handed beta helix region/periplasmic copper-binding protein	
		Proteasome activator protein pa26	
		Peroxidoxin	
		Eukaryotic translation initiation 5A	
		Leucine-rich repeat protein	
		Putative CAS/CSE/importin domain protein	
		Glucosamine 6-phosphate n-acetyltransferase	
		Mannosyl oligosaccharide glucosidase	
		Cytochrome b5-like haem/steroid binding domain containing protein	
		PFPI/DJ-1-like protein	
		Enolase	
		Eukaryotic initiation factor 4a	
		B-box zinc finger containing protein	

Li, *Leishmania infantum*; Lp, *Leishmania panamensis*.

Table 5. List of records obtained from PubMed and web of science searches for babesiosis, chagas disease, onchocerciasis, and lymphatic filariasis antigen screening via protein microarrays.

Species	Disease	Antigen candidate identified/validated, description	References
Bmi	Babesiosis	Herpes_BLLF1 domain	[26]
		GCC-2 GCC-3 domain	[26,27]
		2A & PRK domain	[26–28]
		S3 Ae domain	
		PTZ domain	
		BmGPI proteins	
		BMN1–3 protein	
Bb	Bovis babesiosis	Hypothetical protein	[85]
		Membrane protein	
		Subtilisin-like protein	
		Apical membrane antigen 1 (RGSDDTSESSDRYSG)	
		Apical membrane antigen 1 (ASRGQLLNSRRGSDD)	
Tc	Chagas disease	GDH (glutamate dehydrogenase)	[29,30]
		Cytoplasmic antigen (CRA)/calpain cysteine peptidase	[29]
		Cetrotransposon hot spot (RHS) protein, putative	[29,86]
		Kinetoplast DNA-associated protein, putative	[29,86]
		Lectin, putative	[29,86,87]
		Malate dehydrogenase, putative	
		60S ribosomal protein L7a, putative, antigenic protein	
		TSSA (trypomastigote small surface antigen)	
		TryP (Tryparedoxin Peroxidase)	
Ov	Onchocerciasis	OVOC9752 (acpS; holo-[acyl-carrier protein] synthase)	[88]
		OVOC7453 (groEL, HSPD1; chaperonin GroEL)	[89]
		OVOC12400 (PRPF31; U4/U6 small nuclear ribonucleoprotein PRP31)	
		OVOC9748 (GALNT; polypeptide N-acetylgalactosaminyl transferase)	
		OVOC9325 (KIDINS220, ARMS; ankyrin repeat-rich membrane spanning protein)	
		OVOC9988 (ROR2, NTRKR2; receptor tyrosine kinase-like orphan receptor 2)	
		OVOC9592 (SNRP70; U1 small nuclear ribonucleoprotein 70kDa)	
		OVOC8985 (GGPS; geranylgeranyl diphosphate synthase, type II)	
		OVOC12449 (POU3F, OTF; POU domain transcription factor, class 3)	
		OVOC9475 (SUMO, SMT3; small ubiquitin-related modifier)	
		OVOC4612 (PRCC; proline-rich protein PRCC)	
		OVOC6327 (MYO5; myosin V)	
		OVOC5718 (RBM7; RNA-binding protein 7)	
		OVOC7381 (EPRS; bifunctional glutamyl/prolyl-tRNA synthetase)	
		OVOC11487 (GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase)	
		OVOC10067 (K08473; nematode chemoreceptor)	
		OVOC10103 (ECE; endothelin-converting enzyme)	
		OVOC5823 (DCTN5; dynactin 5)	
		OVOC9990 (ROR2, NTRKR2; receptor tyrosine kinase-like orphan receptor 2)	
		OVOC2486 (NDUFA1; NADH dehydrogenase(ubiquinone)1 alpha subcomplex S)	
		OVOC10995 (ALDH5A1; succinate-semialdehyde dehydrogenase)	
		OVOC3203 (CAP1_2, SRV2; adenylyl cyclase-associated protein)	
		OVOC11847 (NOVA; RNA-binding protein Nova)	
		OVOC1213 (EDD1, UBR5; E3 ubiquitin-protein ligase EDD1)	
		OVOC12448 (SF3A1, SAP114; splicing factor 3A subunit 1)	
		OVOC7430 (SOS; son of sevenless)	
		OVOC5419 (HSD17B10;3-hydroxyacyl-CoA dehydrogenase/3-hydroxy-2-methyll)	
		OVOC1897 (RP-L5e, RPL5; large subunit ribosomal protein L5e)	
		OVOC11218 (glgB;1,4-alpha-glucan branching enzyme)	
		OVOC10638 (APPBP1; amyloid beta precursor protein binding protein 1)	
		OVOC10982 (ARFGAP2_3; ADP-ribosylation factor GTPase-activating protein 2/3)	
		OVOC9384 (MRD1, RBM19; multiple RNA-binding domain-containing protein 1)	
		OVOC10469 (hypothetical secreted protein precursor)	
		OVOC10602 (Conserved secreted protein precursor - signalP detected)	
		OVOC11950 (hypothetical secreted protein precursor)	
		OVOC3261 (hypothetical secreted protein precursor)	
		OVOC5127 (DNA-binding protein of the nucleobindingfamily - signalP detected)	
		OVOC8491 (fatty acid retinoid binding protein 2)	

