Parasitol Res (2016) 115:459–468
DOI 10.1007/s00436-015-4824-1

REVIEW

Review on the identification and role of *Toxoplasma gondii* antigenic epitopes

Yanhua Wang[1] · **Guangxiang Wang**[1] · **Jianping Cai**[1] · **Hong Yin**[1]

Received: 24 September 2015 / Accepted: 10 November 2015 / Published online: 19 November 2015

Abstract *Toxoplasma gondii* is an obligate intracellular protozoan parasite with a broad range of hosts, and it causes severe toxoplasmasis in both humans and animals. It is well known that the progression and severity of a disease depend on the immunological status of the host. Immunological studies on antigens indicate that antigens do not exert their functions through the entire protein molecule, but instead, specific epitopes are responsible for the immune response. Protein antigens not only contain epitope structures used by B, T, cytotoxic T lymphocyte (CTL), and NK cells to mediate immunological responses but can also contain structures that are unfavorable for protective immunity. Therefore, the study of antigenic epitopes from *T. gondii* has not only enhanced our understanding of the structure and function of antigens, the reactions between antigens and antibodies, and many other aspects of immunology but it also plays a significant role in the development of new diagnostic reagents and vaccines. In this review, we summarized the immune mechanisms induced by antigen epitopes and the latest advances in identifying *T. gondii* antigen epitopes. Particular attention was paid to the potential clinical usefulness of epitopes in this context. Through a critical analysis of the current state of knowledge, we elucidated the latest data concerning the biological effects of epitopes and the immune results aimed at the development of future epitope-based applications, such as vaccines and diagnostic reagents.

Keywords *Toxoplasma gondii* · Epitopes · Identification and role · Epitope-based vaccines · Epitope-based diagnostic reagents

Introduction

Toxoplasma gondii is a globally distributed obligate intracellular protozoan parasite that causes zoonotic parasitosis (Raizman and Neva 1975; Sharif et al. 2015). *T. gondii* has a complex life cycle with many antigenic compositions, and each antigen can induce distinct immune responses in the body (Dubey et al. 1995). It is also known that the immunological effects of monovalent subunit vaccines and diagnostic reagents involving single antigens are not ideal (Beghetto et al. 2003a; Mévélec et al. 2005; Wang et al. 2013c). Therefore, the development of multi-epitope-based vaccines and diagnostic reagents will be necessary (EL-Malky et al. 2014; Grzybowski et al. 2015; Wang et al. 2013a; Wang and Yin 2014 b). Epitope is through the recognition of foreign or nonself epitopes that the immune system can identify and hopefully destroy pathogens (Toseland et al. 2005). Epitopes would be potentially useful as effective vaccine and diagnostic reagents. For these reasons, studies of *T. gondii* antigen epitopes are receiving increasing attention from researchers. This article reviews the immune mechanisms induced by antigen epitopes, the latest advances in identifying *T. gondii* antigen, and the potential clinical usefulness of epitopes.

✉ Yanhua Wang
wangyh061001@163.com

[1] State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China

Studies on antigenic B cell epitopes

B cell epitope prediction based on bioinformatics

The adoption of immunoinformatics methods for the prediction of antigenic epitopes has become an indispensable tool for epitope localization. These methods can reduce the blindness and improve the accuracy of epitope identification. In addition, such techniques are economical and effective and can substantially reduce experimental costs (Wang et al. 2009).

Predictive study on the linear epitope of B cells

Following significant developments in bioinformatics, a variety of parameters and algorithms including schemes of hydrophilicity, flexibility, accessibility, and antigenicity (Chou and Fasman 1987; Emini et al. 1985; Garnier and Robson 1989; Hoop and Wood 1981; Karplus and Schulz 1985; Kyte and Doolittle 1982) has been developed, which have played a significant role in promoting the study of linear B cell epitopes. To improve predictions of epitopes, it is often necessary to combine a variety of algorithms and results from multi-level analysis. Based on the efforts of many researchers, new analytic software programs have been developed for these applications, such as the ABCpred software program developed by Saha and Raghava (2006). B cell epitopes from the main antigenic molecules (GRA1, GRA2, GRA4, and MIC3) from *T. gondii* have been studied using software-based prediction in combination with IgG avidity assay (Maksimov et al. 2012).

