

## Short Communication

# IgE antibodies from schistosomiasis patients to recognize epitopes in potato apyrase

**Michéla Antônia do Nascimento Gusmão<sup>[1]</sup>, Nayara Braga Emídio<sup>[1]</sup>,  
Danielle Gomes Marconato<sup>[1]</sup>, Priscila Silva Grijó Farani<sup>[1]</sup>, Luiz Felipe Maniezzi<sup>[1]</sup>,  
Paulo Marcos Zech Coelho<sup>[2]</sup>, Rodrigo Correa Oliveira<sup>[2]</sup>, Eveline Gomes Vasconcelos<sup>[1]</sup>  
and Priscila de Faria-Pinto<sup>[1]</sup>**

[1]. Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.

[2]. Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, MG, Brasil.

### 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 eosinophilia<sup>1,2</sup>. 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 isoforms<sup>3</sup>. 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 parasitism<sup>4</sup>.

*Schistosoma mansoni* has two genes encoding NTPDase isoforms, the ATP diphosphohydrolases (SmATPDases) 1 and 2<sup>5</sup>, which have cross-immunoreactivity with potato apyrase<sup>3,6,7,8,9,10</sup>. Both experimentally infected mice<sup>7</sup> and schistosomiasis patients<sup>8</sup> showed elevated levels of IgG reactivity with the shared epitopes between *S. mansoni* ATP diphosphohydrolase

isoforms and potato apyrase<sup>7,8</sup>. These shared epitopes were detected by *in silico* analysis<sup>3</sup>, 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 patients<sup>9</sup>. 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 eggs<sup>10</sup>.

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 described<sup>6</sup>. 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 ELISA<sup>3</sup> and western blots<sup>6</sup>.

In ELISA, IgE antibody reactivity was tested in potato apyrase coated microplates using isotype-specific anti-human

**Corresponding author:** Priscila de Faria-Pinto.

**e-mail:** priscila.faria@ufjf.edu.br

**ORCID:** 0000-0002-7244-6357

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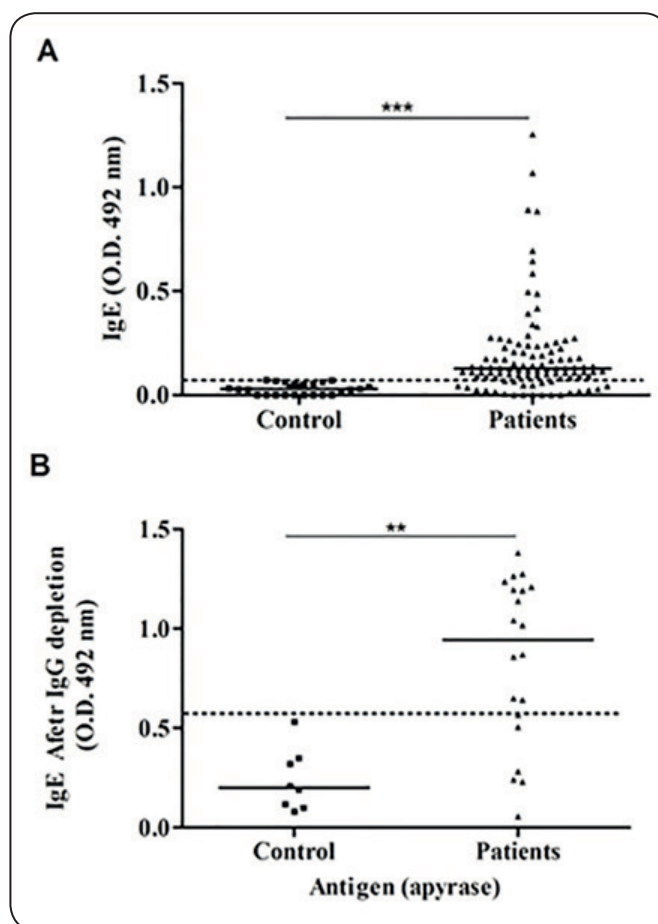
IgE peroxidase-conjugated (Zymed) or isotype-specific anti-mouse IgE peroxidase-conjugated (Zymed) diluted 1:10,000, and o-phenylenediamine/H<sub>2</sub>O<sub>2</sub> (Sigma Chemical Co, USA) as the substrate. The subsequent color reaction was read at 492 nm wavelength on a microplate reader (Molecular 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 ( $0.189 \pm 0.217$ ; 1:50) of serum samples diluted 1:50 from schistosomiasis patients ( $n = 102$ ; 78% seropositivity) to potato apyrase (1 $\mu$ g/ml) is significantly ( $p < 0.001$ ) higher than those found in healthy individuals ( $n = 26$ ;  $0.030 \pm 0.026$ ).