(*Continued*)

Table 5. (Continued).

Species	Disease	Antigen candidate identified/validated, description	References
Bma	Lymphatic filariasis	Bm1_21705 (actin 1, putative) Bm1_45215 (intermediate filament protein, putative) Bm1_40320 (Disorganized muscle protein 1, putative) Bm1_19805 (small heat shock protein, putative) Bm1_04450 (Paramyosin, putative) Bm1_02615 (Paramyosin, identical) Bm1_48810 (EF hand family protein) Bm1_13015 (Nematode cuticle collagen N-terminal domain containing protein) Bm1_01235 (Tropomyosin-related) Bm1_49075 (Calponin homolog OV9M, putative) Bm1_40715 (myosin heavy chain, putative) Bm1_54705 (Nematode cuticle collagen N-terminal domain containing protein) Bm1_50805 (Myosin tail family protein) Bm1_40465 (Cuticular glutathione peroxidase precursor, putative) Bm1_00935 (myosin heavy chain B (MHC B), putative) Bm1_16060 (carbohydrate phosphorylase, putative) Bm1_14060 (myosin heavy chain B (MHC B), putative) Bm1_17485 (Nematode cuticle collagen N-terminal domain containing protein) Bm1_02060 (Tropomyosin family protein) Bm1_53470 (glutamine synthetase, putative)	[90,91]

Bmi, *Babesia microti*; Bb, *Babesia bovis*; Tc, *Trypanosoma cruzi*; Ov, *Onchocerca volvulus*; Bma, *Brugia malayi*.

3.6. Protein microarrays for identifying other VBPD antigens

Studies on antigen discovery and/or testing for other VBPDs, including those for lymphatic filariasis [90,91] and onchocerciasis [88,89], have also been performed (Table 5). A previous study highlighted an integrated immunoproteomic approach that identified adult female *O. volvulus* antigens for the development of serodiagnostics for human onchocerciasis. In that study, the authors identified 241 highly immunoreactive proteins, which included 33 new proteins that are promising serodiagnostic antigens [88]. In addition, a further study identified seven biomarkers of active patent infection (OVOC10469, OVOC10602, OVOC11950, OVOC3261, OVOC5127, OVOC8491, and OVOC9988) that have not been previously reported based on IgG4 responses in infected individuals. These highly antigenic volvulus proteins could serve as specific and sensitive biomarkers of patent infection [89].

4. Conclusion

This review highlights research on B-cell epitopes of surface antigens of major vector-borne parasites, including *Plasmodium*, *Schistosoma*, *Leishmania* and *Babesia*, to provide insights for the rational design of blood-stage malaria vaccine candidates. Additionally, the potential applications of biomarkers in infections for early diagnosis and prognostic purposes were explored. These findings collectively contribute to advancing our understanding of host – parasite interactions, antigen discovery, and immune responses in vector-borne parasitic diseases, paving the way for improved disease control strategies, diagnosis, therapy, and vaccine design.

5. Expert opinion

The identification of antigen markers via the immunomic approach can be applied to VBPD control in the following ways: vaccine development, diagnosis, disease surveillance, and treatment.