Studies on the prediction of conformational B cell epitopes

It is also known that most of the B cell epitopes (~90 %) are conformational epitopes. Combining computerized prediction algorithms and experimental approaches has led to rapid developments in conformational epitope analysis and localization, and a number of useful prediction programs have been released, including the CEP (Kulkami-kale et al. 2005), DiscTope (Andersen et al. 2006), and MEPS (Castrignano et al. 2007) software programs. The application of techniques to display peptide libraries using phages in combination with computer modeling for conformational epitope prediction is an alternative method for predicting conformational epitopes in B cells. In particular, antibodies are used to filter a phage peptide library to obtain antibody affinity-simulating peptides. This collection of simulating peptides is then compared to obtain a probe sequence, which is used to search for homologous amino acid sequences on the surface of the antigen protein. Scott (Scott and Smith 1990) was the first to use a phage-expressed random peptide library for antigen localization, which has significantly contributed to studies of antigenic protein epitopes. Currently, many types of representative algorithms are available, and web-based predictive services include the Pepitope (Bublil et al. 2007), 3DEX (Schreiber et al. 2005), and MI2MOX (Huang et al. 2006) algorithms.

Expression of *T. gondii* antigens in segments

In addition, some researchers have expressed genes in segments and then used monoclonal or polyclonal antibodies to screen out the segments with positive reactions to determine the antigenic epitope. The antigenicity of SAG1 is primarily controlled by a 1/3 to 4/9 region from the N terminus (Nam et al. 1996). Residues 125–269 encompass all B cell epitopes recognized on the SAG1 protein after infection with the parasite, and the sequence of residues 125–165 is essential for the structural integrity of these B cell epitopes (Velge-Roussel et al. 1994). For GRA protein, the 59 C-terminal amino acids from *T. gondii* GRA2 contain at least three B cell epitopes (Murray et al. 1993); the 11 amino acids at the C terminus and the amino acids 318–334 from *T. gondii* GRA4 proteins contain a major B cell epitope and a second B cell epitope that was present at a relatively low frequency, respectively (Mevelec et al. 1998). In addition, the antigenic epitopes of the GRA6 proteins of *T. gondii* were also identified by Li et al. (2003).

Peptide scanning technique

In the past, several experimental techniques were developed for mapping antibody-interacting residues on an antigen, including the identification of interacting residues from the structure of antibody–antigen complexes (Van Regenmortel 1989). One popular approach is the synthetic peptide technique, which primarily identifies sequential epitopes (Frank 2002). Many researchers have applied this technique to study epitopes (Cardona et al. 2009; Godard et al. 1994; Siachoque et al. 2006; Wang et al. 2013a, b). In murine experimental models, antibody recognition appeared to be more broadly distributed along the SAG1 sequence. In the absence of any carrier protein, the peptide (238–256 aa) induced a B and T cell immune response independent of the route of immunization (oral route or subcutaneous injection). This peptide (238–256 aa) induced multiple antibody isotypes. In contrast to the 238–256 peptide, the 48–67 peptide, either (82–102 aa, 213–230 aa, and 279–285 aa) free or in the form of a multiple antigenic peptide (MAP) construct, or the 279–295 aa peptide elicited antibodies associated with a TH2 response (Godard et al. 1994). However, there were no correlations between antibody production and survival, and significant differences existed in humoral immunogenicity and protection for peptides derived from SAG1 (Siachoque et al. 2006). It is generally known that protection against *Toxoplasma* mainly

depends on a cellular-mediated immune response; thus, antibodies alone do not guarantee protection against this parasite.

