

Letter to the Editor

Fine localization of the minimal B-cell epitopes of *Echinococcus granulosus* rEg.P29 and immunogenicity of epitope-based vaccines

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Dear Editor,

Echinococcus species, which are classified under the phylum Platyhelminthes and the class Cestoda, are parasitic organisms that cause a range of diseases in humans, with cystic echinococcosis (CE) being particularly notable [1]. At present, the implementation of prophylactic vaccination against CE represents a highly appropriate and efficacious preventive measure [2,3]. In recent years, epitope peptide vaccines have attracted increasing attention because of their ease of production, target specificity, safety, and efficacy [4]. The P29 protein is a newly identified 29 kDa antigen derived from the protoscoleces of *E. granulosus* [5]. Our earlier studies confirmed that rEg.P29 has a protective effect against secondary infections of *E. granulosus* in mice and sheep [6,7]. However, the precise epitope of Eg.P29 was unclear. Identifying this epitope is highly beneficial for understanding antibody-antigen interactions and guiding future vaccine design efforts. In previous studies, we identified 10 linear B cell epitopes on the basis of bioinformatics and animal experiments (Supplementary Table S1 and Supplementary Figure S1). We subsequently synthesized two candidate dominant epitopes by using the amino acid overlap regions of the 10 dominant epitopes. (Supplementary Table S2 and Supplementary Figure S2). To pinpoint the minimal residues of the linear B cell epitopes, we performed incremental truncations on peptides B1 (aa 64–89) and B2 (aa 164–186). In our research, shorter N- and C-truncated peptides were created via the sequential elimination of one or three amino acids. All the truncated peptides were analyzed via ELISA using antisera from rEg.P29-immunized mice. The amino acid sequences of the two groups of truncated peptides are presented in Supplementary Tables S3 and S4. The peptide ELISA results demonstrated the reactivity of rEg.P29 antiserum with the truncated peptides (Supplementary Figure S3).

N-truncated peptides effectively bound to the antiserum up to the removal of the Ser76 residue, in contrast to the control group (Supplementary Figure S3A). C-truncated peptides demonstrated robust binding to antiserum until the Gln89 residue was removed, as illustrated in Supplementary Figure S3B. In the case of B2, the binding capacity of the truncated peptides was significantly diminished upon the removal of either the Thr169 or the Glu184 residue, in contrast to the control group (Supplementary Figure S3C, D). This process enabled us to determine that the minimal motifs for the two linear B cell epitopes are ⁷⁶KITTTDKLGTALEQ⁸⁹ and ¹⁶⁹AKTAEQKAKWEAEVRK¹⁸⁴, respectively.

To initially assess the antigenicity of the identified epitopes, we employed ELISA to detect specific antibodies. In addition, we synthesized the tandem peptide P29₇₆₋₈₉₊₁₆₉₋₁₈₄ (KITTTDKLGTALEQSGSGSAKTAEQKAKWEAEVRK) (Supplementary Figure S2). All animal experimental protocols in this study were approved by the Ningxia Medical University Ethics Committee (No. SCXK2016-0006) and performed following the laboratory conditions described in our previous work [8]. All groups that received the epitope-based vaccine exhibited increased levels of specific IgG and IgM antibodies. Notably, the epitope peptide P29₇₆₋₈₉ and the linked peptide P29₇₆₋₈₉₊₁₆₉₋₁₈₄ showed binding affinity to IgE in the rEg.P29 + CFA/IFA antiserum. However, none of the three B cell epitope peptides demonstrated the ability to bind to IgA in the rEg.P29 + CFA/IFA antiserum. Notably, P29₇₆₋₈₉₊₁₆₉₋₁₈₄ appears to show a stronger binding capacity than P29₇₆₋₈₉ and P29₁₆₉₋₁₈₄ (Figure 1A–D). Serum IgG isotype analysis was conducted in mice vaccinated with rEg.P29 enhanced the IgG1/IgG2b response. The isotype ratio (IgG1/IgG2b) was found to exceed 1.0, while control sera exhibited low levels of both IgG1 and IgG2b. IgG3 levels were negligible in all analyzed samples (Figure 1E–I). This pattern was consistent across

