Short Communication

IgE antibodies from schistosomiasis patients to recognize epitopes in potato apyrase

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Abstract

Introduction: High percentages of structural identity and cross-immunoreactivity have been reported between potato apyrase and Schistosoma mansoni ATP diphosphohydrolase (SmATPDases) isoforms, showing the existence of particular epitopes shared between these proteins. Methods: Potato apyrase was employed using ELISA, western blot, and mouse immunization methods to verify IgE reactivity. Results: Most of the schistosomiasis patient’s (75%) serum was seropositive for potato apyrase and this protein was recognized using western blotting, suggesting that parasite and plant proteins share IgE-binding epitopes. C57BL/6 mice immunized with potato apyrase showed increased IgE antibody production. Conclusions: Potato apyrase and SmATPDases have IgE-binding epitopes.

Keywords: ATP diphosphohydrolase. IgE. Potato apyrase.

Schistosomiasis infections are characterized by high levels of specific and non-specific IgE antibodies and eosinophilia1,2. The NTPDase family (ATP diphosphohydrolase-EC 3.6.1.5) shares conserved amino acid sequences that also exhibit antigenic properties, especially between parasite and plant isoforms1. This close structural relationship between antigenic NTPDase domains from different organisms might have a strong link with the physiological role of this protein family in parasites, previously described as being involved in purinergic signaling and contributing to successful parasitism4.

Schistosoma mansoni has two genes encoding NTPDase isoforms, the ATP diphosphohydrolases (SmATPDases) 1 and 25, which have cross-immunoreactivity with potato apyrase3,6,7,8,9,10. Both experimentally infected mice7 and schistosomiasis patients8 showed elevated levels of IgG reactivity with the shared epitopes between S. mansoni ATP diphosphohydrolase isoforms and potato apyrase7,8. These shared epitopes were detected by in silico analysis3, encouraging construction of a recombinant polypeptide (r-Domain B; 6xHis tag polypeptide). This belongs to a conserved domain of 40 amino acids from the potato apyrase, which exhibits immunostimulatory properties and reactivity with IgG subclasses of schistosomiasis patients9. In addition, a specific peptide from SmATPDase 2, shared with potato apyrase was used for studying its immunostimulatory property and for localizing this specific parasite protein in eggs10.

IgE antibody reactivity against potato apyrase has now also been investigated. Highly purified potato apyrase (50 kDa) was obtained in our laboratory from a commercial strain of Solanum tuberosum, as previously described6. Serum samples from schistosomiasis patients and healthy individuals were collected in accordance with Brazilian National Council of Research in Humans guidelines, with approval from the Ethical Committee for Human Research of René Rachou Research Center (CPqRR), Belo Horizonte, Minas Gerais, Brazil, under protocols CEPSH/CPqRR 06/2001 and 04/2005. These serum samples were used in ELISA1 and western blots6.

In ELISA, IgE antibody reactivity was tested in potato apyrase coated microplates using isotype-specific anti-human
IgE peroxidase-conjugated (Zymed) or isotype-specific anti-mouse IgE peroxidase-conjugated (Zymed) diluted 1:10,000, and o-phenylenediamine/H$_2$O$_2$ (Sigma Chemical Co, USA) as the substrate. The subsequent color reaction was read at 492 nm wavelength on a microplate reader (Molecular Devices Corp., Menlo Park, USA). For statistical analysis of ELISA, median and 95% confidence interval were calculated, and data were analyzed using the Mann–Whitney test to compare the two groups (GraphPad Prism Software; version 5). As shown in Figure 1A, IgE antibody reactivity (0.189 ± 0.217; 1:50) of serum samples diluted 1:50 from schistosomiasis patients (n = 102; 78% seropositivity) to potato apyrase (1µg/ml) is significantly (p < 0.001) higher than those found in healthy individuals (n = 26; 0.030 ± 0.026).