Furthermore, Cardona et al. (2009) discovered that in addition to several C-terminal fragments of SAG1 (181–200 aa, 241–260 aa, 261–280 aa, and 301–320 aa), the sera of *T. gondii*-infected humans recognized a N-terminal fragment (61–80 aa), although the 301–320 aa had the highest reactivity. Similarly, our laboratory used sera from *T. gondii*-infected pigs to screen B cell epitopes of SAG1 and showed that fragments composed of 91–120 aa, 151–180 aa, 271–300 aa, and 301–336 aa were all specifically recognized by the porcine sera and that the 271–300 aa fragment displayed the strongest reactivity (Wang et al. 2013b). A comprehensive analysis of the above findings indicated that the sera from *T. gondii*-infected humans and pigs and SAG1-immunized mice exhibited some differences in serum recognition patterns. This mapping of antigenic epitopes could advance the development of diagnostic reagents and epitope vaccines for *T. gondii*. Although this method has been used successfully to identify certain B cell epitopes, epitopes in overlapping areas may have been omitted. Therefore, some B cell epitopes from the targeted protein could not be identified.

Phage display technique

The phage display technique is a powerful tool for studying antigenic epitopes (Parmley and Smith 1988; Smith 1985; Scott and Smith 1990). Using a random peptide library, linear and conformational antigenic epitopes can be simultaneously obtained, and this method does not require the predetermination of protein amino acid sequences. Candidate gene fragments are directly inserted into the phage coat gene locus, leading to the expression of exogenous polypeptides that are presented and displayed on the surface of the phage while maintaining specific spatial conformations. Then, the proteins or polypeptides are screened for specificity and affinity. This technology has been widely used in studies of the antigenic epitopes of *T. gondii*, and many epitopes from SAG1, GRA1, GRA3, GRA7, GRA8, MIC3, MIC5, and SAG2A were obtained (Beghetto et al. 2001; Beghetto et al. 2003b; Cunha-Júnior et al. 2010; Lin et al. 2004; Robben et al. 2002). And, these results indicate that epitope-displaying phage can induce partial protection against *T. gondii* (Lin et al. 2004) and would be very useful in understanding the host–pathogen relationship (Grimwood and Smith 1995).

X-ray diffraction studies of complexes between antigen and fab fragments

Several studies, based mainly on chemical or recombinant approaches, have been carried out to identify the different T and B cell epitopes. However, chemical or recombinant peptide sequences contained only linear protein epitopes, which undoubtedly omitted important conformational information (Cason 1994). Little is known about the conformational B cell epitopes of antigen proteins from *T. gondii*. Only one conformational epitope of SAG1 was identified. This epitope present on the surface of SAG1 and located within the SAG1 N-terminal domain did not overlap with the proposed ligand-binding pocket. This study provided the first structural description of the monomeric form of SAG1 and significant insights into its dual role of adhesin and immune target during parasite infection (Graille et al. 2005).

Many tools for identifying and predicting B cell epitopes have been developed previously. However, conformational epitope selection relies on the determination of the tertiary structure of an antigen to identify residues that interact with antibodies. The experimental techniques required to determine the tertiary structure of the antigen, such as crystallography, are expensive and time-consuming, and the mapping of conformational epitopes has been severely hampered. The majority of methods and databases have focused on the identification of linear epitopes to date (Saha and Raghava 2007; Vita et al. 2010). Several identified linear epitopes that have potential clinical applications have been summed up in Table 1.

Studies on T cell epitopes

T cell epitope identification based on bioinformatics and the synthesized peptide technique

The prediction of T cell epitopes can be divided into two categories: cytotoxic T lymphocyte (CTL) epitope prediction and T helper (Th) epitope prediction. CTL epitope prediction primarily involves the prediction of major histocompatibility complex (MHC)-I type molecules, and Th epitope prediction involves the affinity-based prediction of MHC-II type molecules. Epitope prediction for T cells is primarily based on whether the candidate peptide can combine with MHC molecules. The length of multi-peptide-binding MHC-I type molecules is between 8 and 11 amino acids, generally 9. Furthermore, there is an anchor point on the specific location of the sequence. The length of affinity peptide-binding MHC-II molecules can be over 30 amino acids, and there are varying degrees of degradation within the binding motif (Brusic et al. 2004). Consequently, creating algorithms for the prediction of affinity peptides that bind to MHC-II type molecules is more difficult.