all adjuvanted vaccine formulations [9]. PyMOL 1.1 software was used to analyze the locations of two immunodominant epitopes (aa 76–89 marked blue, aa 169–184 marked red) (Supplementary Figure S4A). Using the NCBI website, we compared the amino acid sequences of the *Echinococcus* P29 protein from various sources. We focused on the immunodominant epitopes at aa 169–184 and

aa 76–89 (Supplementary Figure S4B). Nevertheless, these two epitopes have not been reported previously. Location analysis revealed that these two B cell epitopes are situated on the exterior of the P29 protein. Conservation analysis revealed that the epitopes were conserved across various *E. granulosus* strains (Supplementary Figure S4). These findings align with the characteristics typical

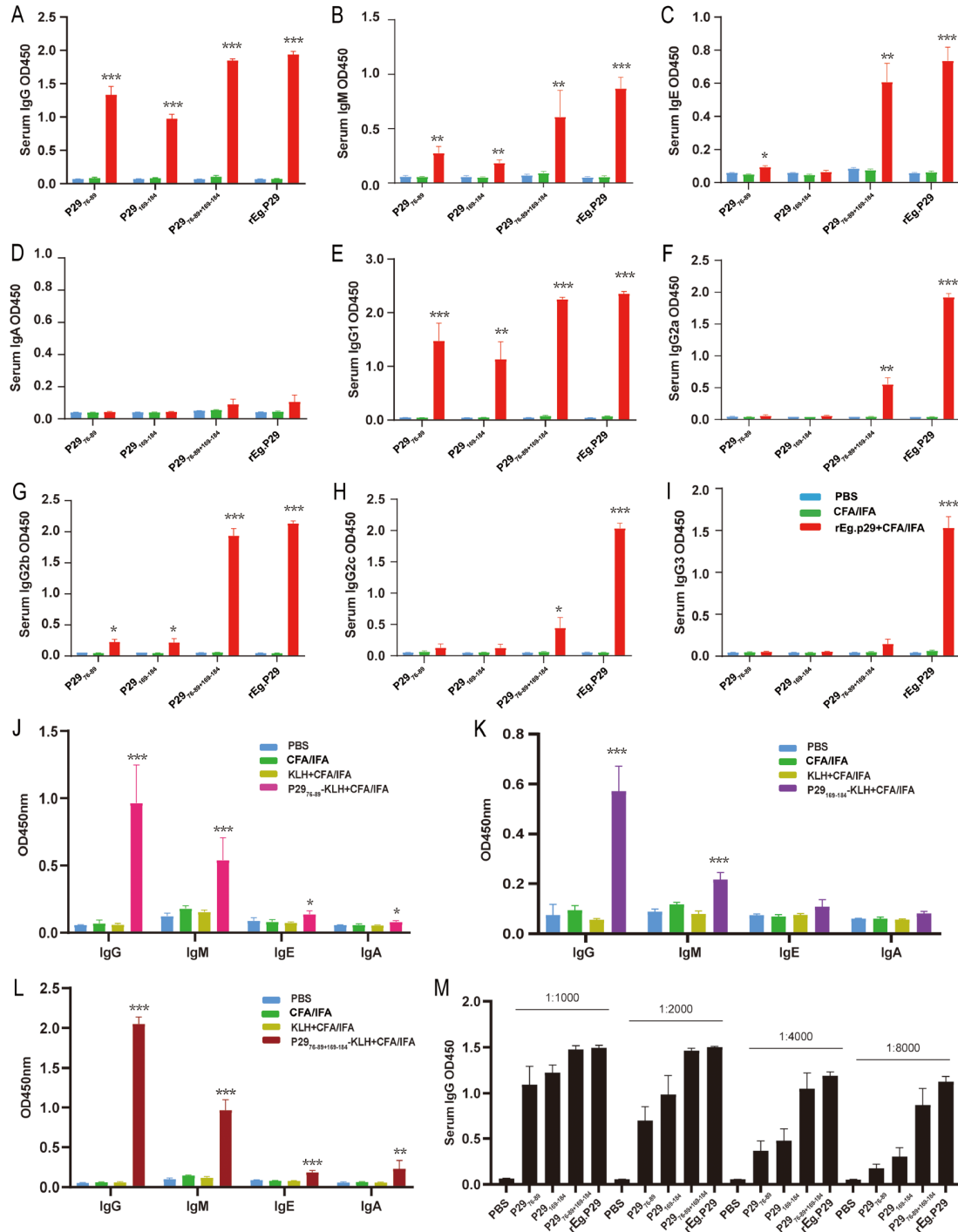


Figure 1. Detection of specific antibodies and antibody responses induced by epitope-based vaccines (A–D) ELISA detection of specific antibodies against the identified epitopes. ELISA plates were coated with P29_{76–89}, P29_{169–184}, and P29_{76–89+169–184}, respectively. (E–I) Detection of IgG subclasses in antiserum from rEg.P29-immunized mice. (J–L) Detection of peptide-specific antibodies in antiserum from mice immunized with KLH-conjugated epitopes (P29_{76–89}, P29_{169–184}, and P29_{76–89+169–184}) plus CFA/IFA. Dominant epitope-specific antibody titers measured at 1:8000 dilution in immunized mouse serum. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of a decoy epitope.

Our investigation revealed that the epitopes P29₇₆₋₈₉, P29₁₆₉₋₁₈₄, and P29₇₆₋₈₉₊₁₆₉₋₁₈₄ serve as the dominant B cell epitopes for the P29 protein. However, the ability of these epitopes to elicit an immune response *in vivo* has yet to be determined. In the course of this study, a prime-boost immunization regimen was used to inoculate C57BL/6 mice. Mice were immunized with KLH-conjugated epitopes (P29₇₆₋₈₉, P29₁₆₉₋₁₈₄, and