As IgG antibody levels were higher than IgE levels, the hypothesis that stated their competition for the same epitopes in ELISA, was ruled out. For this, randomly selected serum samples from healthy individuals (n = 8) and schistosomiasis patients (n = 20) were treated with anti-human IgG. After this treatment, serum samples from schistosomiasis patients (80% seropositivity) showed higher IgE antibody reactivity (0.843 ± 0.416; dilution 1:20) when compared to the control group (0.238 ± 0.154, p < 0.01), confirming the elevated seropositivity of this immunoglobulin isotype against potato apyrase in schistosomiasis patients (Figure 1B). Therefore, cross-immunoreactivity of specific IgE antibodies from schistosomiasis patients with potato apyrase suggests that parasite and plant proteins share IgE-binding epitopes.

To prove the induction of IgE, C57BL/6 mice (n= 4) were immunized with 3 intraperitoneal injections of apyrase (10 µg) with 15-day intervals, the first being emulsified in Freund’s complete adjuvant, and the others in Freund’s incomplete adjuvant. The serum samples obtained 15 days after the third injection were pooled and depleted of IgG antibodies, and used diluted 1:20 in duplicate for ELISA. As shown in Figure 2-I, the IgE antibody level of post-immunized mice (0.344 ± 0.10; p < 0.001) is significantly higher than the control (0.176 ± 0.21). Therefore, pure apyrase induces IgE production and under these experimental conditions, it is not possible to observe an allergic reaction in animals.

In addition, eight-week-old female Swiss mice (n= 5) were subcutaneously infected with approximately 50 S. mansoni cercariae (LE-BH strain) from infected Biomphalaria glabrata snails (Ethical Committee of Federal University of Juiz de Fora - 025/2012). Seven weeks post-infection, sera immune was obtained. Sera samples from sex and age-matched Swiss mice maintained under the same standard conditions during the time of the experiment were used as the healthy control. These samples were pooled, depleted of IgG antibody and were used in western blots (Figure 2-II).

Aliquots of potato apyrase (7 µg) were separated by SDS-PAGE 10%, electroblotted onto a nitrocellulose membrane, and subjected to western blots (Figure 2-II) using pooled serum samples depleted of IgG, as previously described. IgE antibodies from potato apyrase post-immunized (C) C57BL/6 mice, (D) infected-Swiss mice and (E) schistosomiasis patients recognized the potato apyrase, a polypeptide of approximately 50 kDa (Figure 2-II), and no reactivity was observed when serum samples of healthy-Swiss mice were tested (Figure 2-II, A). As a positive control, a polyclonal immune serum anti-potato apyrase developed in a New Zealand white rabbit was used (Figure 2-II, B), which identified only this protein, confirming its identity and purity. Together, these results again suggest the presence of IgE-epitopes shared between potato apyrase and SmATPases 1 and 2.

Recently, a six amino acid sequence from the SmATPDase 1 isoform was characterized as an IgE epitope. The synthetic peptide derived from this sequence had significant reactivity with IgE antibodies from serum samples of murine schistosomiasis, which also identified the SmATPDase 1 using western blots, confirming the hypothesis of IgE induction by this parasite protein. A search in the allergen protein...
database (http://www.allergome.org/) failed to detect potato apyrase, as apyrase isoforms was found only in insects such as Aedes aegypti (P50635 UNIPROT access number), Aedes albopictus (E0D877), and Tabanus yao (B3AON5). These have no structural relationships with potato apyrase or SmATPDases proteins. Here, we demonstrated cross-immunoreactivity for the first time between potato apyrase and IgE antibody, and stimulatory property of this protein to induce IgE production. Possibly, the IgE-binding epitope on the potato integral apyrase is not sufficient to cause an allergic reaction. On the contrary, elucidation of shared amino acid sequences between these parasite and plant proteins, which are among the previously identified by in silico analyses, could reveal new epitopes with potential therapeutic for treating clinical symptoms of allergic diseases.

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