There are five main prediction methods for MHC molecular affinity peptides: sequence similarity prediction, molecular modeling method, binding motif method, quantitative matrix method, and machine learning method. Sequence similarity prediction has relatively low accuracy and is rarely used.

Table 1 Identified linear B cell epitopes from *T. gondii* antigens

Proteins	Position	References
SAG1	A 1/3 to 4/9 region from the N terminus	Nam et al. 1996
	125–165 aa	Velge-Roussel et al. 1994
	48–67aa, 82–102 aa, 213–230 aa, 238–256 aa, and 279–285 aa	Godard et al. 1994
	61–80 aa, 181–200 aa, 241–260 aa, 261–280 aa, and 301–320 aa	Cardona et al. 2009
	91–120 aa, 151–180 aa, 271–300 aa, and 301–336 aa	Wang et al. 2013b
SAG2A	137–141 aa	Cunha-Júnior et al. 2010
GRA1	162–177	Maksimov et al. 2012
	134–153 aa, 164–183 aa, and 214–233 aa	Wang et al. 2013b
	172–186 aa	Beghetto et al. 2001
GRA2	28–43 aa and 61–76 aa	Maksimov et al. 2012
	The C-terminal 59 amino acids	Murray et al. 1993
	46–100 aa	Beghetto et al. 2003a
GRA3	46–126 aa	Beghetto et al. 2003b
	44–85 aa	Robben et al. 2002
GRA4	309–324 aa and 321–346 aa	Maksimov et al. 2012
	318–334 aa	Mevelec et al. 1998
	62–77 aa, 233–252 aa, and 314–333 aa	Wang et al. 2014a
GRA6	43–152 aa	Li et al. 2003
GRA7	24–102 aa	Beghetto et al. 2003a
GRA8	52–87 aa	Beghetto et al. 2003b
MIC3	191–206 aa and 282–297 aa	Maksimov et al. 2012
	234–307 aa	Beghetto et al. 2003a
MIC5	31–163 aa	Beghetto et al. 2003b

The molecular modeling method can reveal interaction mechanisms within molecules, although it is not suitable for high-throughput data processing. The binding motif method is simple and easy to implement, and it is particularly suitable for the prediction of MHC allele-binding peptides without extensive experimental data. The quantitative matrix method uses linear processing, but because it is difficult to add new experimental data into the prediction model, the versatility of predictions using this method is diminished. In contrast, the machine learning method solves the problem of searching for core-binding motifs, and it can integrate information from peptide residue interactions to improve the specificity, accuracy, and applicability of predictions (Shao and Feng 2008). One epitope of SAG1 (238–256 aa) (Godard et al. 1994) and two epitopes of ROP2 (197–216 and 501–524 aa) (Saavedra et al. 1996) have been predicted and identified.

Although many immunoinformatics methods for epitope prediction have been established and applied, some prediction methods are limited by the complexity of the immune system. Therefore, when identifying the T cell epitopes, immunoinformatics can be combined with other methods, such as the peptide scanning technique, to obtain more accurate results.

CD8+ T cell epitope identification based on the caged major histocompatibility complex tetramer technology

The generation of interferon (IFN)-γ by innate NK cells and by CD4+ and CD8+ T lymphocytes is crucially important to host resistance; therefore, some *T. gondii*-derived CD8+ T cell epitopes in some proteins (Tgd057, GRA6, GRA4, and ROP7) have been identified using the caged MHC tetramer technology (Frickel et al. 2008; Wilson et al. 2010). The CTL epitopes defined in three antigens (GRA6, GRA4, and ROP7) are all H-2^{Ld}-restricted, but the CTL epitope defined in Tgd057 is H-2^{Kb}-restricted CTL epitope. Tgd057-specific CTLs were activated during acute infection period or after vaccination with live, irradiated parasites. In contrast, ROP7- and GRA6-specific CTLs correlate with the establishment of chronic infection (Frickel et al. 2008), and tgd057-specific CTLs are immediately available for processing in antigen-presenting cells. Phenotypically, tgd057-specific effector CTLs were generally representative of the total CD8+ T cell population and contained higher frequencies of cells expressing granzyme B and IFN-γ compared to the polyclonal population. And, tgd057-specific CTLs probably also represent an immunodominant population; they are similar to the