As IgG antibody levels were higher than IgE levels, the hypothesis that stated their competition for the same epitopes in ELISA<sup>11</sup>, 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<sup>11</sup>. After this treatment, serum samples from schistosomiasis patients (80% seropositivity) showed higher IgE antibody reactivity ( $0.843 \pm 0.416$ ; dilution 1:20) when compared to the control group ( $0.238 \pm 0.154$ ,  $p < 0.01$ ), 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  $\mu$ g) 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 ( $0.344 \pm 0.10$ ;  $p < 0.001$ ) is significantly higher than the control ( $0.176 \pm 0.021$ ). 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<sup>7</sup> (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<sup>11</sup> and were used in western blots<sup>6</sup> (**Figure 2-II**).

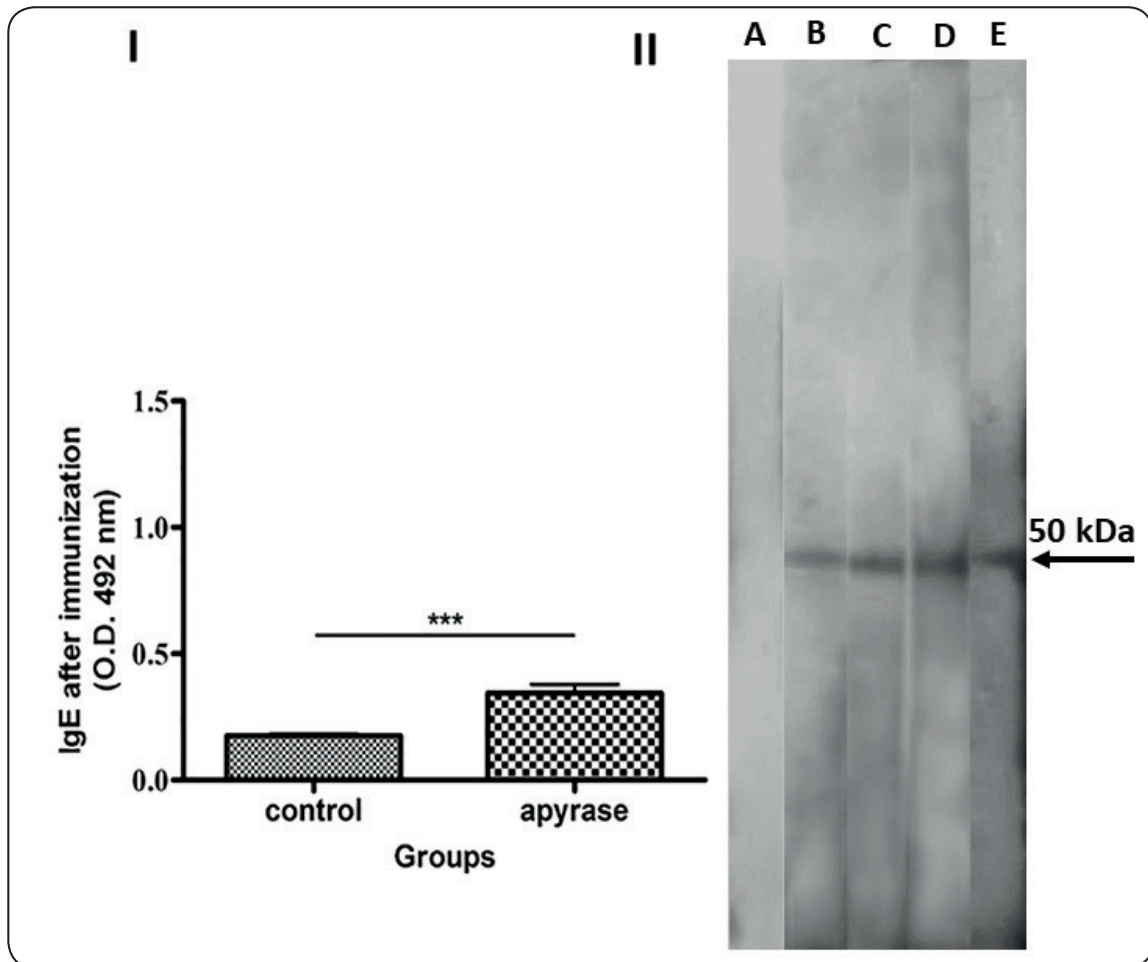
Aliquots of potato apyrase (7  $\mu$ g) were separated by SDS-PAGE 10%, electroblotted onto a nitrocellulose membrane, and subjected to western blots<sup>6</sup> (**Figure 2-II**) using pooled serum samples depleted of IgG, as previously described. IgE antibodies from potato apyrase post-immunized (C) C57BL/6 mice, (D) infected-Swiss mice and (E) schistosomiasis patients