P29₇₆₋₈₉₊₁₆₉₋₁₈₄) along with an adjuvant. The antibody isotypes employed included IgM, IgG, IgE, and IgA. Our study revealed that compared with control immunization, epitope-based vaccine immunization in mice led to higher levels of peptide-specific antibodies, which primarily induced IgM- and IgG-dominated antibody responses. The linking peptide P29₇₆₋₈₉₊₁₆₉₋₁₈₄ demonstrated increased reactivity (Figure 1J–L). Although vaccines based on multiple B cell epitopes are not yet approved for human use, our study suggests that this strategy has the potential to enhance *E. granulosus* vaccine design. Each immunologically dominant epitope in the peptide can individually elicit a specific antibody response. Moreover, vaccines incorporat-

ing linked epitopes induce stronger and more sustained antibody responses. Analysis of titers revealed that at a 1:8000 dilution, the mean antibody titer in the group immunized with the linking epitopes plus the CFA/IFA epitope-based vaccine remained higher than that in the other two groups (Figure 1M).

Analogous findings emerged from the examination of serum IgG subclasses in mice vaccinated with epitopes. Analysis of serum IgG isotypes in peptide-immunized mice revealed a mixed IgG1/IgG2b response, which was intensified by the inclusion of KLH and the CFA/IFA adjuvant. Furthermore, mice immunized with a vaccine based on linked peptide epitopes showed enhanced antisera reactivity, characterized by an isotype ratio (IgG1/IgG2b) greater than 1. In contrast, the control serum samples displayed low levels of both IgG1 and IgG2b. This vaccination approach elicits robust IgG1/IgG2b-specific responses, with a notable emphasis on IgG1, and concurrently promotes elevated levels of IgG2a, IgG2c, and IgG3 (Figure 2A–C). In mice vaccinated with the epitopes, an analysis of serum IgG isotypes revealed an IgG1/IgG2b ratio greater than 1, indicating a Th2-biased immune response.