immunodominant GRA6-specific CTLs. Tgd057-specific CTLs also can mediate significant protective immunity to lethal parasite challenge in adoptive transfer recipients (Kirak et al. 2010). The epitopes that afford protection against parasites may facilitate the development of strategies to vaccinate against or otherwise control pathogen.

Impact factors on antiparasitic CD8 T cell responses

CD8 T cells play a key role in immune-mediated protection against intracellular apicomplexan parasites. Although the *T. gondii* proteome is intricate, CD8+ T cell responses are restricted to only a minority of peptide epitopes derived from a limited set of antigenic precursors. This phenomenon is referred to as immunodominance. One factor that may influence the immunogenicity and immunoprotection of potential CD8 antigens is the intracellular pathway by which pathogen-derived antigens are processed and presented in infected host cells (Grover et al. 2014). Of course, the route of protein trafficking after secretion, the C-terminal position of the epitope within the source antigen (Feliu et al. 2013), the ability of the peptide to bind MHC, and the affinity and precursor frequency of the responding T cells also impact the immunogenicity and immunoprotection of potential CD8 antigens (Moon et al. 2007; Obar et al. 2008). Moreover, although most CD8+ T cell responses appear to be targeted to invaded host cells (Goldszmid et al. 2009), the ability of particular antigens to be efficiently cross-presented by noninvaded bystander cells could potentially promote strong CD8+ T cell responses independently of the mode of secretion from the parasite (Mashayekhi et al. 2011). The knowledge of the mechanisms that enhance immunogenicity and determine the immunodominance hierarchy may help to get better natural immune responses and improve vaccine design against intravacuolar pathogens or other therapies against intracellular pathogens.

Studies of epitope-based vaccines

The principles of epitope-based vaccine design

Immune cells primarily recognize protein epitopes through cell surface receptors. Epitopes are generally 5–7 amino acids in length and rarely exceed 30 amino acids. The MHC combines with T cell epitopes and presents polypeptide fragments to T cells to destroy pathogeny. This complex of MHC-I type molecules and the antigenic peptide stimulates CD_8+ T cells to differentiate into CTL, which directly kills infected cells (Pinilla et al. 1999). MHC-II type molecules combine with the surface receptors of CD_4+ T cells after presenting the T epitope, and together, they stimulate CD_4+ T cells to proliferate and differentiate into Th cells. Helper T cells stimulate B cells, activate and recruit numerous other immune cells, and, via the secretion of interleukin (IL)-2 and other cytokines, provide auxiliary to cytolytic T cells, which, in turn, exert their effector function by killing infected cells (Sospedra et al. 2003). B cells combine with the B cell epitope of the exogenous protein antigen through surface B cell receptors to phagocytize the protein, which they then process to present the Th epitope and the MHC-II molecular complex containing the antigen on the surface of cells, ultimately to be identified by CD_4+ T cells. B cells are activated by the secretion of cellular molecules and through interactions with co-stimulatory molecules, eventually producing specific antibodies (Garside et al. 1998). Figure 1 illustrates the process of immune cells to eliminate pathogens. Therefore, epitopes are the basic unit of the immunological response, which is the basis for the design of epitope-based vaccines.