Protein/peptide arrays enable the rapid discovery of new vaccine candidate antigens for preclinical and clinical development. The identification of antigens can aid in the development of more effective vaccines by targeting specific antigens that are essential for the survival of the pathogen. Applications of this technology for vaccines include (1) the discovery of candidate antigens for subunit vaccines through the analysis of correlates of protection, (2) immunogenicity studies for the optimization of vaccine formulations and dosing regimens, (3) subtyping of immune responses (e.g., antibody isotypes and subclasses), and (4) the quantification of adjuvant effects on the immune response after vaccination. However, a large repertoire of potential antigen candidates for VBPDs are genetically variant, as they undergo host immune selection. Therefore, research on combinations of vaccines that target different parasite life cycle stages may be needed to achieve high efficacy. For example, the combination of multivalent vaccines and conserved epitope vaccine approaches can be used to select vaccines with broad reactivity for preclinical evaluation and inclusion in multiantigen vaccines for clinical trials.

The identification of specific antigen markers can be used to develop diagnostic tools for the detection of VBPDs. Antibody-based detection of infectious diseases is an ideal and cost-effective platform for diagnostic testing. Protein microarray technology facilitates the discovery of proteins targeted by antibodies that provide both sensitive and specific detection of infection and active disease. With well-characterized samples, a clear pathway from discovery to development of a new serodiagnostic could be established [14]. Under the premise of the application of advanced technologies such as proteomics, the serological diagnosis of insect-borne parasitic diseases has improved, but more sensitive and specific diagnostic methods that meet these standards need to be established to achieve permanent control of these diseases. In addition, large-scale evaluations to assess the advantages and disadvantages of different antigens and diagnostic technologies are needed for a sensitive and specific gold standard. Here,

again, the use of multiple antigen combinations could be helpful for detecting antibodies against concurrent and (recent) past infections caused by VBPDs. Although serological testing still has several limitations, it is still a valuable approach for controlling and accelerating the elimination of VBPDs [93,94].

In disease surveillance and treatment, the identification of antigen markers via the immunomic approach can be used for disease surveillance and monitoring and for developing targeted therapies for VBPDs. This approach can aid in the early detection of outbreaks and in the monitoring of disease incidence. In addition, targeted therapies can be developed by targeting specific antigens that are essential for the survival of the pathogen.

The identification of antigen markers by the use of the immunomic approach for VBPD control can present several challenges. These challenges include the following:

(i) Limited sample size: Antigen discovery often requires the analysis of a large number of samples to establish reliable associations between specific antigens and disease. However, obtaining a sufficient number of samples, especially for rare diseases or in resource-limited settings, can be challenging
(ii) Variability in antigen expression: Antigen expression can vary among individuals, pathogens, and disease stages. This variability can make it difficult to identify consistent antigen markers that can be used for disease control.
(iii) False-positive results: The upregulation of surface markers used as antigen markers can be influenced by factors other than the specific antigen of interest. For example, cell culture contaminants or factors secreted by bystander cells can lead to false-positive results.
(iv) Complexity of omics data: The identification and application of antigen markers often involve the analysis of complex omics data, such as genomic, transcriptomic, or proteomic data. Analyzing and interpreting these large datasets can be challenging, requiring expertise in statistical analysis and bioinformatics.
(v) Validation and reproducibility: Once potential antigen markers are identified, they need to be validated in independent cohorts to ensure their reliability and reproducibility. Validation studies can be time-consuming and resource-intensive.
(vi) Heterogeneity of diseases: Many VBPDs can exhibit heterogeneity in terms of pathogen strains, host immune responses, and disease manifestations. This heterogeneity can complicate the identification and application of antigen markers that are universally applicable for disease control.

Despite these challenges, the identification and application of antigen markers via immunomics remains a promising approach for VBPD control. Advances in technology and data analysis methods should continue to improve the ability to identify and utilize antigen markers for the prevention, diagnosis, and treatment of various diseases.

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Author contributions

X Zhou and QQ Zhang conceived the study, collected and analyzed the data, and drafted the manuscript. JH Chen, JF Dai and K Kassegne conceived the project, provided technical support for data collection and analysis and revised the manuscript. All authors read and approved the final manuscript.

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