**FIGURE 1:** IgE antibody reactivity of schistosomiasis patient's serum samples to potato apyrase by ELISA. **(A)** IgE antibody reactivity of serum samples (diluted 1:50, tested in duplicates) of schistosomiasis patients domiciled in an endemic area ( $n = 102$ ) is represented by the mean optical density (O.D.). The dotted line represents the cut-off (0.082) obtained by the mean optical density plus double the standard deviation of serum samples obtained from healthy individuals from non-endemic areas for *S. mansoni* (control group;  $n = 26$ ). \*\*\* $p < 0.001$ . **(B)** Serum samples from the schistosomiasis patients ( $n = 20$ ) or healthy individuals (control group;  $n = 8$ ) were IgG antibody-depleted. Followed by centrifugation, the supernatants were diluted 1:20 and IgE antibody reactivity with potato apyrase was tested. The dotted line represents the cut-off (0.546). \*\* $p < 0.01$ .

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<sup>6</sup> 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 SmATPase 1 isoform was characterized as an IgE epitope<sup>12</sup>. The synthetic peptide derived from this sequence had significant reactivity with IgE antibodies from serum samples of murine schistosomiasis, which also identified the SmATPase 1 using western blots, confirming the hypothesis of IgE induction by this parasite protein<sup>12</sup>. A search in the allergen protein



**FIGURE 2:** (I) IgE antibody production in C57BL/6 mice induced by potato apyrase immunization, evaluated by ELISA assay. \*\*\* $p < 0.001$ . (II) Western blotting of potato apyrase and IgE antibody of (A) serum sample (1:50) from healthy-Swiss mice; (B) rabbit polyclonal serum anti-potato apyrase (1:3,000); (C) C57BL/6 mice polyclonal serum anti-potato apyrase (1:50); (D) serum samples (1:50) from *S. mansoni*-infected Swiss mice, and (E) pool of serum samples from patients with schistosomiasis. Signals were revealed by chemiluminescence using isotype-specific anti-rabbit IgG (B) or anti-mouse IgE (A, C, D, E) coupled to horseradish peroxidase, luminol as substrate (ECL Western Blotting System; GE Healthcare, Brazil), and can be detected by X-ray film.

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<sup>3</sup>, could reveal new epitopes with potential therapeutic for treating clinical symptoms of allergic diseases.

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**Conflict of Interest:** The authors declare that there is no conflict of interest.

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