Carriers for epitope peptide vaccines

As an immunogen is transmitted from pathogens to protein molecules to epitope polypeptides, its molecular weight gradually decreases and the specificity of the induced immune response is enhanced. Conversely, the likelihood of developing inhibitory effects or pathological epitopes is decreased. However, immunogenicity also exhibits a pattern of gradual decline, and therefore, many vaccines based on small molecules do not stimulate a satisfactory protective response. Indeed, this has become one of the main obstacles for breakthroughs in the field of molecular vaccine research (Ert and Xiang 1996). Therefore, improving polypeptide immunogenicity, affinity, and stability has become the focus of many current studies. Experiments confirm that the immunogenicity of polypeptides can be improved by forming complexes with other polypeptides (Gagnon et al. 2000), connecting with a lysine core, coupling them to protein-type carriers or a VLP-based carrier (Billaud et al. 2005; Gluck et al. 2005; Yang et al. 2005), and/or incubating them with dendritic cells (Lv and He 2001).

Studies on multi-epitope DNA vaccines for T. gondii

The life cycle of *T. gondii* is relatively complex, and its antigenic component can change in specificity or makeup during different development stages; therefore, vaccination with stage-specific antigens may only exhibit stage-specific protection (Alexander et al. 1996). A vaccine that worked against every stage of the life cycle would certainly yield better protective effects (Girish 2003). For this reason, efforts have been made to develop *T. gondii* vaccines combining antigens from multiple development stages or from distinct property (e.g., membrane proteins, microneme proteins, and dense granules). One of the most attractive strategies is to design multi-epitope-based DNA vaccines (Cai et al. 2007; Cong et al. 2008). Multi-epitope-based DNA vaccines, also known

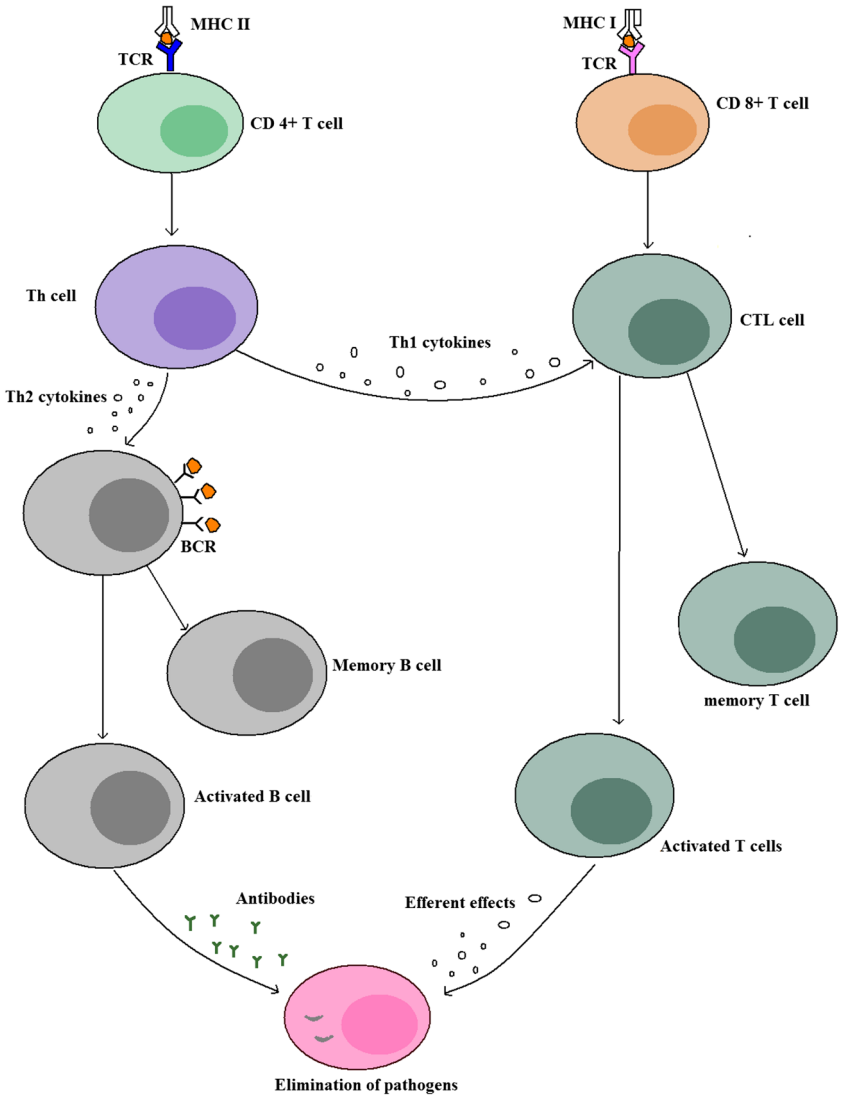

Fig. 1 Activation of CD8+ T/CD4+ T lymphocytes to kill *T. gondii*-infected cells