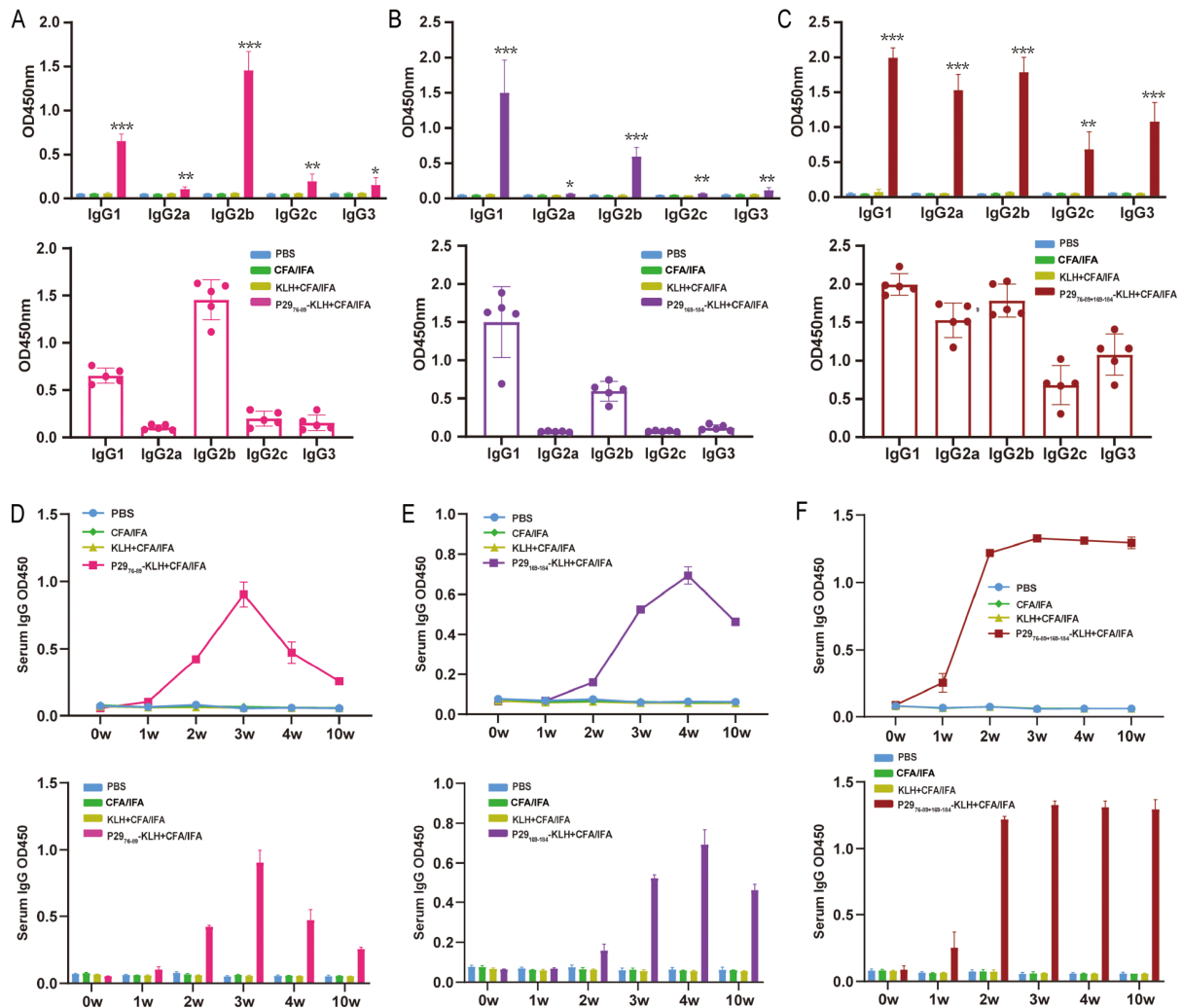


Figure 2. Polypeptide immunization induces specific IgG1 responses and the linked-epitope vaccine enhances antibody responses (A–C) Detection of IgG subclass levels in the antisera of mice immunized with different peptides. (A) P29₇₆₋₈₉ immunized group, (B) P29₁₆₉₋₁₈₄ immunized group, (C) P29₇₆₋₈₉₊₁₆₉₋₁₈₄ immunized group. (D–F) Kinetics of antibody responses induced by different epitope-based vaccines. (D) Epitope peptide 76-89 immunized group. (E) Epitope peptide 169-184 immunized group. (F) Epitope peptide 76-89+169-184 immunized group. The linked-epitope vaccine (F) induced stronger and more sustained antibody responses. *P < 0.05, **P < 0.01, ***P < 0.001.

To establish this concept, three peptide conjugates were synthesized using KLH as a carrier protein: one derived from the sequence of P29₇₆₋₈₉, another from P29₁₆₉₋₁₈₄, and the third from the combination of P29₇₆₋₈₉₊₁₆₉₋₁₈₄. C57BL/6 mice were subjected to immunization with these conjugates following a prime-boost regimen and were treated with CFA/IFA as adjuvants. The immunization process was carried out on three separate occasions, with a one-week interval between each session. Serum samples were collected at 0, 1, 2, 4, and 10 weeks after the initial immunization and subsequently tested for the presence of specific antibodies. As shown in Figure 2D–F, the P29₇₆₋₈₉ and P29₁₆₉₋₁₈₄ immunization groups began to increase their specific antibody levels in the second immunization compared to the PBS and CFA/IFA groups. Antibody levels peaked at weeks 3 and 4 but then decreased thereafter. Moreover, P29₇₆₋₈₉₊₁₆₉₋₁₈₄ immunization resulted in higher levels and a longer duration of specific antibodies compared to the P29₇₆₋₈₉ and P29₁₆₉₋₁₈₄ immunization groups, maintaining, and these levels were maintained for more than 10 weeks.