simultaneously, carry multiple epitopes related to the targeted antigens or helper epitopes. In addition, multi-epitope-based DNA vaccines remove factors that are unfavorable to the protective immune response, and they can often induce immune protection that is highly specific. Multi-epitope-based DNA vaccines are now a popular subject of research, and they have yielded increasing results (Cong et al. 2008; Shi et al. 2008). The multi-epitope antigen can present a diverse array of antigenic compositions, and it is also possible to carry out specific operations due to a variety of advanced methods and improved techniques.

Studies on multi-epitope synthetic peptide vaccines for T. gondii

Monomeric peptides usually cannot induce an immune response. Previously, crosslinking epitope peptides to carrier proteins were mostly performed to improve immunogenicity, although the carrier proteins often had inherent defects. Carrier proteins contain their own epitopes, and the induced immune response is often primarily against the carrier itself. In addition, carriers can sometimes induce epitope inhibition, which is a significant problem. However, MAP constructs can strongly induce the immune response (Tam 1988). Structurally, MAP is a radially branching peptide with an oligomeric lysine at its core, a unique spatial structure that can allow for multiple protective antigenic epitopes to be fully expanded in space, forming the three-dimensional epitope clusters. This structure simplifies the transportation and presentation of antigenic epitopes, which has been confirmed in murine models. The oligomeric lysyl has a low molecular weight and is only weakly immunogenic, which has been confirmed by previous studies. Therefore, MAP does not induce an immune response against itself, but it can significantly enhance the immunogenicity of antigenic epitopes to induce high-level-specific immune responses. The MAP method has been used to develop

experimental vaccines for toxoplasmasis. In our laboratory, we investigated murine immune responses to one linear B cell epitope (derived from conserved regions of SAG1) when conjugated to two other defined T cell epitopes (from conserved regions of GRA1 and GRA4) in a MAP arrangement. The results indicated that MAP construct could trigger strong humoral and cellular responses against *T. gondii* and that this MAP is a vaccine candidate worth further development (Wang et al. 2011).

In addition, nanoparticles (NPs) can modulate the immune response and can be potentially useful as an efficient vaccine adjuvant. Recently, a nanoparticle-based vaccine for *T. gondii* was developed to deliver a *T. gondii* antigen CD8+ T cell epitope peptide (720–28 aa LPQFATAAT) from GRA7 and a universal CD4+ T cell epitope (derived from PADRE). These results indicated that NPs conjugated with groups that permit specific recognition of DCs allow a more precise localization of these cells and that use of these self-assembling nanoparticles as a platform for a vaccine approach to protect against toxoplasmosis is very available (El Bissati et al. 2014).

Compared with traditional vaccines, epitope-based vaccines have many advantages. (1) As they do not contain inactivated pathogens, these vaccines are very safe, completely removing any potential threat of infection. (2) Vaccines based on different combinations of T and B cell antigenic epitopes can induce multiple immune protective responses, making it possible to develop broad-spectrum vaccines that can prevent a variety of diseases. (3) The high specificity of epitope-based vaccines cannot be equaled by subunit vaccines created through genetic engineering. (4) Epitope-based vaccines can also be used as effective therapeutic vaccines. (5) Epitope-based vaccines can eliminate certain unfavorable aspects of the immunological reaction, they can avoid the "immune escape effect" caused by the rapid mutation rates of pathogens, and they can decrease the potential for autoimmune responses due to homology between complete antigens and host molecules. (6) Finally, epitope-based vaccines can also induce very strong cellular immunity within the body by selecting for specific types of immunization, which is particularly important for combating intracellular parasites such as *T. gondii*.

Diagnostic reagents based on antigenic epitopes from T. gondii

Methods for the diagnosis and detection of toxoplasmosis include etiological, immunological, and molecular biological methods. However, immunological methods, particularly ELISA, remain the preferred means of diagnosis and detection.

The primary testing antigens used for ELISA include crude tachyzoite antigen, excreted–secreted antigen, recombinant antigen, and synthetic peptide antigen. The compositions of crude tachyzoite antigen and excreted–secreted antigen are complex, and they are difficult to standardize with respect to quantity and quality. Therefore, there will be differences in the effectiveness of diagnostic tests involving these types of antigens (Beghetto et al. 2006; Hassl et al. 1991; Hofgartner Swanzy et al. 1997; Taylor et al. 1990). At present, the primary recombination proteins including SAG1, SAG2, MIC3, ROP2, GRA1, GRA6, and GRA7, particularly multi-epitope recombinant antigens (Dai et al. 2012; Dai et al. 2013), have been applied in diagnostic assays. But essentially, all recombinant antigens are purified from *Escherichia coli*, which can lead to nonspecific reactions when testing mammalian serum. Moreover, some recombinant antigens show lower reactivity with specific antibodies than the corresponding native antigens, mainly because of the differences in protein folding that can result in altered epitope presentation (Burg et al. 1988; Harning et al. 1996; Holec-Gasior 2013). Variations in sensitivity and specificity were also observed with respect to the level of recognition of recombinant antigens by serum samples (Maksimov et al. 2012). Therefore, several high-quality antigens are necessary for the detection of toxoplasmosis. Synthetic peptide antigens are specific, inexpensive, and can be easily standardized. These peptides can also be produced on a large scale without risk of infection, making them attractive candidates for the detection of toxoplasmosis. To date, synthetic peptide-based ELISA has been used to detect many viruses, bacteria, and parasitic diseases (Blasco et al. 2007; de Oliveiraa et al. 2008; Kannangai et al. 2001; Plagemann 2006; Vordermeier et al. 2001). Recently, researchers have investigated the possible use of multi-epitope synthetic peptide in immunological tests for toxoplasmosis. These results indicate that particular peptides from MIC3 (MIC3–282: GVEVTLAEKCEKEFGI; MIC3–191: SKRGNAKCGPNGTCIV) were recognized with a significantly higher intensity by sera from acutely infected patients than by sera from latently infected patients and that these peptides may be candidates for the diagnosis of acute toxoplasmosis in humans (Maksimov et al. 2012).

The use of multi-epitope antigens in the serodiagnosis of toxoplasmosis would be conducive to improving the standardization of the diagnosis and reducing their production costs (Holec-Gasior 2013). In addition, multi-epitope peptide products may not only facilitate the development of more reliable and more consistent test systems but may also allow the development of new tests capable of discriminating acute infections from latent infections.

Ethical approval

Ethical approval was obtained from the Ethical Committee of the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Conclusions

Despite significant progresses in studying *T. gondii* epitopes, many theoretical and technical hamper epitope-based vaccines or diagnostics from becoming commercial vaccine or diagnostics. (1) Due to the complexity of immune response mechanisms within the body, the design and optimization of epitope-based vaccines or diagnostics have been difficult, hampering the application and promotion of these types of vaccines or diagnostics. (2) The application of epitope-based vaccines or diagnostics is highly dependent on the accurate identification of conformational B cell epitopes and Th epitopes. At present, epitope identification remains at the level of prediction and simulation, and we are not able to completely simulate the natural spatial conformation of epitopes. (3) It is unclear how multiple epitopes are arranged and combined to yield optimal effect, and there is currently a lack of experimental evidence and theoretical models on this subject. Hopefully, the above problems will be resolved and epitope-based vaccines or diagnostics will become widely adopted.

Acknowledgments This study was supported by grants from the Natural Science Foundation of Gansu Province, China (145RJZA234).

Authors' contributions YW and GW drafted the manuscript. JC and HY revised the manuscript. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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