In summary, we identified two previously unreported core residues of immunodominant B cell epitopes in the Eg.P29 protein. We confirmed that these two epitopes strongly induce immune responses in mice, with the linking peptide P29₇₆₋₈₉₊₁₆₉₋₁₈₄ demonstrating superior binding ability. We further showed its ability to elicit robust IgG1 and IgG2b responses, especially epitope-specific IgG1 responses, characterized by an isotype ratio (IgG1/IgG2b) > 1.0, which suggests a Th2-biased response. In addition, epitope-based vaccines containing linked epitopes elicited a more sustained antibody response. These findings indicate that linked peptides may have greater potential as vaccine components. The findings from our work may have significant scientific implications for guiding the design of vaccines in the future. However, our study has several limitations. CFA/IFA has not been approved for human use. The T cell-mediated cellular response to the B cell epitope vaccine was not assessed in our investigation. In future studies, our team intends to identify and evaluate the protective T cell epitopes associated with rEg.P29. We aim to develop more effective *E. granulosus* vaccines by employing a human-approved adjuvant and combining B cell-dominant epitopes with Th2-specific T cell epitopes.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

The data that underpin the findings of this study are accessible within the methods and materials section of this article.

Author Contributions

Shasha Li and Yinqi Zhao contributed equally to this work. Shasha Li conceived and designed the study, performed the animal immunization experiments and antibody detection, analyzed the

data, and drafted the manuscript. Yinqi Zhao performed the epitope truncation analysis, ELISA experiments, and peptide synthesis. Tingrui Zhang assisted with animal experiments, antibody detection, and data analysis. Yazhou Zhu assisted with animal immunization and data collection. Hui Li and Hong Ma assisted with data collection. Wei Zhao supervised the entire study, secured funding, and critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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